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Gibbon Ape Leukemia Virus-Hall's Island: New Strain of Gibbon Ape Leukemia Virus

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Gibbon ape leukemia virus-Hall's Island (GaLV-H), a type C virus related to previous isolates of GaLV and simian sarcoma virus, was isolated from a gibbon ape with lymphocytic leukemia from a small colony of free-ranging gibbon apes on Hall's Island near Bermuda. We show here by molecular hybridization experiments that GaLV-H is approximately 60% related to three previous isolates of GaLV (GaLV-SF, GaLV-SEATO, and GaLV-Br) and is less closely related to simian sarcoma virus. The oligopyrimidine pattern of a transcript of the terminal 135 ± 5 nucleotides of the viral RNA of GaLV-H is similar to that of GALV-Br but distinct from that of GaLV-SF and simian sarcoma virus. GaLV-H thus represents a fifth distinct strain of the infectious primate type C viruses, which among the previously described isolates of GaLV is most closely related to GaLV-Br.

Type C viruses have been isolated from primate neoplasias, including a woolly monkey fibrosarcoma (simian sarcoma virus [SiSV]) (22, 25), gibbon ape lymphosarcoma (gibbon ape leukemia virus [GaLV]-SF) (12, 19), and gibbon ape granulocytic leukemia (GaLV-SEATO) (11). Related isolates were obtained from frozen brain tissue of nonleukemic gibbons (GaLV-Br) (23). All of the above isolates are closely related to but distinguishable from each other by nucleic acid sequence homology and protein serology (1, 10, 14, 16, 17, 23, 24). None is endogenous to primates (1, 16, 26). SiSV is tumorigenic in marmosets (25). GaLV-SEATO induces a myeloproliferative disease when inoculated into gibbons (T. Kawakami, personal communication). These viruses are thus horizontally transmitted viruses which are etiologically linked to hematopoietic neoplasias in nonhuman primates.

We recently reported the isolation of type C virus from a gibbon ape with acute lymphocytic leukemia from a colony near Bermuda (4). This virus, called GaLV-H (For Hall's Island, the site of the gibbon colony), was shown to be related to SiSV and previous GaLV isolates by p30 and reverse transcriptase serology and by analyses of nucleic acid sequences. We compare these viruses here in detail and show that GaLV-H is distinguishable from the previous isolates. GaLV-H thus represents a fifth distinct strain within this group of viruses.

GaLV-H derived from the peripheral blood of a gibbon (6G-1) was either grown in the primary leukemic leukocytes or transmitted to and grown in bat lung fibroblasts (CCL-88). SiSV was grown in the 71AP1 marmoset tumor line or in CCL-88 cells. GaLV-SF was grown either in CCL-88 or in the original primary tumor line, UCD-144. GaLV-SEATO was grown in CCL-88 cells, a human rhabdomyosarcoma cell line (A204) (5), a human osteogenic sarcoma cell line (HOS), or a transformed cell line derived from HOS by nonproductive infection with Kirsten murine sarcoma virus (K-HOS) (15). GaLV-Br was grown in CCL-88 cells.

Viral RNA was prepared either as polyadenylic acid-containing RNA by oligodeoxythymidylic acid-cellulose chromatography or as 70S RNA by treatment with sodium dodecyl sulfate-Pronase, phenol extraction, and velocity gradient sedimentation as described previously (4). Cytoplasmic RNA was prepared by digestion with sodium dodecyl sulfate-Pronase and extractions with phenol-cresol-chloroform, pH 9 (PCC9) and chloroform-isoamyl alcohol as described previously (4). Cell DNA was prepared in a similar manner, except that cell extracts were digested with alkali (0.3 N KOH for 20 min at 95°C) to remove RNA (4). ^{3}H-labeled complementary DNA (cDNA) was prepared by published conditions (4). Specific activity of [^{3}H]cDNA was 2×10^7 cpm/μg. [^{3}H]cDNA protected [^{125}I]RNA from RNase digestion to 55 to 60% at a 1:1 weight ratio and essentially com-

pletely (86%) at a 5:1 ratio. Viral RNA was labeled with ^{125}I to a specific activity of 5×10^7 to 10×10^7 cpm/μg.

