Type C Virogenes: Modes of Transmission and Evolutionary Aspects

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Introduction

Extensive evidence has demonstrated that type C RNA viruses are active agents in the causation of naturally occuring cancers. Type C RNA viruses are a distinct class of vertebrate viruses which share a common morphology, protein composition, and viral life cycle. They are spherical particles containing a large single-stranded RNA as their viral genome complexed with a RNA-directed DNA polymerase (reverse transcriptase) in a central, symmetric, electron dense core surrounded by a unit membrane. During viral replication the nucleoid condenses beneath the surface of the cytoplasmic cell membrane with subsequent "budding" of the virus from the cell surface.

Type C RNA viruses have been isolated from many vertebrate species. They have been shown to cause a variety of naturally occurring vertebrate neoplastic diseases, including leukemias and sarcomas of chickens, lymphomas and related hematopoietic neoplasms and sarcomas of mice, lymphosarcomas and fibrosarcomas of domestic cats, and leukemias and sarcomas of some primates. Type C viruses have also been isolated from other mammalian species such as rats, guinea pigs, hamsters, cattle, domestic pigs, woolly monkeys, gibbon apes, and baboons (Table 1). Recently there have been reports of isolates from human tissues (see below). As yet, in some of these species, the relationship between these viruses and neoplastic diseases of their host species has not been clarified. There have also been reports of electron microscopic observations of typical type C viral particles in tissues from some other mammalian species, including dogs, horses, rhesus monkeys, and in certain human tissues, but such viruses have not yet been isolated in vitro and biochemically characterized. Type C RNA viruses exhibit varying biological activity. Some have no known pathological effect and others are extremely efficient in producing neoplasias. Also, transformation may occur either with complete or incomplete virus expression. Type C viruses have also been detected in normal tissues; embryonic and placental tissues show more type C viral expression than other differentiated tissues. The viruses produced by both normal and tumorigenic

Table I: Mammalian type C RNA virus isolates

Species	Description
Mouse *(Mus musculus)*	Many well-studied laboratory strain leukemia and sarcoma viruses (MuLV, MSV). Large variety of endogenous viruses.
Mouse *(Mus caroli)*	Antigenically related to gibbon and woolly monkey viruses (see text).
Rat *(Rattus norvegicus)*	Endogenous viruses released from numerous rat cell lines in culture. Poorly infectious.
Chinese hamster *(Cricetulus griseus)* and Syrian (or Golden) hamster *(Mesocricetus auratus)*	Poorly infectious viruses released from cells in culture.
Guinea pig *(Cavies spp.)*	Type C virus induced from cultured cells and associated with spontaneous and transmissable leukemia. Some consider it more like a type B virus.
Domestic cat *(Felis catus)*	Two distinct classes: (a) Feline leukemia and sarcoma viruses (FeLV, FeSV); (b) RD-114/CCC family of endogenous feline viruses.
Pig *(Sus scrofa)*	Endogenous viruses released from both normal and leukemic cell lines in culture. Poorly infectious.
Cattle *(Bos taurus)*	Infectious type C viruses isolated from lymphosarcoma tissue.
Woolly monkey *(Lagothrix ssp.)*	Simian sarcoma virus (SSV-1).
Baboon *(Papio cynocephalus* and other *Papio* species)	Endogenous viruses which replicate well in cells of heterologous species.
Gibbon ape *(Hylobates lar.)*	Gibbon lymphosarcoma virus (GLV).

tissues are very similar to one another in their morphology, biochemical and immunological properties (1, 2).

Transmission of virogenes

The spontaneous appearance of complete, infectious type C RNA viruses in animals of certain mammalian species and in cultured cells derived from these animals led to the hypothesis that the information for the production of such viruses might be transmitted genetically from parent to progeny along with other cellular genes (virogene-oncogene hypothesis) (3, 4). Activation of this normally repressed, genetically transmitted, type C endogenous virogene information, rather than infection from outside the animal was proposed as the most common mechanism by which type C RNA tumor viruses produce naturally occurring cancers.

