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Analysis of the Virogenes Related to the Rhesus Monkey Endogenous Type C Retrovirus in Monkeys and Apes

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Molecular hybridization studies were carried out by using a [^{3}H]complementary DNA (cDNA) probe to compare the endogenous type C retrovirus of rhesus monkeys (MMC-1) with other known retroviruses and related sequences in various primate DNAs. The genomic RNA of the endogenous type C retrovirus of stumptail monkeys (MAC-1) was found to be highly related to the MMC-1 cDNA probe, whereas the other retroviral RNAs tested showed no homology. Related sequences were found in Old World monkey DNAs and to a lesser extent in gorilla and chimpanzee DNAs. No homology was detected between MMC-1 cDNA and DNA of gibbon, orangutan, or human origin. Restriction endonuclease analysis of genomic DNA indicated that many of the several hundred sequences related to MMC-1 in rhesus monkey DNA differed from that integrated into DNA of infected canine cells. Gorilla and chimpanzee DNAs contained a specific restriction endonuclease fragment of the MMC-1 genome.

Endogenous retroviruses have been isolated from tissue of many sources by cocultivation with cell lines from heterologous species (16) or by induction with halogenated pyrimidines (1, 8). The first reported endogenous type C retrovirus from a rhesus monkey was obtained by cocultivation of a rhesus monkey (*Macaca mulatta*) esophageal carcinoma cell line (816A) (11) with a canine osteosarcoma cell line (D17) (10). Sequences related to the genome of this virus, called MMC-1, were shown by molecular hybridization to be present in the DNA of rhesus monkeys. MMC-1 has the morphology of a type C virus and contains the expected RNA-dependent DNA polymerase activity. In addition, MMC-1 reacts with a broadly reactive interspecies antiserum to the p30 protein of mammalian type C viruses (10). A highly related retrovirus has been isolated from *M. artoides* (15).

I report here the presence and characteristics of MMC-1-related sequences in the genomes of other primates, as well as the relationship of the MMC-1 genome to that of other primate retroviruses. Molecular hybridization showed that MMC-1-related sequences were present in DNAs isolated from Old World monkeys and to a lesser extent in DNAs of gorillas and chimpanzees. In addition, MMC-1-related sequences were found in cat liver DNA.

To better understand the observed hybridization of MMC-1 cDNA with gorilla and chimpanzee DNAs, restriction endonuclease analyses were carried out. MMC-1 genomes present in the chromosomal DNA of infected canine cells were mapped by hybridization of [^{32}P]complementary DNA (cDNA) to nitrocellulose filters bearing immobilized DNA fragments produced by seven restriction endonucleases. This pattern was then compared with cleavage patterns obtained with DNA from normal rhesus monkey (which contains several hundred MMC-1-related sequences [10]); significant differences were found. A similar analysis of DNAs from gorillas and chimpanzees, which contain MMC-1-related sequences, indicated that a specific restriction fragment from the MMC-1 genome had been conserved in these apes. This conserved fragment was distinct from that observed when restriction fragments from gorilla and chimpanzee DNAs were hybridized to a baboon endogenous virus (BaEV) cDNA probe. No such related region was found in human DNA.

MATERIALS AND METHODS

Viruses and cells. MMC-1-infected canine thymus cells (Cf2Th) were propagated in Earle modified Eagle medium containing 10% fetal calf serum, 2 mM glutamine, 100 μg of streptomycin per ml, and 100 U of penicillin per ml. Cells were passaged at a 1:10 dilution weekly. Virus was harvested at 24-h intervals after the cells had reached confluency.

Virus was purified as described by Benton et al. (2). Other viruses used were: Rauscher murine leukemia virus grown in JLS-V9 BALB/c mouse bone marrow cells; Mason-Pfizer monkey virus grown in the human rhabdomyosarcoma cell line A204; BaEV strain M7 grown in the human osteosarcoma cell line HOS; RD114 grown in RD cells; equine infectious anemia virus grown in equine fetal kidney cells; bovine leukemia virus grown in fetal lamb kidney cells; reticuloendotheliosis virus grown in transformed chicken bone marrow cells; gibbon ape leukemia virus grown

in 6G-1 gibbon T lymphoblasts; feline leukemia virus grown in FEA feline cells; rat leukemia virus grown in Fisher rat embryo cells; squirrel monkey retrovirus grown in Cf2Th canine thymus cells; and *M. artoides* endogenous virus grown in A549 human cells.