The relationship of different isolates of the GaLV-SiSV group of viruses was analyzed by nucleic acid hybridization experiments. ^{125}I-labeled viral RNA was hybridized to excess DNA from cells experimentally infected with different viruses from the SiSV-GaLV group. GaLV-H [^{125}I]RNA hybridized best to the homologous infected cell DNA (Fig. 1). Hybridization to DNA from cells infected with other strains of

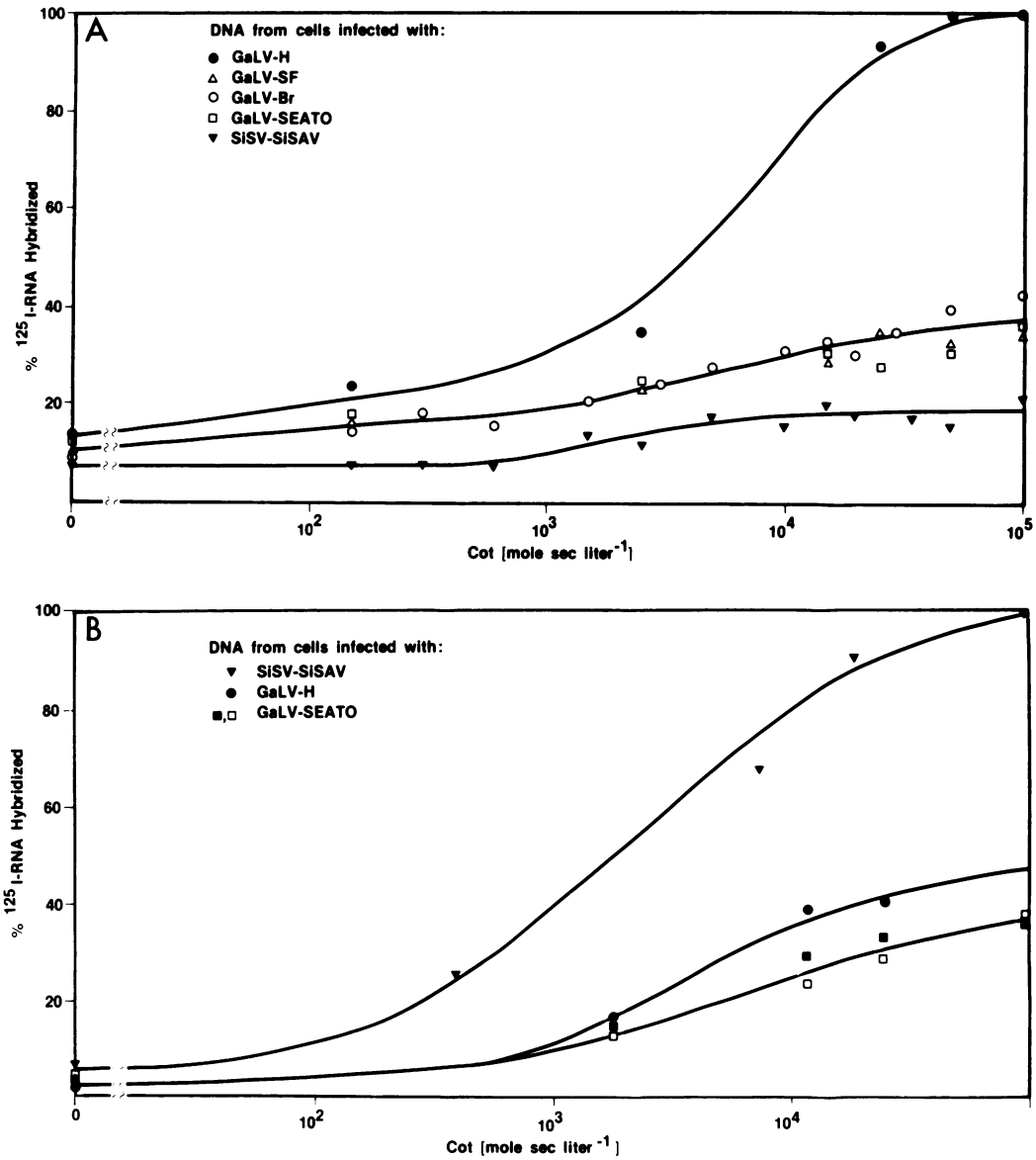

FIG. 1. *Kinetics of hybridization of viral RNA to infected cell DNA. Hybridization of [^{125}I]RNA to infected cell DNA was performed with a DNA:RNA ratio of at least 2×10^7 in 0.4 M phosphate buffer, pH 7, at 60°C. Hybridization was assayed by digestion with RNase A (50 μg/ml for 2 h at 37°C in 0.5 M NaCl) as described previously (4). Symbols: ●, DNA from the primary gibbon ape leukemic lymphoid line 6G-1 producing GaLV-H; ▼, 71AP1 marmoset tumor cells producing SiSV-SiSAV; △, CCL88 cells producing GaLV-SF; ○, CCL88 cells producing GaLV-Br; □, A204 cells producing GaLV-SEATO; ■, KHOS cells producing GaLV-SEATO. (A) [^{125}I]RNA from GaLV-H was labeled RNA; (B) [^{125}I]RNA from SiSV-SiSAV (71AP1) was the labeled RNA.*

GaLV (GaLV-Br, GaLV-SEATO, and GaLV-SF) was about 40% relative to the homologous DNA, with no substantial differences within this group. Hybridization to DNA from cells infected with SiSV was only about 20% of the value obtained with DNA from GaLV-H-infected cells, indicating that GaLV-H is more distantly