Table II: Species where a COMPLETE virogene is known to be present in normal cells

Chicken	Mouse *(Mus musculus)*	Cat
Chinese Hamster	Mouse *(Mus caroli)*	Pig
Syrian Hamster	Rat	Baboon

Vertical transmission from generation to generation rather than infection from animal to animal was postulated to be the primary means by which the viral genes have been maintained in animal populations. Much subsequent experimental work supports this, the most important being that "virus-free" cell cultures (Table 2) derived from chicken, mouse, hamster, rat, pig, cat, and baboon tissues (reviewed in 5) can begin to secrete either spontaneously or after treatment with chemical inducing agents, typical complete type C viruses (6, 7). Cocultivation of the virus producing cell cultures with appropriate permissive cell lines from heterologous species has been needed to detect and increase virus production in several of these systems (8–10). The properties characterizing such endogenous mammalian type C RNA viruses which are products of the genetically transmitted virogenes are summarized in Table 3.

Table III: Properties of endogenous type C virogenes

1. DNA of all somatic and germ cells of all the animals in a species contain viral gene sequences.
2. Multiple related but not identical copies present in the cellular DNA, more than DNA from a heterologous cell that is actively producing virus.
3. Virus expression (RNA, gs antigen, polymerase, complete particles) under cellular control. Expressed in certain tissues at certain times during development.
4. Clonal lines either spontaneously or after induction are capable of releasing complete virion.
5. Cells generally resistant to exogenous infection by the homologous endogenous virus.

The endogenous type C virogenes are those sets of gene sequences that are an integral part of the host species' chromosomal DNA and code for the production of type C viruses. These gene sequences contained in normal cellular DNA should be distinguished from type C viral DNA sequences which can be added to the animal's genome from the outside by "exogenous" viral infection and subsequent integration (provirus formation) (11). Endogenous type C virogenes should also be distinguished (Table 4) from those gene sequences not originally present in the genome, that are postulated to arise by gene duplication and/or recombination mediated by the reverse transcriptase mechanism (12, 13) (protovirus formation (14)).

The endogenous virogenes and the oncogenes (those cellular genes responsible for transforming a normal cell into a tumor cell which may or may not be present

Table IV: Major differences between virogene and protovirus models

Virogene	*Protovirus*
1. Viral copies present in germ cells and somatic cells.	1. Germ cells lack virus information. Generated in rare somatic cells by chance.
2. Genes maintained in population by normal cellular replication. Reverse transcriptase *not required.*	2. Reverse transcriptase plays essential role in generating new viruses.
3. Transformation results from activation of normally latent cellular genes associated with and/or part of the viral gene sequences.	3. Transformation results from the generation of new gene sequences that do not preexist in normal cellular DNA.

as a part of the genome of type C viruses (4)) are normally repressed, but can be activated by a variety of intrinsic (genetic, hormonal) as well as extrinsic (radiation, chemical carcinogens, other infecting viruses) factors (Table 5). Regulatory genes and environmental factors determine the extent of virogene transcription.

Table V: Implications of the virogene-oncogene hypothesis

Virogenes

1. All somatic cells of a species have DNA homologous to type C virus RNA of that species (virogenes).
2. Type C viruses derived from closely related species should have closely related specific antigens, e. g., gs antigens, polymerase and their nucleic acid sequences should be more related to one another than are those viruses released by distantly related species (virogene evolution).

Oncogenes

3. The transformation specific sequences of RNA tumor viruses should be present in normal cellular DNA (oncogenes).
4. Spontaneous, chemically induced and viral induced transformed cells and tumor cells should have RNA as well as DNA sequences homologous to the transforming specific sequences found in tumor viruses (oncogene expression).