Synthesis of MMC-1 [^{3}H]cDNA. MMC-1 [^{3}H]-cDNA was synthesized in a 0.250-ml reaction mixture containing 1 mM dATP, dCTP, and dGTP, 0.15 mM [^{3}H]dTTP (44 Ci/mmol), 0.04% Nonidet P-40, 0.05 M Tris-hydrochloride (pH 8.3), 5 mM magnesium acetate, 0.05 M NaCl, 0.01 M dithiothreitol, 0.100 mg of actinomycin D per ml, and approximately 2.3 mg of virus. After 2 h, sodium dodecyl sulfate (SDS) was added to 1%, and the mixture was centrifuged at 40,000 rpm for 2.5 h at 10°C in an SW41 rotor through a 15 to 30% sucrose gradient. A sharp peak of trichloroacetic acid-insoluble radioactivity was observed in the middle of the gradient. The pooled fractions were incubated in 0.3 N NaOH for 3 h at 37°C, neutralized, and extracted with phenol followed by chloroform. The [^{3}H]cDNA was collected by ethanol precipitation. These conditions generate probes with a high degree of representation and a specific radioactivity of 3×10^7 dpm/μg (12).

Preparation of nucleic acids for molecular hybridization. Viral RNA was prepared as described by Schlom et al. (13), and DNA was prepared according to the urea-phosphate method (5). Hybridizations were performed with 1×10^3 to 3×10^3 cpm of [^{3}H]-cDNA and 100 to 500 μg of DNA or 0.5 μg of RNA in 1 M sodium phosphate buffer, pH 6.8, at 65°C. After appropriate times, the samples were diluted to 0.14 M phosphate buffer and 0.01% SDS, applied to hydroxyapatite columns at 50°C, and eluted at 10°C increments. Fractions were assayed for ^{3}H counts per minute after addition of 2 volumes of Aquasol II. The extent of hybridization is defined as the percentage of total radioactivity eluting from the column above 50°C, and C_0t values are corrected to the standard conditions of 0.12 M phosphate buffer (5).

Preparation of high-molecular-weight DNA for Southern blot analysis. Packed cells were diluted 1:10 in 50 mM Tris-hydrochloride (pH 7.4)–100 mM NaCl–1 mM EDTA (TNE), and proteinase K (100 μg/ml). SDS was added to 1%, and the mixture was incubated at 42°C for 2 h. After two extractions with phenol-chloroform (1:1), the aqueous phase was extracted once with chloroform and dialyzed three times against TNE. RNase A (boiled for 2 min to inactivate DNase) was added to 25 μg/ml, and the solution was incubated at 42°C for 2 h. The resulting mixture was extracted as before with phenol-chloroform and dialyzed against 10 mM Tris-hydrochloride, pH 7.4, and 1 mM EDTA. The above preparation was modified for tissue DNA by first homogenizing the minced tissue in a Brinkmann Polytron homogenizer at 0°C, using 1 g of tissue to 10 ml of TNE.

Probe synthesis for Southern blot analysis. MMC-1 [^{32}P]cDNA was prepared in a reaction mixture containing 80 μg of 70S virion RNA per ml, 400 μg of calf thymus DNA primers per ml, 0.1 mM dGTP, dATP, and dTTP, 50 mM Tris-hydrochloride, pH 8.3, 50 mM KCl, 8 mM $MgCl_2$, 8 mM dithiothreitol, 10 mCi of [α-^{32}P]dCTP per ml (~400 Ci/mmol), and 1,000 U of avian myeloblastosis virus reverse transcriptase per ml. After 8 h at 37°C, the reaction mixture was phenol extracted and the aqueous phase was treated with 0.3 M NaOH for 5 min at 100°C. The cDNA was purified by Sephadex G-25 chromatography in 3× SSC (SSC = 0.15 NaCl plus 0.015 sodium citrate). The specific radioactivity of this cDNA probe was 3×10^8 dpm/μg.

Restriction endonuclease digestion. Restriction enzymes, obtained from New England Biolabs and Bethesda Research Laboratories, were used under reaction conditions as specified by the manufacturer. Completeness of digestion was monitored by including phage λ DNA in each reaction and analyzing the fragmentation patterns on gels. Electrophoresis was carried out in 40 mM Tris acetate, pH 7.5, 5 mM sodium acetate, and 1 mM EDTA, using 1% agarose (Seakem ME grade). Phage λ DNA or ϕX174 replicative-form DNA cut with various restriction enzymes was used as a size marker. Eucaryotic DNAs were used at 1 to 7 μg per lane. Blot analysis was carried out by the method of Southern (14). Nitrocellulose filters (Schleicher & Schuell Co.) were hybridized for 16 to 24 h in a solution containing 5× SSC, 0.2% bovine serum albumin, 0.2% Ficoll 400, 0.2% polyvinylpyrrolidone, 20 μg of calf thymus DNA per ml (sonicated and denatured), 20 μg of Cf2Th canine cell total RNA per ml, 0.1% SDS, and 20 ng of [^{32}P]cDNA per ml. Washed nitrocellulose filters were analyzed by radioautography, using Kodak XR-5 film and du Pont Lightning Plus intensifying screens.

