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Distribution of endogenous type B and type D sheep retrovirus sequences in ungulates and other mammals

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ABSTRACT **The jaagsiekte sheep retrovirus (JSRV), which appears to be a type B/D retrovirus chimera, has been incriminated as the cause of ovine pulmonary carcinoma. Recent studies suggest that the sequences related to this virus are found in the genomes of normal sheep and goats. To learn whether there are breeds of sheep that lack the endogenous viral sequences and to study their distribution among other groups of mammals, we surveyed several domestic sheep and goat breeds, other ungulates, and various mammal groups for sequences related to JSRV. Probes prepared from the envelope (SU) region of JSRV and the capsid (CA) region of a Peruvian type D virus related to JSRV were used in Southern blot hybridization with genomic DNA followed by low- and high-stringency washes. Fifteen to 20 CA and SU bands were found in all members of the 13 breeds of domestic sheep and 6 breeds of goats tested. There were similar findings in 6 wild *Ovis* and *Capra* genera. Within 22 other genera of Bovidae including domestic cattle, and 7 other families of Artiodactyla including Cervidae, there were usually a few CA or SU bands at low stringency and rare bands at high stringency. Among 16 phylogenetically distant genera, there were generally fewer bands hybridizing with either probe. These results reveal wide-spread phylogenetic distribution of endogenous type B and type D retroviral sequences related to JSRV among mammals and argue for further investigation of their potential role in disease.**

The genomes of most or all vertebrates contain multiple copies of endogenous retroviral sequences that are related to sequences found in infectious retroviruses (1, 2). These sequences represent a large reservoir of viral genes that may be activated by mutations caused by radiation or chemical agents or through recombination with exogenous retroviruses. Studies of endogenous retroviral sequences have contributed not only to knowledge of their biological and pathological roles (3, 4) but also to mammalian evolution generally (5). Our laboratory has been investigating the etiologic role of jaagsiekte sheep retrovirus (JSRV) in ovine pulmonary carcinoma (OPC) (6, 7). Although not yet propagated *in vitro*, RNA of this virus has been isolated from OPC-affected sheep in South Africa and Peru and its nucleotide sequence has been determined (8, 9). By using viral-capsid-specific DNA probes, we found about 20 copies of this retroviral sequence in tumor-cell DNA of OPC-affected sheep and in the genomic DNA of healthy sheep (9). Furthermore, restriction fragment length polymorphisms of these viral sequences could be followed in sheep families, indicating fixation in the genome. JSRV is chimeric with respect to the morphologic classification of retroviruses, because it contains type D capsid sequences and type B envelope sequences, based on deduced amino acid homologies (8). Thus, it was of interest to learn whether JSRV represents a new genus of Retroviridae (2) and the extent of its distribution among mammalian taxa. Mouse mammary tumor virus, the prototype type B retrovirus, exists in endogenous and infectious exogenous forms and causes mammary neoplasms in mice (10). Type D retroviruses are found primarily in primates, both as endogenous and exogenous viruses. A simian type D retrovirus has been shown to cause immune deficiency (11). Among ruminants, only one infectious type D-like retrovirus has been isolated, from bovine cells (12). To learn whether there are lines of domestic sheep that lack the JSRV-related endogenous viral sequences and to study the distribution of these sequences among various groups of mammals, we undertook a survey of sheep and goat breeds, other wild and domestic ungulates, and representatives of diverse mammalian groups. We used nucleic acid probes derived from Peruvian and South African isolates of JSRV to screen Southern blots for viral capsid protein (CA) and virus surface envelope envelope protein (SU) sequences. The results indicated that all sheep carry about 20 sequences related to these viruses and that most of the viral copies have been fixed in the genome prior to the breeding of domestic sheep. An equally large number of viral sequences were found in goats, especially in goats of the domestic lineage. Among wild ruminant genera tested, only the mountain goat was free of capsid-related sequences under conditions of low stringency. A survey of additional ungulates and mammals revealed that related sequences were broadly distributed among the genera tested, correlating partially with the presumed phylogenetic history of these species (13).