Type C virogene sequences offer several distinct advantages for the study of evolutionary relationships. As cellular genes, type C virogenes are subject to the pressures of mutation and selection; thus, closely related animal species would be expected to have closely related, but not identical, endogenous type C virogenes. Type C virogenes are unique from all other known cellular genes in their ability to give rise to the production of infectious type C virus particles. The complete expression of virogenes, at least in some species, with concomitant production of type C viruses containing specific viral proteins, a reverse transcriptase, and a high molecular weight RNA, offers a unique possibility for the isolation of a discrete

set of cellular genes and their products. Single-stranded ^{3}H-DNA transcripts that represent the viral RNA sequences, synthesized in vitro by the viral reverse transcriptase, can be used to detect information in the cellular DNA of related species. Mammalian type C viruses are present in cellular DNA in multiple complete copies (five to fifteen per haploid genome) as a family of related, but not identical, gene sequences (15). These sets of type C virogenes appear to evolve more rapidly than the unique sequence cellular genes, possibly because of their presence in multiple copies in each genome (16). This apparent faster rate of evolutionary divergence of the primate type C viral genes allows a fine degree of discrimination among the various primate species. It is thus possible to establish taxonomic relationships among closely related species that are not revealed by methods involving the annealing of *entire* unique sequence DNA. The use of such viral probes clearly indicates that virogene evolution has followed the pattern of overall species evolution (16). In contrast, infectious, horizontally transmitted primate viruses spread from animal to animal and are completely unrelated by molecular and antigenic criteria to endogenous, genetically transmitted primate viruses. The properties of infectious viruses traveling from animal to animal can become rapidly altered, thereby obscuring their origin. Genetically transmitted viruses have remained stable enough to make it possible to detect events which occurred millions of years ago, and precisely determine the species from which they originated. The inability to detect viral-related sequences in more distantly related species reflects extensive changes in base sequences that have accumulated in the virogene since divergence (17).

Endogenous primate type C viruses

It has only been within the last year or two that endogenous type C viruses have been successfully propagated from primates, man's closest relatives. Several isolates from different tissues and from different species of baboons have been obtained in this laboratory. They are morphologically and biochemically typical of mammalian type C viruses, are closely related by host range, viral neutralization and interference and by immunologic and nucleic acid hybridization criteria, but are distinctly different from all other previously studied type C viruses (10, 18). ^{3}H-DNA transcripts prepared from three of the baboon type C virus isolates hybridize completely to DNA extracted from various tissues of several different healthy baboons (18). These type C virus isolates satisfy all the criteria for endogenous, genetically transmitted viruses of primates. The finding of DNA sequences in normal tissues is one of the strongest pieces of evidence that the viral information is maintained in the population as cellular genes.

If the baboon type C viruses were truly endogenous primate viruses (10) and had evolved as the species evolved, then it appeared reasonable to suspect that other Old World monkeys that are close relatives to the baboon would have related virogene sequences in their DNA. Primate species more distantly related taxonomically to baboons would be expected to have more extensive mismatching of their virogene DNA sequences as measured by the thermal stability of nucleic acid hybrids formed or by the final extent of hybridization (19, 17).

The study of the evolutionary relationships of type C viral gene sequences is especially favorable in primates since much is known about the evolutionary rela-

tionships between primates: the fossil record has been intensively studied as *Homo sapiens* have been particularly interested in their own origins. The Old World monkeys (which include the baboon species) have been separated from the great apes and man for 30 to 40 million years. The New World monkey branch diverged from the common stem leading to both the apes and the Old World monkeys, approximately 50 million years ago while the prosimians evolved from primitive mammalian stock roughly 60 to 80 million years ago.

Hybridization studies employing a DNA copy of the baboon virus RNA were used to detect type C viral nucleic acid sequences in primate cellular DNA. Multiple copies of viral gene sequences related to the RNA genomes of the baboon type C viruses are found in all other Old World monkey species, higher apes, and are also found in man. However, no homology can be detected in various New World monkey DNAs (17). The degree of relatedness of the virogene sequences closely correlates with the txonomic relatedness of the monkey species based upon anatomic criteria and the fossil record. The results establish that, within the primates, type C viral genes have evolved as the species have evolved, with virogenes from more closely related genera and families showing more sequence homology than those from distantly related taxons. That such species as the baboon and rhesus monkey, which have diverged genetically and have been geographically separated for several million years, still retain related virogene sequences, and the low, but consistently observed, hybridization to ape (chimpanzee) DNA with the baboon viral probe, demonstrates that this virogene information has been conserved in the primate stock during the course of evolution as stable cellular elements for at least 30 to 40 million years (17). The ubiquitous presence of endogenous type C virogenes among anthropoid primates and their evolutionary preservation suggest that such genes provide functions with a selective advantage to the species possessing them.