RESULTS

Molecular hybridization. The relationship of MMC-1 to other known viruses was studied by nucleic acid reassociation, using MMC-1 [^{3}H]cDNA and RNA extracted from purified virions. When assayed by hydroxyapatite chromatography, the MMC-1 [^{3}H]cDNA probe was homologous to MMC-1 and MAC-1 viral RNAs (10). Under these conditions, no hybrid formation was observed with RNA extracted from other retroviruses, including bovine leukemia virus, BaEV strain M7, equine infectious anemia virus, Mason-Pfizer monkey virus, feline leukemia virus, feline sarcoma virus, rat leukemia virus, gibbon ape leukemia virus, Rauscher murine leukemia virus, and the squirrel monkey retrovirus.

To assay for the presence of viral gene sequences related to MMC-1 in other animal species, the [^{3}H]cDNA transcript was annealed to highly purified DNA preparations. The MMC-1 [^{3}H]cDNA hybridized essentially completely to canine cells infected with MMC-1 (Table 1), whereas these cells contained no related nucleic acid sequences before infection. In addition, high levels of hybridization were observed with DNAs from various Old World monkeys (i.e., rhesus monkeys, stumptail monkeys, baboons, and Sykes monkeys). These results are qualitatively similar to those of Todaro et al. (15) in the study

TABLE 1. *Hybridization of MMC-1 [^{3}H]cDNA to cell and tissue DNAs*

DNA source	% Hybridization
Old World monkeys	
Macaca mulatta liver (rhesus monkey)	100
M. mulatta spleen (rhesus monkey)	99
M. mulatta kidney (rhesus monkey)	90
M. arctoides MA250 cells (stumptail macaque)	89
Papio hamadryas kidney (Russian baboon)	90
Cercopithecus alboqularis kidney (Sykes monkey)	81
New World monkeys	
Lagothrix logothricha kidney (woolly monkey)	4
Aotus trivirgatus kidney (owl monkey)	4
Apes	
Gorilla gorilla machi cells (gorilla)	38
Pan troglodytes kidney (chimpanzee)	31
Homo sapiens A204 cells (human)	3
Hylobates lar kidney (gibbon ape)	4
Orangutan CP81 cells	4
Nonprimates	
Mus musculus kidney (BALB/c mouse)	8
M. musculus liver (AKR mouse)	8
Canis familiaris liver (dog)	4
Felis catus (cat)	42
Bos taurus (calf)	4
Salmon	4
Control cell lines	
D17 canine cells	3
D17 canine cells producing MMC-1	97
Cf2Th canine cells	3
Cf2Th canine cells producing MMC-1	90

of another macaque endogenous virus isolate, MAC-1. DNAs from New World monkeys were also tested for sequence relatedness to the MMC-1 genome, but neither woolly nor owl monkey DNAs showed hybrid formation. MMC-1 [^{3}H]cDNA reassociated with DNA purified from some ape tissues and cells. Chimpanzee and gorilla DNAs hybridized to a similar extent (31 and 38%, respectively) with the MMC-1 probe. No hybrid formation was observed with DNA from humans, orangutans, or gibbon apes, even under these conditions of low stringency.

DNAs extracted from various nonprimates, i.e., mice, dogs, calves, and salmon, were also examined for sequence relatedness to the MMC-1 genome, but no homology was observed. Similar to MAC-1 (4), a surprisingly high level of homology was found in DNA extracted from domestic cat liver (42%).

Nucleic acid reassociation kinetics were used to determine the reiteration frequency of MMC-1-related sequences in the DNA of primates and MMC-1-infected cells. These sequences were present in multiple copies (200 per haploid genome) in the genome of the species of origin, rhesus monkeys (10). In addition, DNA samples from other Old World monkeys were tested; baboons, Sykes monkeys, and African green monkeys all contained MMC-1-related sequences in multiple copies ranging from approximately 40 to 300 copies per haploid genome. In the MMC-1-infected Cf2Th canine cell line, MMC-1 sequences occurred at a frequency of about 30 copies per haploid genome. DNA from human HOS cells productively infected with MMC-1 virus were found to contain about 20 copies of MMC-1-related viral genes per haploid genome. In agreement with Bonner and Todaro (4), who found MAC-1-related sequences present in multiple copies in cat DNA, MMC-1 DNA sequences were found to be reiterated in cat liver DNA at approximately 150 copies per haploid genome.

As reported previously (10), the MMC-1 genome shared extensive sequence homology with the endogenous virus of stumptail macaques, MAC-1. The thermal stabilities of the hybrids formed between the MMC-1 probe and genomic RNAs from these macaque endogenous viruses were found to be identical within experimental variation (data not shown). This is consistent with the observed similarity of the proteins of the two viruses as analyzed on polyacrylamide gels (10).