MATERIALS AND METHODS

Samples from domestic sheep and goats were collected from flocks in Wyoming, Colorado, Texas, and Kenya. Tissue or DNA samples from other animal species and humans were provided by the staff of research institutes, zoos, and ranches. Genomic DNA was isolated (14), and 8–10 μg was digested with 50 units of *Eco*RI or other restriction enzymes, electrophoresed for 17.5 h at 50 V through 0.8% agarose, and, transferred to nylon membranes. The integrity of the DNA of each sample was evaluated and each had an average size greater than 50 kb. Two molecular probes were used in this study. The 604-bp CA probe was designed from pCA1, a Peruvian type D retrovirus isolate with more than 90% nucleotide homology to JSRV between nt 1203 and 1806 (9). The SU probe was generated using JSRV clone JS107, provided by G. Querat and D. York (8), by priming at bp 5539 and 6102 of the JSRV sequence. Probes were labeled with [α-^{32}P]dCTP (15), amplified, and purified as described (9). Each Southern blot included reference DNA from the same sheep as a positive control, and λ DNA and DNA samples that did not hybridize served as negative controls. Hybridized blots were prewashed two or three times for 20-min periods at room temperature in 1× SSC/0.1% SDS, followed by stringency washes at one of two temperatures. Low stringency consisted

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Abbreviations: JSRV, jaagsiekte sheep retrovirus; OPC, ovine pulmonary carcinoma; CA, viral capsid protein; SU, virus surface envelope protein; MPMV, Mason–Pfizer monkey virus.

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of two or more washes in 2× SSC/0.1% SDS at 55°C (≈30°C below the calculated melting temperature T_m of the probes used). T_m = 81.5 + 16.6 log[Na^+] + 0.41(%GC) (16). High stringency consisted of two or more washes at 65°C in 1× SSC/0.1% SDS (≈20°C below the T_m of the probes used). Blots were autoradiographed for optimal periods at −70°C. No hybridization bands were observed with control Southern blots of Mason–Pfizer monkey virus (MPMV), a type D retrovirus with 57% homology to JSRV in the CA region. Searches of GenBank with the CA and SU probe sequences did not reveal any known sequence with more than 57% homology to these probes.

RESULTS

Southern Blot Analysis of Mammals. Genomic DNA was isolated from animals, digested with *Eco*RI, and separated by agarose gel electrophoresis. Southern blots of these gels were hybridized with probes from the CA and SU regions of JSRV. Digests with *Bam*HI, *Sac* I, *Eco*RI, and *Pvu* II suggested that *Eco*RI cuts the virus into two integrated fragments as predicted by the JSRV sequence (8). Table 1 summarizes the distribution of JSRV-like sequences in diverse species of mammals (13), indicating the number of bands seen in each taxonomic group. The order of the species in Table 1 corresponds with the order of phylogenetic groups in the text and in the figures; Figs. 1–5 show representative results. By using both the CA and SU probes, differences in stringency were exploited to gain an indication of the homology between the probes and the JSRV integrations. The number of sheep DNA sequences that hybridized to either CA and SU probes did not vary when washed at low stringency (≈30°C below probe T_m) or higher stringency (≈20°C below probe T_m), whereas hybridizing bands in other species were often lost or diminished at the higher stringency.

Domestic and Wild Sheep (Genus: *Ovis*). As shown in Fig. 1, genomic DNA isolated from sheep of 13 domestic breeds was probed in Southern blot hybridization with the CA probe. Two or three animals from each breed were probed with similar results (data not shown). Fifteen to 20 bands were seen in each lane (Fig. 1*A*). Most of the similarly sized hybridizing bands between 5.5 and 20 kb were conserved in distantly related sheep breeds. For example, also shown in Fig. 1, a band profile similar to that of domesticated sheep of North America was seen in Jacob sheep, a Northern European four-horned breed with wool, and Red Masai, a breed of fat-tailed hair sheep from East Africa. This also was true of bighorn sheep, mouflon sheep, and dall sheep, all wild sheep genera (Fig. 1 *B* and *C*).