Virogene information is not only present in other Old World primates, but is also normally expressed. Probes from the baboon virus isolates have detected viral-specific RNA in rhesus monkey, stumptail and green monkey liver tissue; and p30 antigen has been found in normal stumptail spleen tissue and in a rhesus ovarian carcinoma (20). Two human tumors, an ovarian carcinoma and a lymphocytic lymphoma, have also been found to contain primate type C viral p30 antigen (21). These genes, therefore, are not inactive, but are normally expressed; the level, however, varies from animal to animal and from tissue to tissue in a given animal.

Interspecies transfer of type C virogenes

Type C viruses have also, under natural conditions, been transferred between species that are only remotely related phylogenetically. In some instances, type C virogenes have escaped host control as virus particles infectious to other species. These viruses can be transmitted from one species to another with integration of their information into the DNA and subsequent perpetuation through the germ line of the recipient species. Because of the stability of the viral gene sequences when they are incorporated into cellular DNA, events that have occurred millions of years ago still can be recognized by examining the genetic information of the virus and that of the host cell. One can assess the relatedness of a given virus to the host

it is associated with by comparing (using molecular hybridization) the match between the viral RNA genome and the DNA of cells from an animal of the species with which the virus is associated. Endogenous viruses from one species horizontally transmitted to another species are related to, but distinct from, one another by many different criteria: nucleic acid sequence homology, antibody inhibition of polymerase activity, antigenicity of the p30 protein, viral interference and viral neutralization. Three known examples of trans-species infections by endogenous type C genes are discussed below.

One example involves the transfer of an endogenous primate type C virus into the germ line of the ancestor of the domestic cat (22, 23). Results have shown that domestic cat DNA contains sequences partially related to endogenous baboon type C viral sequences, even though unique sequence baboon and cat *cellular* DNA show no homology. Since other mammals do not contain those related sequences, the finding of baboon type C viral sequences in the distantly related domestic cat *(Felis catus)* cannot be explained strictly on evolutionary grounds (17).

Domestic cat DNA contains type C virogenes which can lead to the production of endogenous RD-114/CCC viruses (24, 25). In comparing the endogenous primate viruses to this feline group of viruses we found that they are related to each other, but can be distinguished by biologic and immunologic criteria and by partial nucleic acid sequence homology. Endogenous viruses from one group of mammals (primates) are concluded to have infected and become a part of the germ line of an evolutionary distant group of animals, progenitors of the domestic cat (22, 23) and thus have had a common ancestor even though they now behave as endogenous viruses of two taxonomically distant mammalian species.

Genes related to the nucleic acid of an endogenous domestic cat type C virus (RD-114/CCC) are found in the cellular DNA of anthropoid primates while at the same time many members of the cat family Felidae lack these sequences (Table 6).

Table VI: Relationship between cat and baboon endogenous type C virus

1. The cat (RD-114/CCC) and baboon virus groups are *related but distinct* from one another by:
   a. Viral DNA-RNA hybridization,
   b. Inhibition of polymerase activity by antibody,
   c. Antigenicity of the p30 protein,
   d. Viral interference,
   e. Viral neutralization.
2. Cat and baboon unique sequence DNA markedly different, species diverged from one another over 80 million years ago.
3. Cat (RD-114/CCC) virus DNA transcripts hybridize to the DNAs of *all* Old World Monkeys and apes, and to the DNAs of domestic cats and certain other *Felis* species.
4. Baboon (M7/M28) virus DNA transcripts hybridize to the DNAs of all Old World Monkeys, higher apes, and man, and to DNAs of those *Felis* species which contain RD-114 related sequences.

From the relatives of the domestic cat that have RD-114/CCC viral genes and from those that did not acquire them, we have concluded that the infection occurred 3 to 10 million years ago, in Africa or in the Mediterranean Basin region before the Old World monkeys had significantly diverged. This absence of RD-114/CCC related information in other cats is consistent with acquisition of this virus relatively recently in feline evolution.