Genomic organization of integrated MMC-1 proviruses. High-molecular-weight DNA isolated from canine cells chronically infected with MMC-1 was fragmented by using restriction enzymes and analyzed by hybridization to Southern blots, using a [^{32}P]cDNA probe. The specificity of the MMC-1 [^{32}P]cDNA probe was tested by comparison of the hybridization to DNA from uninfected and infected canine cells cleaved with either *Eco*RI or *Hin*dIII. No bands were apparent when uninfected cell DNA was analyzed (Fig. 1). In contrast, analysis of infected cell DNA yielded a single intense band for each of these enzymes. The *Eco*RI fragment was 2.2 kilobase pairs (kbp), and the *Hin*dIII fragment was 6.4 kbp.

*Hin*dIII, *Eco*RI, and *Xho*I cleaved each of the integrated MMC-1 genomes twice, generating single intense bands with molecular sizes of 6.4, 2.2, and 1.3 kbp, respectively (Fig. 2). When *Eco*RI and *Hin*dIII were used in combination,

bands of 2.4, 2.2, and 1.8 kbp were observed. The 2.2-kbp band represented the *Eco*RI cleavage product band, and the 2.4- and 1.8-kbp fragments were on either side of it, derived from the *Hin*dIII 6.4-kbp band. When *Xho*I and *Hin*dIII were used in double-digestion experiments, the 1.3-kbp *Xho*I band was observed along with two other fragments of 3.55 and 1.55 kbp (Fig. 2), which were on either side of the 1.3-kbp fragment within the *Hin*dIII 6.4-kbp fragment. When *Eco*RI and *Xho*I were used in double-digestion experiments, a doublet band of 1.1 kbp was observed. Therefore, the *Xho*I had one cleavage site in the middle of the *Eco*RI 2.2-kbp fragment and one site very close to the one *Eco*RI cleavage site, yielding a fragment too small to be observed. When *Eco*RI, *Hin*dIII, and *Xho*I were used in triple-digestion experiments, bands were observed at 2.4 and 1.55 kbp, as well as the *Eco*RI-*Xho*I 1.1-kbp doublet. The 1.55-kbp fragment arose from *Xho*I-*Hin*dIII cleavage. The 2.4-kbp fragment was generated by cleavage by *Eco*RI and *Hin*dIII and must have come from the region of the *Xho*I-*Hin*dIII 3.55-kbp fragment.

The restriction enzyme *Bam*HI cleaved the genome three times to yield two fragments, 2.65 and 0.95 kbp (Fig. 3). In double-digestion experiments with *Hin*dIII and *Bam*HI, two additional fragments, 1.15 and 1.65 kbp, were observed (Fig. 3). These arose from sequences between the outside *Bam*HI sites and the two *Hin*dIII sites. When *Bam*HI and *Xho*I were used in combination (Fig. 3), three major bands were detected, at lengths 1.45, 1.25, and 0.95 kbp. Therefore, the *Bam*HI fragment at 2.65 kbp was cleaved by *Xho*I into two fragments, of lengths 1.45 and 1.25 kbp. The 1.25-kbp band arose from a *Bam*HI site within the *Xho*I 1.3-kbp fragment. This fragment (lane 3) had a slightly slower mobility than the 1.25-kbp fragment.

*Sac*I cleaved the genome four times, generating three fragments of lengths 5.6, 1.83, and 0.7 kbp (Fig. 4). When the *Sac*I digest was further cleaved by *Xho*I, the 0.7-kbp fragment remained unchanged. A new 0.95-kbp fragment appeared from cleavage of the 5.6-kbp by *Xho*I. The remaining 0.38-kbp band resulting from cleavage of the *Sac*I 1.83-kbp band into the 1.45-kbp band was only faintly observed after prolonged exposure. When the *Sac*I digest was treated with *Sma*I, the 1.83-kbp fragment was cleaved into two fragments of 1.3 and 0.53 kbp (Fig. 4). *Sma*I also cleaved the *Hin*dIII 6.4-kbp fragment once, yielding a 4.95-kbp and a 1.35-kbp band. When

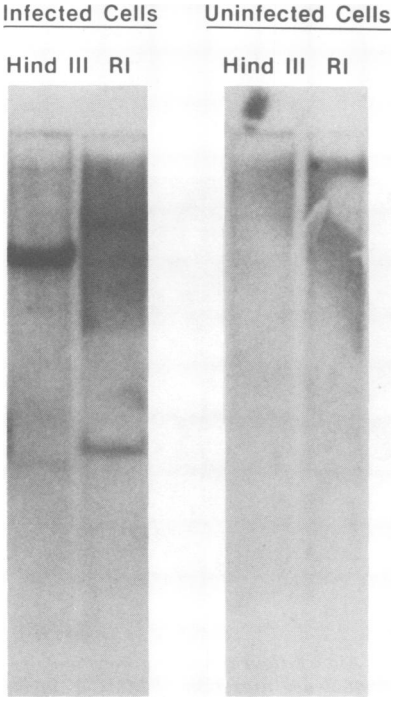

FIG. 1. *Autoradiograph of Southern blot of restriction endonuclease digestion of MMC-1-infected and uninfected canine Cf2Th cells. RI, EcoRI.*