The same blots in Fig. 1*C* were treated to remove the CA probe and hybridized with the JSRV sequence. Fig. 1*D* shows band profiles of a bighorn, a mouflon, and the domestic Suffolk breed. The sheep genomic DNA samples had similar numbers of JSRV CA and SU sequences. Some higher molecular weight SU bands were similar in size to the CA bands and may represent the same virus, while lower bands may be subviral sequences. However, it is impossible to predict the restriction patterns of the endogenous sequences, and variations in the banding patterns could be due to deletions or changes in sequences affecting restriction sites.

Table 1. Endogenous retroviral sequences related to JSRV type D CA and type B SU regions in various groups of mammals detected by Southern blot-hybridization at two levels of stringency

Group	Genus and species	Common name	No. studied	No. of bands: Type D CA Low	Type D CA High	Type B SU Low	Type B SU High
Order: Artiodactyla							
Family: Bovidae							
Subfamily: Caprinae	*Ovis aires*	Domestic sheep	40	15–20	15–20	15–20	15–20
	Ovis canadensis	Bighorn sheep	3	15–20	15–20	15–20	15–20
	Ovis musimon	Mouflon	3	15–20	15–20	15–20	15–20
	Ovis dalli	Dall sheep	1	16	16	16	16
	Capra hircus	Domestic goats	18	15–20	15–20	15–20	15–20
	Capra aegagrus	Cretan goat	1	15	15	15	ND
	Capra ibex	Siberian ibex	1	15	15	15	ND
	Capra falconeri	Markhor	1	17	17	12	6
	Psuedois nayaur	Bharal	1	2	2	1	ND
	Capricornis crispus	Japanese serow	1	12	8	2	ND
	Nemorhaedus goral	Goral	2	6	6	5–6	4
	Hemitragus jemlahicus	Himalayan tahr	1	11	11	14	7
	Ammotragus lervia	Aoudad	1	7	0	0	ND
	Budorcas taxicolor	Takin	1	7	6	3	1
	Oreamnos americanus	Mountain goat	5	0	0	±	0
Subfamily: Bovinae	*Bos taurus*	Domestic cattle	22	2–4	1–2	8–10	1–3
	Bison bison	Bison	2	7	1	5–7	2–4
	Bosfrontalis gaurus	Gaur	1	2	1	10	4
	Bos javanicus	Banteng	2	2	ND	7	ND
	Bos grunniens	Yak	1	9	2	7	2
	Syncerus caffer	Cape buffalo	1	4	0	4	1
	Boselaphus tragocamelus	Nilgai	1	6	0	0	0
Subfamily: Other	*Tragelaphus imberbis*	Lesser kudu	1	5	1	0	ND
	Tragelaphus angasi	Nyala	1	7	2	1	1
	Antidorcas marsupialis	Springbok	1	9	4	6	0
	Aepyceros melampus	Impala	1	4	0	7	0
	Connochaetes gnou	Gnu	1	3	3	2	0
	Oryx dammah	Scimitar	1	8	0	10	0
	Damaliscus dorcas	Blesbok	1	2	0	5	ND
	Cephalphus maxwelli	Maxwell's duiker	1	13	6	6	2

Table 1. (*Continued*)