Experiments have shown that, besides the RD-114/CCC cat viruses which were transmitted from primates to cats (as described above), another distinct class of type C RNA virus was acquired by cats and is now present in their germ line. These feline leukemia viruses (FeLV) were transmitted from an ancestor of the rat to ancestors of the domestic cat and their close relatives (26). The relationships observed between FeLV and the endogenous viruses of rodents are similar to those between endogenous feline viruses of the RD-114/CCC group and endogenous primate type C viruses. FeLV-related gene sequences are found not only in the cellular DNA of domestic cats but also in the DNA of three other closely related Felidae *(Felis sylvestris, F. margarita, F. chaus).* More distantly related *Felis* species lack FeLV-related virogenes, while the cellular DNA of rodents, in particular rats, contains related virogene sequences. This suggests that FeLV-related genes were introduced into the *Felis* lineage following trans-species infection(s) by type C viruses of rodent origin. The absence of FeLV-related DNA sequences in most of the Felidae indicates that these genes were acquired subsequent to the initial Felidae divergence in evolutionary history but prior to the radiation of the above four *Felis* species. It is interesting that cats which contain sequences related to RD-114/CCC genes also contain FeLV-related genes, while other members of the *Felis* species lack both sets of sequences. Both groups of viral genes appear to have been introduced to the cat germ cells from distinctly different groups of animals (rodents and primates) (26).

The third example of trans-species infection is that of an endogenous virus acquired by an ancestor of the domestic pig from an ancestor of the mouse (27). Pig cell cultures produce type C viruses (28–31) that are genetically transmitted and present in all pig tissues in multiple copies in the cellular DNA (31, 15). Partially homologous viral gene sequences are also found in rodent, in particular Muridae, cellular DNA (27). Close relatives, such as the European wild boar and the African bush pig, have closely related viral genes in their DNA. The nucleic acid homology between the endogenous pig type C viral RNA and murine cellular DNA suggests that the endogenous viruses had a common ancestor. It can be shown that this virus was acquired by an ancestor of the pig from a small rodent related to the mouse (27). From the extent of hybridization of the pig type C viral DNA probes to rodent cellular DNA, the type C virogenes were introduced into the Suidae lineage by trans-species infection from members of the family Muridae after the mouse had separated from the rat, but before the different species of mice had diverged from each other. Rodent viral genes thus gave rise to infectious particles that became incorporated into the porcine germ line. The rate of evolution of the virogene sequences in the pig appears to be much slower than that of genes that have remained in the rodent lineage; this may be a consequence of transfer from a shorter-lived animal (the rodent) to a longer-lived one (the pig) (27). The time of gene transmission is estimated as occurring 5 to 10 million years ago and it is concluded

Table VII: Examples of transmission of type C virus genes between species

Donor	Recipient	Genetically Transmitted in Recipient
Primate (Old World monkey)	*Felis* (Ancestor of the domestic cat)	Yes
Rodent (Mouse ancestor)	Pig ancestor	Yes
Rodent (Rat ancestor)	*Felis* (Ancestor of domestic cat)	Yes (but also horizontally transmitted in *Felis catus)*
Rodent (*M. caroli* or close relatives)	Primates	No

that the present-day porcine type C virogenes most closely approximate the viral genes as they were 4 to 6 million years ago in the rodent lineage (27).

The data as summarized in Table 7 demonstrate that *viral genes from one group of animals can give rise to infectious particles that not only can integrate into the DNA of animals of another species, but can also be incorporated into the germ line (germ line inheritance of acquired virus genes).* Clearly, if viral gene sequences can be acquired in this way, it is possible that type C viruses have served to introduce other genes from one species to another, and may provide an important mechanism by which species stably acquire new genetic information.

The infectious primate type C RNA virus group

Infectious primate type C viruses have recently been recovered from several colonies of gibbon apes with various hematopoietic neoplasms, especially myelogenous and lymphoid leukemias (32), and from one woolly monkey with a spontaneous fibrosarcoma (a New World primate) (33, 34). GALV (gibbon ape leukemia virus) and SSV-SSAV (simian sarcoma virus-simian sarcoma associated virus) spread from animal to animal under natural conditions and induce tumors when inoculated into other primates (34–36). These viruses are related to one another by several immunologic criteria and contain related RNA genomes (37). Gene sequences homologous to those of the RNAs of GALV and SSAV have not been detected in the cellular DNA of normal primates studied thus far (38, 19). Thus, unlike the baboon type C virus, these two viruses are *not* endogenous viruses of primates.