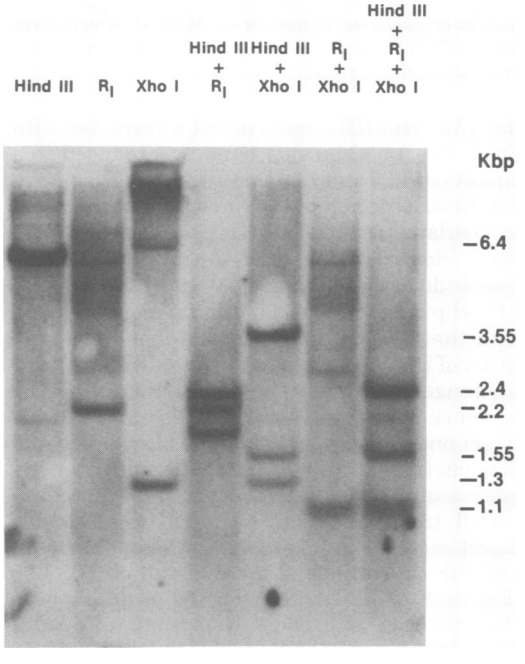

FIG. 2. *Autoradiograph of Southern blot of restriction endonuclease digestion of MMC-1-infected canine Cf2Th cells. Kilobase pair size estimates are indicated on the right. RI, EcoRI.*

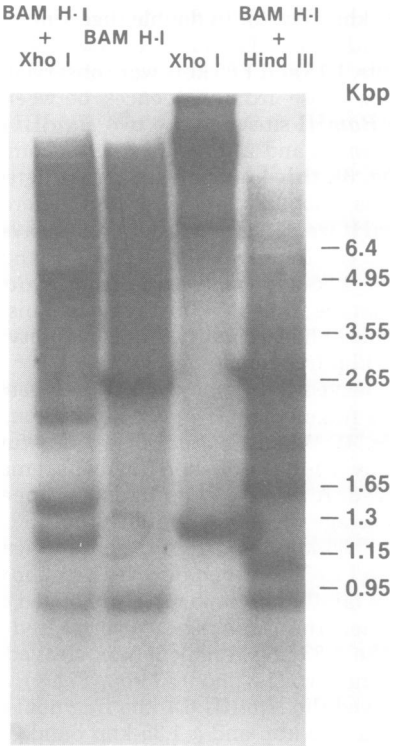

FIG. 3. *Autoradiograph of Southern blot of restriction endonuclease digestion of MMC-1-infected canine Cf2Th cells. Kilobase pair size estimates are indicated on the right.*

the *Xho*I-*Hin*dIII double digest was treated with *Sma*I, the 1.55-kbp side band was reduced to a doublet of 1.35 and 1.3 kbp (Fig. 5), thus confirming the orientation of the *Sac*I 1.8-kbp fragment as overlapping the *Hin*dIII-*Xho*I 1.55-kbp fragment. *Sma*I treatment of the *Bam*HI-*Hin*dIII double digest resulted in cleavage of the flanking 1.65-kbp band into a 1.35-kbp fragment identical with the *Hin*dIII-*Sma*I 1.35-kbp band. The position of the *Sac*I 0.7-kbp fragment was arbitrarily assigned to the right end of the map. The presence of *Sac*I sites at the ends of the MMC-1 genome was confirmed by Lovinger and Schochetman (7), who found the site in a sequence of the MMC-1 strong stop DNA.

*Pst*I treatment of the *Hin*dIII-*Xho*I double digest resulted in loss of the 3.55-kbp fragment and the appearance of a new band of 3.05 kbp (Fig. 6). Further evidence for the position of the *Pst*I cleavage site came from *Pst*I and *Sma*I double digestion, which yielded one band of 4.55 kbp (data not shown). From these data, a restriction enzyme map was constructed for much of the genome integrated in infected cells (Fig. 7).