Group	Genus and species	Common name	No. studied	No. of bands: Type D CA Low	Type D CA High	Type B SU Low	Type B SU High
Family: Antilocapridae	*Antilocapra americana*	Pronghorn	3	4	0	5	0
Family: Cervidae	*Cervus elaphus*	Wapiti	2	8–9	1–2	4–6	0
	Elaphurus davidianis	Pere David's deer	3	6–7	1–3	10–11	1–3
	Alces alces	Moose	1	6	5	2	2
	Odocoileus virginianus	Whitetail deer	1	12	0	6	1
	Odocoileus hemionus	Mule deer	2	3–7	0–2	4	1
	Rangifer tarandus	Reindeer	2	0	0	1–2	1
Family: Giraffidae	*Giraffa cameloparalis*	Giraffe	3	5	0	3	0
Family: Camelidae	*Lama glama*	Llama	5	6–9	1–2	6	2
Family: Hippopotamidae	*Hippopotamus amphibus*	Nile hippopotamus	2	2	0	3	0
Family: Tayassuidae	*Tayassu pecari*	Collared peccary	1	±	0	0	0
Family: Suidae	*Sus scrofa*	Old World boar	1	±	0	0	0
Order: Perissodactyla							
Family: Equidae	*Equus caballus*	Domestic horse	1	0	0	4	0
	Equus caballus przewlaski	Przewlaski's horse	1	2	0	3	0
	Equus grevyi	Zebra	1	1	1	4	1
Family: Rhinocerotidae	*Ceratotherium simum*	Rhinoceros	1	0	0	0	0
Order: Carnivora							
Family: Felidae	*Panthera tigris*	Bengal tiger	2	2	0	3–6	0
	Panthera pardus	Spotted leopard	1	3	0	4	0
	Felis concolor	Mountain lion	2	3–8	0	3–6	0
Family: Canidae	*Chrysocyon brachyurus*	Maned wolf	1	0	0	1	0
Family: Ursidae	*Ursus americanus*	Black bear	1	±	0	2	0
Family: Procyonidae	*Ailuropoda fulgens*	Lesser panda	2	2	0	11–12	0
Order: Rodentia							
Family: Muridae	*Mus musculus*	Mouse (BALB-c)	1	8	0	11	0
	Crethrionomys rutilis	Vole	2	0	0	11–13	0
Order: Primates							
Family: Ellithricidae	*Saguinus oedipus*	Cotton-topped tamarin	1	2	0	3	0
Family: Cebidae	*Allouatta caraya*	Black howler monkey	1	3	1	1	0
Family: Cerocopithecidae	*Macaca mulatta*	Macaque	2	±	0	1	0
Family: Hominidae	*Homo sapiens*	Human	8	0	0	2	0

Low stringency is equal to ≈30°C below calculated probe T_m; high stringency is equal to ≈20°C below calculated probe T_m. ND, not done; ±, faint band(s) in some animals.

Other Sheep and Goats (Family Bovidae, Subfamily Caprinae). Similar to sheep, 15–23 type D and B sequences were found in domestic goats (Fig. 2 *A* and *D*). Profiles of endogenous sequences in this animal group were different from the sheep: an intense band about 3 kb that hybridized with the probe suggested an internal band shared by several viruses. The Cretan goat, the Siberian ibex, and the markhor, all *Capra* genera, displayed patterns similar to domestic goats (Fig. 2 *B* and *C*). Among other genera of Caprinae (Fig. 2), the Japanese serow (Table 1) and the goral had patterns somewhat similar to domestic goats, especially at low stringency. However, the aoudad (Barbary sheep, Table 1) had only light bands at low stringency. Himalayan tahrs had a pattern different from the domestic goats. The bharal had only two hybridizing bands, fewest among the members of Caprinae examined, but these hybridized strongly. A takin had multiple but weakly hybridizing bands. Interestingly, none of the five Rocky Mountain goats tested (Table 1), phylogenetically grouped with takin in Caprinae, had any hybridizing bands with the CA probe at low or high stringency and only showed one or two faint bands with low-stringency SU probing.