related to SiSV than to other isolates of GaLV. DNA from other infected cell lines, including GaLV-SF in the original UCD-144 gibbon lymphoma line, GaLV-SEATO in CCL-88, HOS, and KHOS cells, and SiSV in NRK rat kidney cells, gave similar results (Table 1). The hybridization of labeled RNA from SiSV-SiSAV (71AP1) to GaLV-H-infected cell DNA (40% relative to the homologous value) is in reasonable agreement with the reciprocal heterologous cross. SiSV-SiSAV RNA hybridizes approximately equally to DNA from cells infected with other strains of GaLV (Fig. 1B).

The divergence of RNA sequences of these viruses is also reflected in the difference in thermal stabilities between the homologous and heterologous hybrids. The T_m of the homologous GaLV-H hybrid in 0.15 M NaCl is 81°C (Fig. 2A). Hybrids of GaLV-H RNA and proviral DNA from GaLV isolates have a T_m about 5 to 6°C lower, and hybrids formed with SiSV proviral DNA give a T_m about 15°C lower. Thermal denaturation experiments with DNA from the other infected cell types gave substantially similar results (Table 1).

Experiments on infected cell DNA were also performed with [^{3}H]cDNA. Although experiments with cDNA have the disadvantage of uneven representation of the viral RNA in the probe, they have the advantage of more extensive hybridization and thus are useful in conjunction with experiments with labeled RNA. As shown in Table 1 and Fig. 2B, these results are similar to those with labeled viral RNA and infected cell DNA.