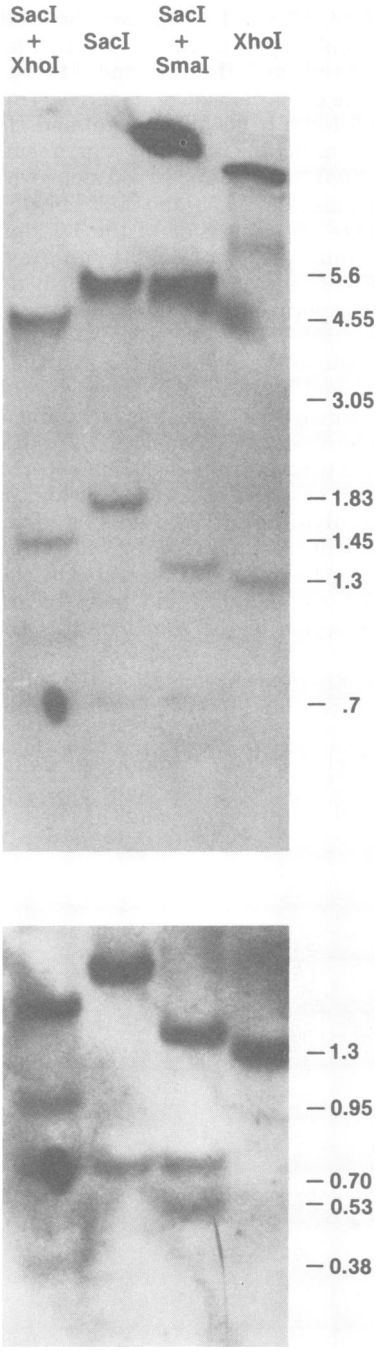

FIG. 4. *Autoradiograph of Southern blot of restriction endonuclease digestion of MMC-1-infected canine Cf2Th cells. Kilobase pair size estimates are indicated on the right. Lower portion was exposed longer to observe weaker bands.*

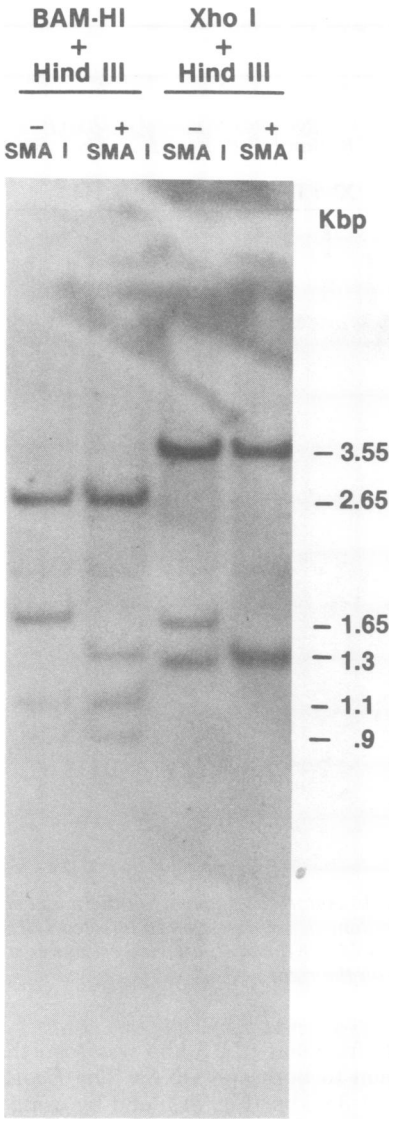

FIG. 5. *Autoradiograph of Southern blot of restriction endonuclease digestion of MMC-1-infected canine Cf2Th cells. Kilobase pair size estimates are indicated on the right.*

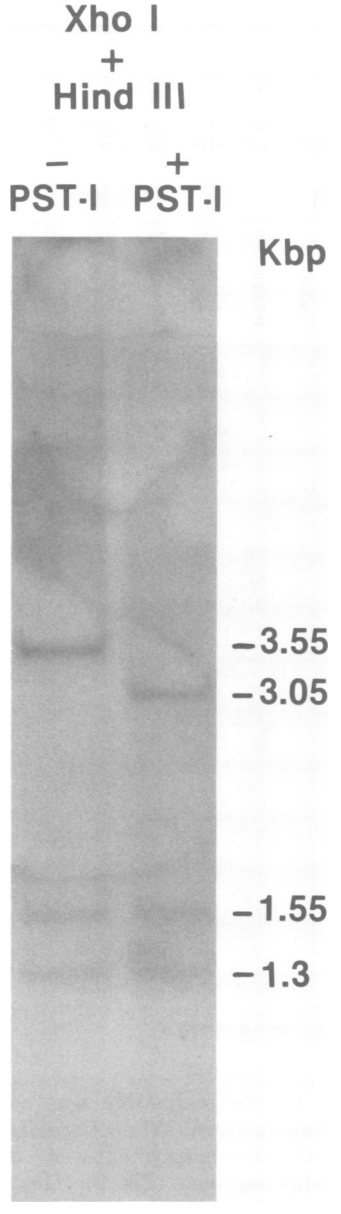

FIG. 6. *Autoradiograph of Southern blot of restriction endonuclease digestion of MMC-1-infected canine Cf2Th cells. Kilobase pair size estimates are indicated on the right.*

Structural analysis of MMC-1-related sequences in primate DNAs. DNA was isolated from a rhesus monkey embryonic kidney cell line (MA104) and digested with *Eco*RI and *Hin*dIII. The major restriction fragments observed previously in infected canine cell DNA were also observed in Southern blots of rhesus monkey cell DNA (Fig. 8). Additional hybridizing high-molecular-weight fragments were observed which could be accounted for by several explanations (e.g., multiple flanking bands, heterogeneity among genomes, etc.). However, no further experiments were carried out to clarify their origin. When baboon DNA was analyzed, the *Eco*RI 2.2-kbp band was observed, but not the *Hin*dIII 6.4-kbp band (data not shown).