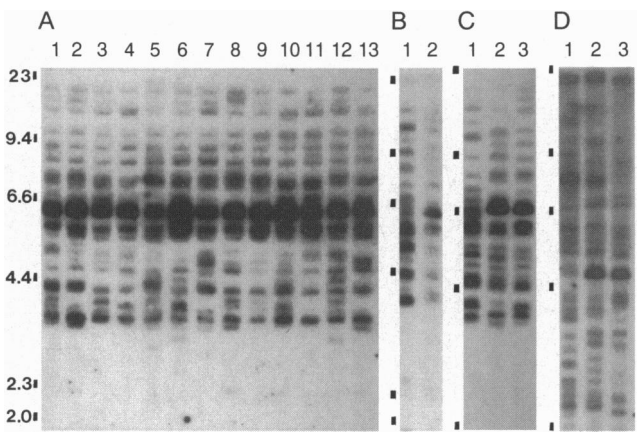

FIG. 1. Endogenous JSRV-related sequences in representative sheep. Genomic DNA of each sample was digested with *Eco*RI and subjected to Southern blot analysis. Hybridization conditions were low stringency (55°C; T_m, −30°C). (*A–C*) Hybridized with the CA probe. (*D*) Same blot as *C* but hybridized with SU probe after stripping. Thirteen breeds of domestic sheep are shown in *A* as follows. Lanes: 1, Barbados; 2, Columbia; 3, Dorset; 4, Hampshire; 5, Jacob; 6, Merino; 7, Polypay; 8, Rambouilet; 9, Red Masai; 10, Romanov; 11, Southdown; 12, Suffolk; 13, Texel. (*B*) Lanes: 1, dall sheep; 2, Suffolk 2. (*C*) Lanes: 1, bighorn sheep; 2, mouflon; 3, Suffolk 2. The size, in kb, of λ DNA digested with *Hin*dIII is shown to the left.

Cattle, Antelopes, and Other Bovids (Family Bovidae). American bison and domestic cattle of two breeds had patterns similar to each other and had fewer bands cross-hybridizing with both probes than did goats (Fig. 3 *A* and *D*). Other members of the Family Bovidae (Fig. 3 *B* and *C*) also had a few CA bands at the lower stringency. Yak, springbok, and Maxwell's duiker, representing different subfamilies of Bovidae, had many bands at both stringencies. Other members of Bovidae, such as the lesser kudu and nilgai, had a few bands that cross-hybridized with one or both probes (Table 1). The

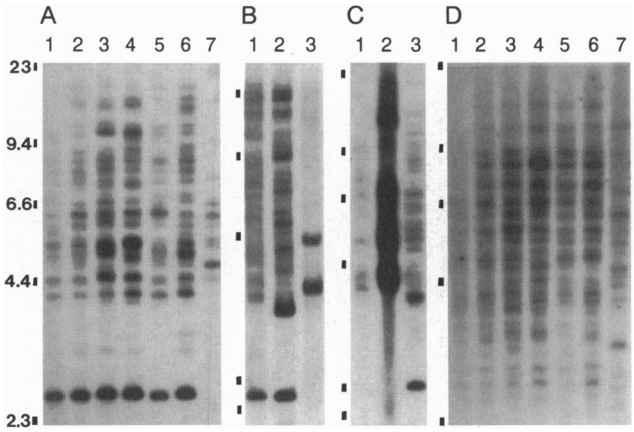

FIG. 2. Endogenous JSRV-related sequences in representative goats. Genomic DNA of each sample was digested with *Eco*RI and subjected to Southern blot analysis. Hybridization conditions were low stringency (55°C; T_m, −30°C). (*A–C*) Hybridized with the CA probe. (*D*) Same blot as *A* but hybridized with the SU probe after stripping. (*A*) Lanes: 1, East African (Kenya); 2, Galla (Kenya); 3, Togenburg (Kenya); 4, Saneen (Colorado); 5, Angora (Texas); 6, Spanish (Texas); 7, Himalayan tahr. (*B*) Lanes: 1, Cretan goat; 2, Siberian ibex; 3, bharal. (*C*) Lanes: 1, takin; 2, goral; 3, markhor. The size, in kb, of λ DNA digested with *Hin*dIII is shown. In *B* only, the top marker shown is 9.4 kb. In *B* and *C*, the bottom markers are 2.0 kb.

CA bands of domestic cattle and bison were intense and subviral, so these may represent internal *Eco*RI fragments of several copies of virus.

Deer and Other Ungulate Families Within Order Artiodactyla. Representatives of other families of Artiodactyla are shown in Fig. 4. The American pronghorn had several bands when hybridized with the CA or SU probes at lower stringency (Fig. 4 *A* and *E*). The Cervidae, considered an early branch of Bovidae, varied extensively. Reindeer had only a few faint bands with the CA probe (Fig. 4*C*) and a few bands with the SU probe (Table 1). Wapiti (American elk), moose, and Pere David's deer all had CA and SU cross-reactive bands (Fig. 4 *A–C*). Giraffes had CA- and SU-related sequences at low