The homology of RNA from these viruses was also analyzed by hybridization of [^{3}H]cDNA to viral RNA or RNA from the cytoplasm of infected cells. These results also distinguish GaLV-H from previous isolates. The relative hybridization of GaLV-H cDNA to RNA from cells infected with other GaLV isolates was relatively higher (60%) (Table 1), but the ΔT_m was still about 5°C (Figure 2C). The somewhat higher heterologous hybridization in experiments with cDNA may partly reflect preferential transcription of cDNA from conserved regions of the genomic RNA. Hybridization to viral RNA is in agreement with the results with cy-

TABLE 1. *Relatedness of GaLV-H to other GaLV isolates as determined by molecular hybridization*

Sources	Hybridization with [^{125}I]RNA[a]		Hybridization with [^{3}H]-cDNA[b]	
	% Hybridization	ΔT_m (°C)	% [^{3}H]cDNA hybridized	ΔT_m (°C)
DNA				
GaLV-H (CCL88)	100		100	
GaLV-H (6G-1)	67.6 ± 2.6	0 ± 0	100	ND[c]
GaLV-SEATO (CCL88)	41.9 ± 5.7	4.1 ± 0.7	50	ND
GaLV-SEATO (A204)	47.7 ± 4.9	5.1 ± 0.2	ND	ND
GaLV-SEATO (KHOS)	ND	5.0 ± 0	46	3
GaLV-SF (UCD144)	33.5 ± 1.7	4.0 ± 0.7	69.7	ND
GaLV-SF (CCL88)	42.8 ± 3.2	5.1 ± 0.7	54.5	5
GaLV-Br (CCL88)	42.4 ± 4.4	4.8 ± 0.8	66.6	5.5
SiSV-SiSAV (NRK)	22.2 ± 0	12.9 ± 1.8	ND	ND
RNA				
GaLV-H (CCL88)			100	
GaLV-H (6G-1)			ND	ND
GaLV-SEATO (CCL88)			ND	ND
GaLV-SEATO (A204)			59	2
GaLV-SEATO (KHOS)			65	ND
GaLV-SF (CCL88)			83	4.5
GaLV-SF (UCD144)			52	ND
GaLV-Br (CCL88)			66	2

[a] GaLV-H [^{125}I]RNA was hybridized to a 2×10^7-fold excess of infected cell DNA to a C_0t of 20,000 as described for Fig. 1. Values given for [^{125}I]RNA are for multiple determinations with average deviations.

[b] GaLV-H [^{3}H]cDNA was hybridized to a 10^7-fold excess of cell RNA or a 2×10^7-fold excess of cell DNA to a C_0t of 20,000 (corrected) as described for Fig. 2. Thermal stability of the resultant hybrids was measured as described for Fig. 2.

[c] ND, Not determined.