The type C viruses of the GALV-SSAV group are poorly controlled by the primate host and appear readily capable of producing neoplastic disease. Infection by such viruses can cause local epidemics of lymphoproliferative tumors in infected gibbon colonies (39). The ability to isolate viruses from gibbons, however, is not restricted to animals with tumors. Recently, three isolates have been obtained from

the brains of normal gibbons (animals without tumors) from a single colony in the United States (37). Based on immunologic assays and interference tests, the group of infectious type C viruses of primates contains many members, all partially related to one another. At present, the infectious primate type C viruses can be classified into four distinct subgroups (see Table 8) based on hybridization studies

Table VIII: Infectious primate type C viruses; isolation and partial characterization

Proposed Subgroup	Isolates	Reference
A Woolly monkey	SSV/SSAV	(34)
B Gibbon type 1	GALV-1	(32)
C Gibbon type 2	GALV-SEATO	(39)
D Gibbon type 3	GBr-1, GBr-2, GBr-3	(37)

which show extensive mismatching of the gene sequences when the different gibbon isolates were compared to one another and to SSAV (37). It is probable that additional subgroups will be defined as new isolates are obtained.

In studying the relationships between the various mammalian type C viruses using nucleic acid hybridization it was noted that the infectious primate viruses, GALV and SSAV, share a significant degree of nucleic acid sequence homology with endogenous type C viruses from the laboratory mouse, *Mus musculus* (40). Several homologous proteins of these two major groups of viruses also share unique interspecies determinants (41). These unexpected findings suggested the possibility that the infectious primate viruses of the GALV-SSAV group were derived from endogenous mouse viruses or from a type C virus of a rodent closely related to the mouse. Primates can, therefore, possess both endogenous and exogenous type C viruses. The ease with which type C viruses can be isolated from an Asian primate, the gibbon, and their relationship to *Mus musculus* cellular DNA suggested that an Asian species of *Mus* might have a more closely related endogenous virus. For these reasons, we chose to study type C viruses from several feral Asian subspecies of *Mus musculus*. Ten of thirteen single cell clones of the distantly related Thai mouse species *Mus caroli* are inducible for a xenotropic type C virus. This virus, unlike the isolates from other *Mus musculus* subspecies, was found to be closely related antigenically to a group of infectious primate type C viruses (gibbon and woolly monkey type C viruses) and only weakly related to and distinctly different from previously studied type C viruses of *Mus musculus*. The polymerase of the *Mus caroli* virus is antigenically more similar to the primate viral enzymes than to the enzymes of all *musculus* type C viruses tested (Table 9). It shares cross-reactive p30 antigens, and cross-interferes with the infectious primate type C viruses (42). The p30 protein of the *Mus caroli* virus is more closely related antigenically to viruses of the GALV-SSAV group than to *Mus musculus* type C viruses. By immunologic and interference criteria, then, the virus isolated from *Mus caroli* cells is unique among the murine viruses characterized thus far in its close relationship to infectious viruses isolated from primates. These results lead to the conclusion that

Table IX: Inhibition of viral reverse transcriptase Activity by antisera to viral polymerases

Virus From:	µg Needed For 30 % Inhibition	
	Anti-MuLV	Anti-SSAV
Mouse		
Rauscher	[0.8]	>30
Moloney	0.8	>30
AKR	1.2	>30
BALB/c	1.0	>30
Mus musculus (wild mouse)	0.9	>30
Mus caroli	>60	3.6
Primate		
SSAV	>60	[0.7]
GALV-1	>60	1.2
GALV-SEATO	>60	1.2
GBr-1	>60	1.0
GBr-2	>60	0.9
GBr-3	>60	1.3

a group of infectious, type C viruses horizontally transmitted among primates originated by trans-species infection(s) of certain primates (gibbon, woolly monkey, and perhaps other apes and monkeys) by an endogenous type C virus from *Mus caroli* or another closely related species. This trans-species infection appears to be a relatively recent, perhaps contemporary, event with the viruses not yet being incorporated into the genomes of the recipient primate species.