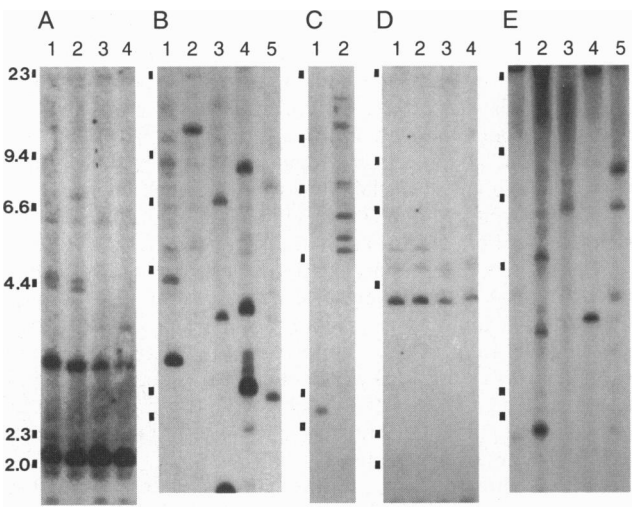

FIG. 3. Endogenous JSRV-related sequences in representative Bovidae. Genomic DNA of each sample was digested with *Eco*RI and subjected to Southern blot analysis. Hybridization conditions were low stringency (55°C; T_m, −30°C). (*A–C*) Hybridized with the CA probe. (*D* and *E*) Hybridized with the SU probe, after stripping blots in *A* and *B*, respectively. (*A*) Lanes: 1, bison; 2, bison 2; 3, domestic cow (Charolais); 4, domestic cow (Holstein). (*B*) Lanes: 1, duiker; 2, nyala; 3, gnu; 4, yak; 5, cape buffalo. (*C*) Lanes: 1, impala; 2, springbok. The size, in kb, of λ DNA digested with *Hin*dIII is shown.

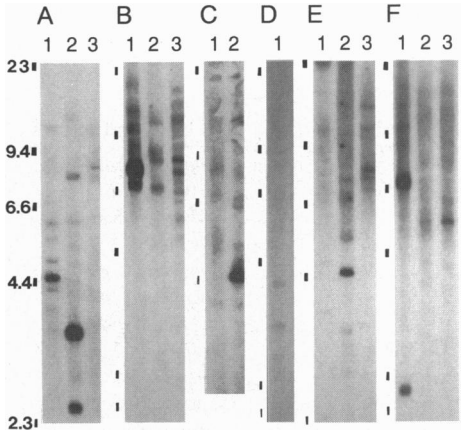

FIG. 4. Endogenous JSRV-related sequences in representative Artiodactyla. Genomic DNA of each sample was digested with *Eco*RI and subjected to Southern blot analysis. Hybridization conditions were low stringency (55°C; T_m, −30°C). (*A–C*) Hybridized with the CA probe. (*E* and *F*) Hybridized with the SU probe after stripping blots in *A* and *B*, respectively. (*A*) Lanes: 1, wapiti; 2, llama; 3, pronghorn. (*B*) Lanes: 1, moose; 2, mule deer; 3, whitetail deer. (*C*) Lanes: 1, reindeer; 2, Pere David's deer. (*D*) Hippopotamus. The size, in kb, of λ DNA digested with *Hin*dIII is shown. The 2.0- and/or 2.3-kb markers are not shown in *A, C*, and *E*.

stringency only (Table 1). Llamas (Camelidae) had cross-hybridizing sequences for both probes (Fig. 4 *A* and *E*), and even one or two high-stringency bands (Table 1). Of the suiformes tested, only the hippopotamus had definitive bands (Fig. 4*D*).

Horses, Carnivores, Rodents, and Primates (Other Orders Within Mammalia). Ungulates outside Artiodactyla and various other mammals were also surveyed (Table 1 and Fig. 5). The domestic horse, Przewlaski's horse, zebra, and rhinoceros had only one or no bands at high stringency. At lower stringency, more SU-cross-hybridizing sequences were seen in Equidae (Fig. 5 *C* and *D*). Most other animals showed bands only at low stringency, more often with the SU probe than the CA probe. Several carnivores, including Bengal tigers, spotted