In *Xho*I digests, the 1.3-kbp fragment ob-

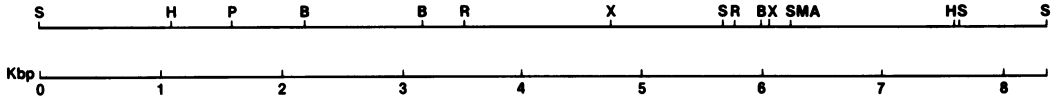

FIG. 7. *Restriction endonuclease cleavage map of MMC-1 proviral genome integrated in DNA from infected canine Cf2Th cells. Abbreviations: S, SacI; H, HindIII; P, PstI; B, BamHI; R, EcoRI; X, XhoI; SMA, SmaI.*

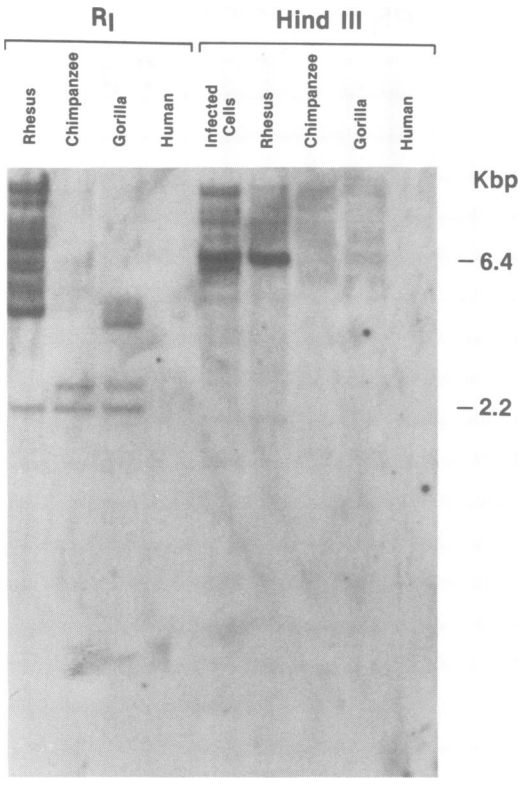

FIG. 8. *Autoradiograph of Southern blot of restriction endonuclease digestion of infected cell and primate DNAs. RI, EcoRI.*

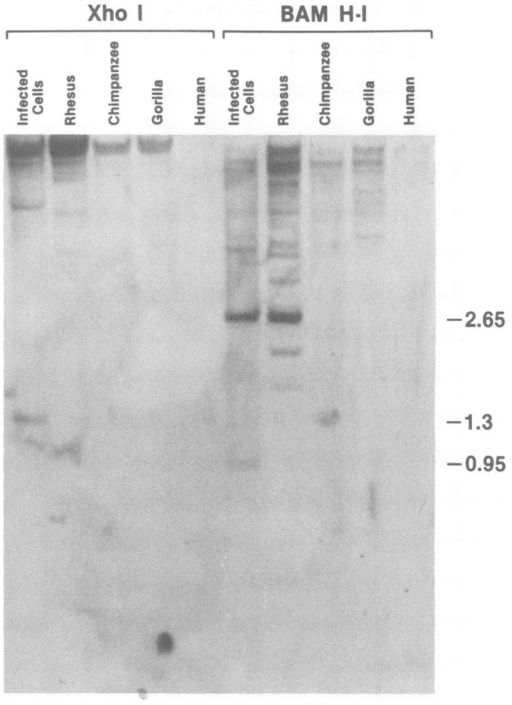

FIG. 9. *Autoradiograph of Southern blot of restriction endonuclease digestion of infected-cell and primate DNAs. Kilobase pair size estimates are indicated on the right.*

served in infected cell DNA was not seen in rhesus monkey DNA. When *Bam*HI was used, the *Bam*HI 2.65-kbp fragment was seen, but not the 0.95-kbp fragment (Fig. 9). The additional *Bam*HI fragments observed indicated the heterogeneity of the MMC-1-related sequences in rhesus monkey DNA. No bands were observed when rhesus monkey DNA was digested with *Sac*I (data not shown).