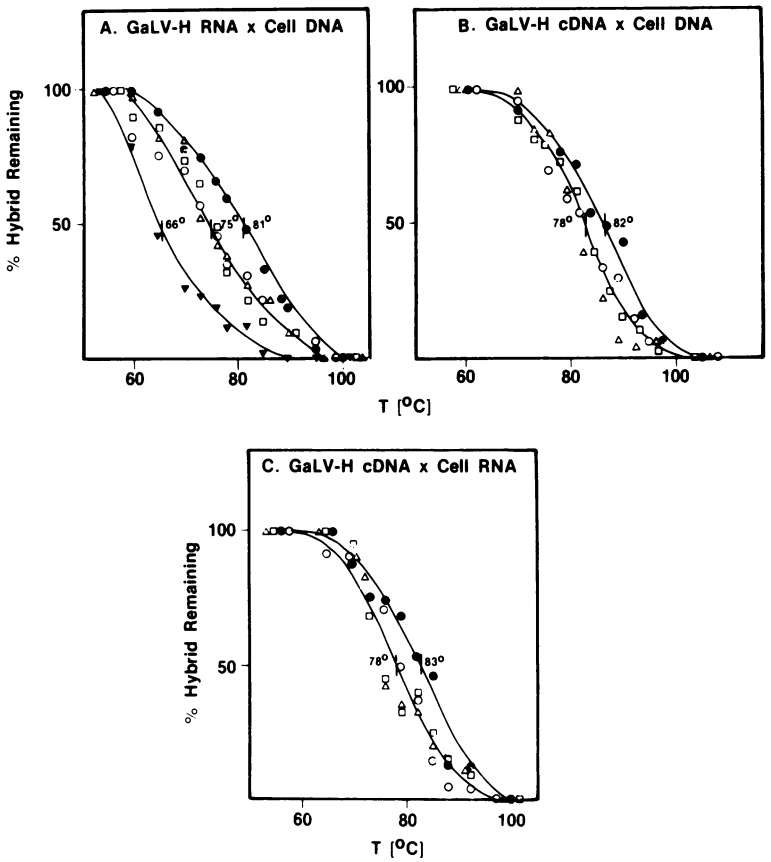

FIG. 2. *Thermal stability of hybrids with GaLV-H viral nucleic acid probes and infected cell nucleic acids. [^{125}I]RNA was hybridized as described for Fig. 1 to DNA from cells infected with different isolates of GaLV or SiSV. [^{3}H]cDNA-cell DNA hybridizations were carried out in 0.4 M NaCl at 65°C with a DNA:cDNA ratio of at least 10^{7}. Hybridization of cDNA to RNA was at 37°C with 0.45 M NaCl in 50% formamide. Cell RNA was present at a concentration of 200 μg/80 to 120 μl; viral RNA was present at 1 μg/80 to 120 μl. Hybridization was assayed by digestion with S1 nuclease from Aspergillus oryzae (Miles, Elkhart, Ind.) (250 U in 0.4 ml at 50°C for 1 h, 0.3 M NaCl) as described elsewhere (4). The resultant hybrids were incubated for 5 min at the indicated temperature in 0.15 M NaCl for [^{125}I]RNA or 0.3 M NaCl for [^{3}H]cDNA. Source of nucleic acids: ●, 6G-1 cells producing GaLV-H; ▼, CCL88 cells producing SiSV-SiSAV; △, CCL88 cells producing GaLV-SF; ○, CCL88 cells producing GaLV-Br; □, A204 (A) or KHOS (B and C) cells producing GaLV-SEATO. (A) [^{125}I]RNA was hybridized to cell DNA. (B) [^{3}H]cDNA was hybridized to cell DNA. (C) [^{3}H]cDNA was hybridized to cell RNA.*

toplasmic RNA (data not shown).

When genomic 70S RNA of retroviruses is used as a template for avian myeloblastosis virus reverse transcriptase in vitro, DNA synthesis is initiated with a tRNA primer bound to a unique site near the 5′ end of the genome (2, 21). The initial DNA product synthesized, called strong stop cDNA, is a copy of the RNA between the tRNA and the 5′ end of the genome (2, 8, 9, 18, 20). The number of nucleotides between the tRNA primer and the 5′ end of the genome is 135 ± 5 for the murine and woolly gibbon type C viruses, 101 ± 2 for the avian type C virus, and 120 ± 5 for the baboon endogenous type C virus (10). The oligopyrimidine composition of this DNA transcription product provides sequence information about the 5′ end of the genome. The strong stop DNA of the SiSAV, GaLV-SF, and GaLV-Br isolates was found to be 135 ± 5 nucleotides long. The three viruses could be distinguished from one another by the composition of the oligopyrimidines that contained deoxycytosine, SiSAV (C, C_2, C_3, C_5, TC, TC_2, TC_6, T_2C_2, T_3C_3); GaLV-Br (C, C_2, C_3, C_5, TC, TC_2, TC_6, T_2C, T_2C_2, T_3C_2); and GaLV-SF (C, C_2, C_3, C_5, TC, TC_2, T_2C, T_2C_2, T_2C_5, T_4C_2).