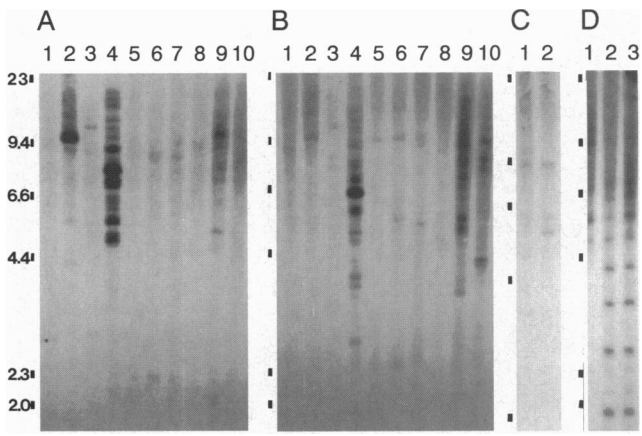

FIG. 5. Endogenous JSRV-related sequences in mammals other than members of Artiodactyla. Genomic DNA of each sample was digested with *Eco*RI and subjected to Southern blot analysis. Hybridization conditions were low stringency (55°C; T_m, −30°C). (*A*) Hybridized with the CA probe. (*B*) Hybridized with the SU probe after stripping blot in *A*. (*C* and *D*) Probed with SU. (*A*) Lanes: 1, human; 2, howler monkey; 3, cotton-topped tamarin; 4, Suffolk sheep control; 5, Bengal tiger; 6, spotted leopard; 7, mountain lion; 8, maned wolf; 9, mouse (BALB/c); 10, vole. (*C*) Lanes: 1, horse; 2, zebra. (*D*) Lanes: 1, Przewlaski's horse; 2, lesser panda; 3, lesser panda 2. The size, in kb, of λ DNA digested with *Hin*dIII is shown. The 2.0-kb marker is not shown in *C*.

leopards, and mountain lions appeared to have similarly sized bands that weakly hybridized with each probe (Fig. 5 *A* and *B*), suggesting that at least one endogenous virus was integrated before the divergence of felid species. Only a few rodents, in which type B viruses were originally described, were examined. A mouse and a vole each had many SU-related bands and fewer CA-related bands (Fig. 5 *A* and *B*). Primates, in which type D retroviruses have been characterized, only had a very few weakly hybridizing bands with the type D CA probe (Fig. 5*A*). Humans clearly had only type B bands, but a howler monkey exhibited some strongly hybridizing, equivalent-sized bands with both probes (Fig. 5 *A* and *B*). A cotton-topped tamarin also had a few bands. Even the two lesser pandas examined had several related type B sequences (Fig. 5*D*).

DISCUSSION

The present results show that domestic sheep of widely varying breeds share many endogenous proviruses at similar integration sites. The breeds studied were chosen based not only on availability but also on their diverse breeding histories (17). Interestingly, wild members of the genus *Ovis* shared integration site patterns with domestic breeds, as did domestic goats with other species in the genus *Capra.* Since both probes bind at high stringency, the sheep and goat viruses must be closely related, but the differences in restriction pattern suggest that much of the amplification from founding viruses within the respective genomes occurred after the divergence of goats and sheep from 4 to 10 million years ago (18). This conservation of endogenous viral numbers and presumed chromosomal sites among sheep and goats is quite different from the variation seen in endogenous type B viruses of wild mice (19) and in avian viruses of chickens, jungle fowl, and pheasants (20). However, it is reminiscent of the endogenous type C viruses shared by *Mus* species (21).

Both type D and B sequences were found in other ungulates as well, with clear demarcations in relationship at several places. The first demarcation is between the domestic goat and sheep lineage and other members of the Caprinae family. The Himalayan tahr, aoudad, and Japanese serow had clear differences in copy numbers and homology, as detected by the probe binding stringency. More type D CA sequences were conserved, which may reflect the tendency to higher conservation of the *gag* region over the envelope sequences before fixation in the germ line. Domestic cattle and bison shared a similar pattern containing very few copies; these were subviral and may reflect a viral group with a conserved *Eco*RI internal fragment but with dissimilar integration patterns or numbers (a caveat also applicable to some deer and llama results). In general, SU and CA sequences had similar distributions, implying that the type D and type B viruses have the same phylogenetic distribution or that a recombinant B/D virus infected the ancestor species. It is possible that JSRV may have arisen through recombination of type B and type D retrovirus prototypes.