Type C RNA viruses and human neoplasia

The studies of type C virogenes in primate populations as described above are unusually significant: first, they are the first isolates of type C viruses from primates; second, some of these viruses have been proven to be oncogenic; third, they provide the closest model of animal neoplasia for man; and fourth, it is possible that one, the other, or both of these two primate virus groups (GALV and SSAV) may be involved in human neoplasia.

Since the horizontally transmitted primate viruses described above are infectious for and can cause tumors in primates, the possibility exists that this group of viruses may be involved in the etiology of human cancer. This is supported by data obtained using different experimental procedures in a number of laboratories. An enzyme with biochemical properties related to those of type C viruses and with antigenic properties similar to polymerases of the woolly monkey type C virus (SSAV) and the gibbon ape leukemia virus (GALV) has been detected in human acute leukemia cells (43, 44). The DNA products of endogenous reactions from the "virus-like" particulate fraction of acute leukemia cells hybridize preferentially to viral RNA from SSAV and GALV (45, 46). Using radioimmunoassays, antigens

related to the major structural proteins (p30) of type C viruses have been detected in peripheral white blood cells from five patients with acute leukemia (47). These results suggest that viruses of this group, known to be infectious for and tumorigenic in other primates, may also be associated with acute leukemia in man.

Recently, several laboratories have reported the isolation of complete infectious type C viruses from human materials (48–51). Most information is available on the isolate designated HL-23, obtained from a cell culture derived from a woman with acute myelogenous leukemia. It appears to be closely related to the woolly monkey virus, SSAV (50), and thus may belong to one of the four previously described subgroups of infectious primate viruses. A virus closely related to baboon type C viruses was also isolated from patient HL-23 (52). Since two different type C viruses also related to the same primate viruses as HL-23 have recently been found in the human embryo cells described by Panem et al. (49), isolation of *one* infectious virus from human material now appears to be an unusual rather than common occurrence. Additional isolates of HL-23 virus have recently been reported from separate clinical specimens obtained at different intervals from the same patient (53). The significance of these isolations, however, requires further evaluation. The careful characterization of additional isolates made by other laboratories from human tissues and cell cultures, then, is awaited with keen interest.

Primates, including man, are known to contain endogenous type C viral sequences in their genome which are related to those found in endogenous baboon viruses (16). Endogenous virogenes may be partially expressed in humans and other primates as evidenced by the detection of RNA sequences (20), and antigens related to the p30 proteins (20, 21) of endogenous baboon viruses. The expression of endogenous viral-related antigens is found in carcinomas and lymphomas (21) as well as in leukemias (47); viral p30 antigen expression has also been reported in certain normal human tissues (54).

If infectious type C RNA viruses are important agents in cancer causation in man, it is critical to know how the viral information is transmitted, normally controlled, and maintained in the population. Are they contained in an animal reservoir or do they spread solely from primate to primate? Finding this reservoir(s), if it exists, provides a chance of disrupting the process. If human leukemia involves the spread of an infectious agent from individual to individual as is clearly shown to be the case for cat leukemia (55) and bovine leukemia (56), then identification of the agent and its mode of spread would provide one set of approaches to prevention of the disease. If, on the other hand, activation of genetically transmitted virus by extrinsic (chemical and physical agents) as well as by various intrinsic factors leads to tumor development and there is no contagious virus involved, the approaches to the prevention of the disease would be quite different. The endogenous primate type C virogenes, present in human cells, would appear to be the more logical candidate virus for involvement in the generality of human cancer.