Similar experiments were done with 70S RNA prepared from GaLV-H. The length of the strong

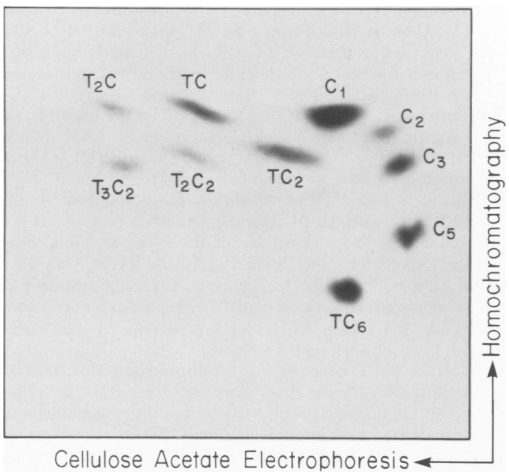

FIG. 3. *Oligopyrimidine tracts containing dC of the strong stop cDNA of GaLV-H. Strong stop cDNA was synthesized in 200-μl reactions that contained 0.05 M Tris (pH 8.3), 0.01% Nonidet P-40, 0.006 M $MgCl_2$, 0.005 M dithiothreitol, 1 mM each dATP, dGTP, and dTTP, 100 μM α-[^{32}P]dCTP (specific activity, 100 C/mmol), 100 μg of actinomycin D per ml, 100 μg of viral 70S RNA per ml, and 10 U of AMV reverse transcriptase per ml. The reaction was incubated for 30 min at 43°C. Extraction of the nucleic acids, preparation of the DNA products for electrophoresis and electrophoresis of the samples were as described previously (8). After electrophoresis the DNA molecules of discrete length were located by autoradiography and excised from the gel. The DNA samples were then depurinated, and the oligopyrimidine composition was determined by electrophoresis on cellulose-acetate followed by homochromatography as described by Ling (13).*

stop DNA of GaLV-H was determined to be 135 ± 5 by analysis of the DNA product of a denaturing 22% polyacrylamide-urea gel. Moloney murine leukemia virus strong stop DNA (135 ± 5 nucleotides long) was used as a size marker on a parallel slot. The oligopyrimidine composition of the strong stop DNA was determined as previously described (7). The oligopyrimidine pattern deduced for GaLV-H (shown in Fig. 3) was (C, C_2, C_3, C_5, TC, TC_2, TC_6, T_2C, T_2C_2, T_3C_2), the same as that of GaLV-Br. By this criterion, the GaLV-H is more closely related to the GaLV-Br isolates than it is to GaLV-SF and SiSAV, although hybridization data distinguish GaLV-H and GaLV-Br. RNase T1 oligonucleotide fingerprints of SiSAV, GaLV-SF, GaLV-Br, and GaLV-H also support the conclusion that among the previously described infectious type C viruses of primates GaLV-H is most closely related to GaLV-Br (Sahagan and Haseltine, unpublished data).

It seems likely that as more isolates of GaLV are reported, additional subgroups will be defined and that a large number of distinct variants of GaLV may exist, as is the case with chickens, cats, and mice. It appears that infectiously transmitted retroviruses (as opposed to the endogenous retroviruses) change relatively rapidly, due to errors in reverse transcription, recombination (either with other viruses of similar type or with endogenous cellular elements), and evolutionary pressures exerted on replicating populations of virus.

The similarity by nucelotide sequence of the 5′ terminus of GaLV-H and GaLV-Br, along with the antigenic similarities to other viruses in the SiSV-GaLV group of both *gag* (p30) and *pol* (reverse transcriptase) gene products (4), makes it plausible that a substantial portion of the observed difference in hybridization between RNA of GaLV-H and the other isolates is due to differences in RNA sequences coding for the envelope protein (gp70). Consistent with this idea, the gp70 of GaLV-H, unlike the *gag* and *pol* gene products, differs substantially by competition radioimmunoassay from that of all the other GaLV isolates (S. Aaronson, personal communication). Among the murine viruses the gp70 appears to be highly variable and appears to recombine at a relatively high frequency (3). Changes in gp70 have been linked to the pathology of murine viruses (6). GaLV-SEATO originated from a gibbon ape with myelogenous leukemia, GaLV-H and GaLV-SF originated from gibbon apes with acute lymphocytic leukemia, and GaLV-Br originated from gibbons apparently free of leukemia. It is possible that differences in the gp70 of GaLV strains, as in strains of murine leukemia virus may help determine their pathological effect or their tissue tropism.

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