As reported here, DNAs from two apes, gorillas and chimpanzees, hybridized with MMC-1 [^{3}H]cDNA; however, those from humans, orangutans, and gibbon apes did not. Restriction analysis of ape DNAs indicated that the *Eco*RI 2.2-kbp fragment found in rhesus monkey and infected cell DNA was also present in gorilla and chimpanzee DNAs (Fig. 8). In addition, an *Eco*RI fragment of 2.5 kbp was seen that was common to both ape DNAs. The *Eco*RI fragment at 3.9 kbp (Fig. 8) found by using gorilla muscle DNA was also observed in analysis of DNA from a herpes gorilla-transformed gorilla cell line (data not shown) and thus appeared to be gorilla specific. *Hin*dIII (Fig. 8), *Bam*HI, and *Xho*I (Fig. 9) cleavage of the ape DNAs did not reveal any DNA fragments which could be correlated with the integrated genome from infected canine cells (Table 2). It should be noted that those ape DNAs which hybridized with [^{3}H]cDNA in solution hybridization experiments were positive on Southern blots also. Even with enzymes for which no internal bands were observed, some hybridization with high-molecular-weight DNAs was seen. In contrast, human DNA was uniformly negative both in blots and in solution hybridization.

TABLE 2. *Summary of restriction fragments observed in infected cells and primate DNAs*

DNA source	*Eco*RI 2.2 kbp	*Hin*dIII 6.4 kbp	*Bam*HI 2.65 kbp	*Bam*HI 0.85 kbp	*Xho*I 1.3 kbp
Infected Cf2Th cells	+	+	+	+	+
Rhesus MA104 cells	+	+	+	–	–
Chimpanzee kidney	+	–	–	–	–
Gorilla muscle	+	–	–	–	–

Both MMC-1 and the BaEV probes hybridize to African ape DNAs (3). To determine whether the homology is to identical ape DNA sequences, Southern blots of restriction enzyme DNA digests of primate DNAs were annealed with BaEV (M7) cDNA probe. Although this probe hybridized to some fragments, the 2.2- and 2.5-kbp fragments were not detected in digests of any of the primate DNAs. Therefore, the retrovirus-related sequences in the African apes fall into at least two subsets: those which are related to MMC-1 and those which are related to BaEV.

DISCUSSION

One property of a genetically transmitted retrovirus is the presence of cross-hybridizing nucleic acid sequences in closely related species. MMC-1-related sequences were found in multiple copies in all Old World monkeys thus far analyzed. In addition, MMC-1-related nucleic acid sequences were present in chimpanzees and gorillas. No homology was observed in DNA from humans, gibbon apes, or orangutans. It still remains possible that a moderately mismatched retrovirus-related sequence present in human DNA has eluded analysis because of the insensitivity of the present methodologies. Since mismatching affects the stability of nucleic acid hybrids, one does not know whether the 38 and 31% hybridization found in gorillas and chimpanzees, respectively, is related to the fact that some of the regions of MMC-1-related sequence diverge extensively, with others being relatively conserved, or to the fact that the entire region diverges to an intermediate extent (assuming that these sequences and MMC-1 arose from a common progenitor). A cleavage map of the integrated genome from infected canine cells was prepared to compare the MMC-1 genome with related sequences observed in various primates.

The observation that a small but discrete region of the MMC-1 proviral genome, the *Eco*RI 2.2-kbp fragment, was found in Southern blot analysis of both chimpanzee and gorilla DNA could reflect a specific regional conservation in these ape DNAs. This was consistent with solution hybridization data with ape DNA. The absence of *Xho*I and *Bam*HI fragments from the same region of the viral genome as the *Eco*RI 2.2-kbp fragment indicates at least some divergence within these specific sequences in DNA from these apes. Structural analysis of the endogenous virogenes related to MMC-1 in rhesus monkey DNA revealed some differences in restriction endonuclease cleavage patterns when compared with integrated proviral genes from infected canine cells. The precise determinants for these differences are as yet undefined. A possibility is that the endogenous virogene copies in rhesus monkey DNA are heterogeneous with respect to at least some DNA sequences. These small differences manifest themselves within this analytical framework by alterations in the recognition sequences at restriction endonuclease cleavage sites such that they are no longer cleaved. Therefore, one could postulate that the copy of the large number of endogenous virogenes (200) which gave rise to an infectious virus was a copy present in low frequency. If this putative infectious virogene were even at the level of a few percent of the total number of endogenous copies, it would not be detected in the present analysis. An alternative hypothesis for the difference in cleavage patterns between MMC-1-infected canine cell and rhesus monkey cell DNAs is that methylation-specific regions inhibit endonuclease cleavage in some loci of MMC-1-related sequences of rhesus monkey DNA. Cloning of these DNA fragments followed by sequence analysis will enable resolution of the above alternatives.

Hybridization of MMC-1 probes to DNAs from chimpanzees and gorillas and not to DNA from humans, gibbon apes, or orangutans follows an interesting pattern similar to that seen with the baboon viruses (3). These nucleic acid sequences from rhesus monkeys are homologous to DNAs from two African apes, gorillas and chimpanzees, but not to DNAs from the Asian apes, orangutans and gibbon apes. Humans are believed to have arisen in Africa and, with respect to the presence of MMC-1-related sequences, are different from the African apes. It is possible that low levels of homology exist in all apes and that the present hybridization methods are too insensitive for such an analysis.

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