Results with certain pecorans (Antilocapridae, Cervidae, and Giraffidae) ranged from nearly negative in reindeer, to a few low-stringency bands in the pronghorn and giraffe, to several high- and low-stringency CA and SU integrations in the Pere David's deer. There was similar diversity within Cervidae, as wapiti and Pere David's deer appeared to share certain conserved proviral regions that were dissimilar to those of whitetail or mule deer, which were, in turn, quite distinct from each other. These results suggested a more recent horizontal viral transfer event rather than phylogenetic passage, consistent with the isolation of type C retroviruses from deer (22). Suiformes (e.g., Tayassuidae and Suidae) were generally negative, whereas llamas (Camelidae) had several cross-hybridizing bands. These integrations also may represent a recent horizontal viral transfer when sheep and llamas were herded together in South America. Camels were not tested in this study. The cladistics of Bovidae are unclear (18), and analysis of conserved subviral fragments may aid in assessing relationships among diverse subfamilies.

Whether the JSRV-related viruses represent a unique class of retroviruses with a type B envelope region and a type D capsid region is difficult to determine. In several cases, the number of SU and CA hybridizing sequences correlated, but experiments employing additional restriction enzymes that do not digest within the viral sequences would be required to clarify the issue. The JSRV envelope region is only minimally related to the mouse mammary tumor virus type B sequence (27% at the amino acid level) (8). Type B and D viruses seem to be closely related to each other (23), and viruses such as JSRV may represent intermediates between events leading to the two morphologic types. Type D viruses are found in primates (MPMV, Po-1-Lu, squirrel monkey retrovirus, and simian retrovirus) (11), prosimians (24), and carnivores (skunks) (25). Type B viruses are reported in mice (mouse mammary tumor virus) (10) and guinea pigs (26), and related sequences extend to other mammals, including humans (27). The hybridization conditions used in this study should not have detected these viruses, since the lower-stringency probing probably allowed no more than a 20–30% mismatch; this was previously confirmed for MPMV and squirrel mouse retrovirus (9). Earlier attempts to use MPMV as a DNA probe to find related sheep sequences were futile, again because of the 40–50% potential mismatch (8, 9).

The observation that this large family of endogenous viruses has integrated in sheep and goats before development of domestic breeds also has implications for the study of diseases with suspected retroviral etiology in these animals. OPC has an extremely variable incidence worldwide: it is insignificant in North America and has not been identified in Australia, but it is economically important in South Africa, Scotland, and Peru (6). This epidemiologic pattern of disease is consistent with an infectious etiology, and exogenous horizontally transmitted JSRV is the presumed etiologic agent of OPC. Evidence has recently been obtained that unique U3 long terminal repeat sequences distinguish JSRV from several JSRV-related endogenous sequences (28). If the endogenous viruses contribute to the induction of OPC through recombination with JSRV or are reactivated to cause disease, other factors may govern the observed geographic variation in incidence of OPC. Since all sheep apparently carry the JSRV-related sequences, the prospect is remote for finding endogenous virus-negative sheep for experimental transmission studies. However, other species such as the Rocky Mountain goat may be useful in such experiments. The results of the present work also have implications for the etiopathogenesis of a type D virus-associated nasal adenocarcinoma of sheep and goats (ref. 29 and unpublished data).

Finally, OPC has been considered as a model for human bronchioloalveolar carcinoma (6). To discover whether a related virus may be associated with the human neoplasm, DNA was extracted from bronchioloalveolar carcinoma tissue of nine humans, probed with the JSRV probes, and compared to the band patterns of concordant nontumor lung tissue from six of these patients (data not shown). As in the case of sheep with OPC, there were no differences between the tumors and nontumors under the hybridization and washing conditions used. If JSRV is the etiologic agent of OPC, it remains possible that similar pathways of oncogenesis are involved in sheep and humans, such as the same oncogene. These possibilities remain to be determined.

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