Possible normal functions of type C viruses

The presence of genetically transmitted viral genes in so many vertebrate species and the evidence that they have been conserved through evolution in several distinct vertebrate lineages suggests that they may provide normal function(s)

Table X: Possible functions of genetically transmitted virogenes in normal cells

1. Activation of oncogenic information, while inappropriate in adult tissue, plays a normal role during differentiation and development.
2. The integrated virus serves to protect the species against related, more virulent infectious type C viruses.
3. Virus activation, being linked to transformation, protects the animal by altering the cell membrane. The released virus could alert the immune system making the transformed cells more susceptible to immunologic control.
4. They may have had an evolutionary role as conveyors of genetic information not only within a species but also between species. Only this group of viruses has been shown to transmit genes between germ cells of different species under natural conditions.

advantageous to the species carrying them (Table 10). The first suggested role, derived from studies on the expression of viral antigens during the course of development, was that such viral expression during the early stages of differentiation was a normal part of the developmental process (3). If this were the case, the expression of cancer genes later in life would be an inappropriate manifestation of a normal developmental function. If viral genes provide a function critical for normal development, they clearly would be conserved during evolution.

The acquisition of viral genes by cats from both primates and rodents, and by pigs from rodents, along with the fact that they have been maintained for millions of years suggests the possibility that the newly acquired viral genes, once integrated, might have been beneficial to the recipient species if they were able to provide resistance to related, but more virulent viruses. Animals that successfully integrated the genomes would have been at a selective advantage relative to those that did not, if the integrated genome protected against infection, and if infection led to cancer or other type C viral-mediated diseases. Genes that provide protection against disease, especially against epidemic diseases, would be at a strong selective advantage in natural populations. This may well explain the success of the transmission between species as described above. For example, in our laboratory we have shown that those species of the genus *Felis*, including the domestic cat, that have acquired primate type C viral genes are resistant to infection by the endogenous baboon viruses, while those *Felis* species that have not acquired the viral information are still susceptible to baboon viral information.

A third possible role for endogenous viruses arises if viral activation was closely linked to the transformed state in the cell. Expression of the endogenous virus under natural circumstances, may be protective on an immunological basis against cancer, rather than the virus acting as the etiological agent. The activated virus could alter the cell membrane and thus alert the host immune system, conveying information as to the number and location of transformed cells in the body. This possibility is supported by the observation that transformed cells in culture, whether transformed spontaneously, by chemical carcinogens, or by other viruses, release their endogenous type C viruses more readily than do their normal, untransformed counterparts (57–59). Transformed cells that are releasing high titers of

type C virus have been reported to be much less able to produce tumors when inoculated into immunocompetent animals of the same species (60). Partial viral expression where viral antigens are introduced into the cell surface may be sufficient to alter its antigenicity and facilitate rejection of these cells.

One final possibility that should be considered is that type C viruses have played an important evolutionary role as transmitters of genetic information, not only between cells of an animal, and animals of a species, but also between species. That viruses can transmit themselves between the germ cell DNAs of very different species has been established as a result of experiments in the past year. That they can recombine with cellular gene sequences and transmit these genes to new cells of a different species also has been clearly demonstrated (61, 62). That this transmission of cellular gene information between species has been a major force in evolution, however, remains a speculation.

This suggestion that viruses may have had a major role in evolution is not a new one (63). Viruses are unique in that they can serve to carry information between genetically isolated species. Classical Darwinian evolution deals with changes which occur within the genetic information of a species; which can be changed and rearranged by mutation and selection, duplication and rearrangements, but not added to from the outside. Viruses, however, offer the possibility of additions of new gene sequences to a species. The type C viruses as a group, are uniquely suited for this role since they must incorporate into the cellular DNA in order to replicate (14) but they do not kill the cells that they infect. Each time they move from cell to cell they may carry with them host cell genes providing a means of communication between cells of different species and different phyla. They serve to keep a species in contact or in communication with its neighbors-ecologic neighbors as well as genetic neighbors.

Of course they can transmit information that may disrupt normal cellular control, and by so doing, lead to the development of cancer in the individual. Instances of genetic significance, however, occur when new genes are incorporated into the germ line. From this perspective, the fact that these viruses cause cancer would then be viewed as a pathological manifestation of normal processes. While the viral genes may well be etiologic agents in cancer causation, either as exogenous or endogenous viruses, and this may be of profound significance to the affected individuals, these relatively rare and sporadic cases may not be of great evolutionary significance.

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