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Highly Lytic and Persistent Lentiviruses Naturally Present in Sheep With Progressive Pneumonia Are Genetically Distinct

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Ovine and caprine lentiviruses share the capacity to induce slowly progressive and inflammatory diseases of the central nervous system (leukoencephalitis or visna), lungs (progressive pneumonia or maedi), and joints (arthritis) in their natural hosts. Studies on their replication indicated that ovine lentiviruses and caprine arthritis-encephalitis virus (CAEV) recently isolated in the United States establish persistent infection in ovine and caprine fibroblasts, whereas older prototype ovine lentiviruses such as Icelandic visna virus or American progressive pneumonia virus irreversibly lyse fibroblast cultures. Since all of the recent isolates were found to be persistent, Narayan et al. (J. Gen. Virol. 59:345–356, 1982) concluded that the highly lytic viruses were only tissue-culture-adapted strains. In the present report, we isolated new ovine lentiviruses from French sheep with naturally occurring progressive pneumonia which are either highly lytic (five isolates), as are the Icelandic strains of visna virus, or persistent (one isolate), as are CAEV or American persistent ovine lentiviruses. Protein and nucleic acid content analyses of these new highly lytic (type I) and persistent (type II) isolates indicated that type I and type II ovine lentiviruses were genetically distinct, type I and type II viruses being closely related to the Icelandic strains of visna virus and to CAEV, respectively. We conclude that (i) highly lytic ovine lentiviruses, such as the Icelandic prototype strains of visna virus and persistent lentiviruses more related to CAEV, are naturally present in the ovine species, and (ii) irreversible cell lysis induced by highly lytic viruses does not result from a tissue culture adaptation of field isolates that were originally persistent but is instead the consequence of a genetic content distinct from that of persistent viruses.

Lentiviruses, members of a subfamily of the family Retroviridae, are agents of naturally occurring, nonmalignant diseases in sheep and goats after prolonged incubation periods and slow progressive development of clinical signs. The three diseases that the lentiviruses induce are progressive leukoencephalitis (visna), progressive pneumonia (maedi), and chronic arthritis. The first two are classical examples of slow virus diseases in sheep (28, 29, 32). Arthritis in joints is predominant particularly in adult goats (6).

Ovine lentiviruses causing visna and maedi, respectively, the Icelandic strains of visna virus and the American strains of progressive pneumonia virus (PPV), replicate through a lytic cycle in cell cultures of ovine choroid plexus (OCP), producing prominent cytopathic effects in the cell monolayers and high amounts of infectious viral particles in the supernatant fluids (12). They contain three major internal proteins (p30, p16, and p14) and a major glycoprotein (gp135) (13) and have in common a large part of their nucleic acid sequences (11, 37).

Caprine lentiviruses causing arthritis in adult goats, such as the American strains of caprine arthritis-encephalitis virus (USA-CAEV), differ biologically and biochemically from the ovine lentiviruses causing maedi and visna. USA-CAEV induces a persistent infection of goat cells without resulting in complete cell lysis as in maedi-visna virus infections (18, 20). It shares with maedi-visna viruses p30 and gp135-related antigens (4, 26) but contains internal proteins, smaller than p30, which are probably antigenically distinct from maedi-visna viruses p16 and p14 (7). USA-CAEV and Icelandic visna virus have in common only 20 to 30% of their nucleic acid sequences (11, 26).

In addition to the clearly distinguished maedi-visna viruses and USA-CAEV, American field viruses were recently isolated from organs of sheep with visna-like disease, progressive pneumonia, and arthritis (23). They appear to replicate slowly and cause persistent infections in OCP cells in an analogous manner to that of USA-CAEV in goat fibroblasts but have not yet been biochemically characterized (20, 22). Since all of the new isolates were found to be persistent, highly lytic viruses such as Icelandic visna virus strains could represent tissue-culture-adapted strains and not natural maedi-visna agents.

In this context, the following questions could be raised. (i) Are highly lytic and persistent viruses naturally present in the ovine species? (ii) If yes, are the two types of virus genetically distinct as suggested by the genetic differences recently observed between prototype maedi-visna viruses and CAEV?

To answer to these questions, we isolated viruses from tissue explants of French sheep with naturally occurring progressive pneumonia and studied their biological and biochemical properties. We found five isolates which replicate very efficiently in OCP cells via a lytic cycle as do Icelandic visna virus strains and one isolate which causes a persistent infection of OCP cells similar to USA-CAEV in caprine synovial membrane cells. Biochemical analysis of these viruses showed that their genetic contents are markedly different. We concluded that two genetically distinct

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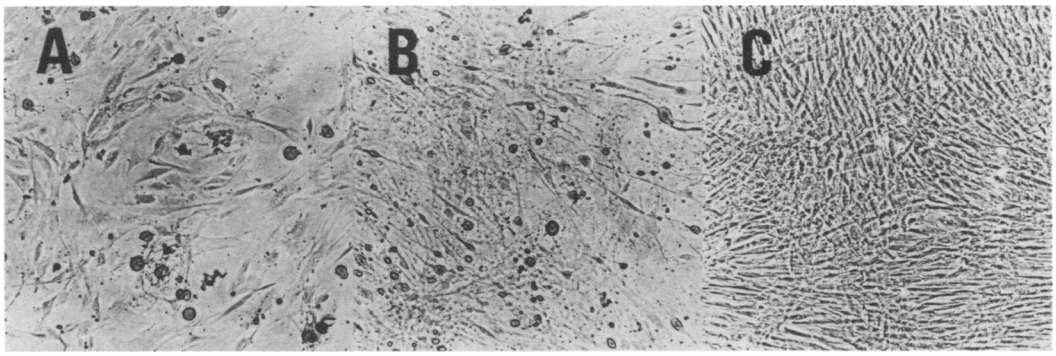

FIG. 1. Morphological aspects of ovine cell cultures infected by new lentiviruses isolated from French sheep with progressive pneumonia. Ovine fibroblasts chronically infected by the new isolate from sheep 2150-81 (A), ovine fibroblasts lytically infected by the new isolate from sheep 1982-81 (B), and normal ovine fibroblasts F-OCP_1 (C) photographed with bright field optics (A and B) or phase-contrast optics (C).

classes of lentiviruses, either highly lytic or persistent, may be present in sheep with naturally occurring progressive pneumonia.

MATERIALS AND METHODS

Viruses. The prototype strains of ovine lentiviruses causing leukoencephalitis are the Icelandic strains K796 and K1514 of visna virus (24). The American strain of PPV isolated in Montana by Kennedy et al. (17) is the prototype strain of ovine viruses causing progressive pneumonia. USA-CAEV isolated by Cork et al. (5) is the prototype of caprine lentiviruses causing arthritis in goats. Field ovine lentiviruses were obtained from several naturally infected French sheep with clinical signs of pneumonia and lentivirus-positive serology (27, 36). Viruses were obtained from these animals by harvesting supernatant fluids from tissue explants cocultivated with lentivirus-negative OCP fibroblasts (cell line F-OCP_1). After propagation in ovine or caprine fibroblasts, they were cloned by endpoint dilution, using cytopathic effects to detect infectivity (18, 20, 21).

Cell lines and tissue explants. F-OCP_1 was used to propagate highly lytic viruses such as Icelandic strains of visna virus, American PPV, and most of the new French lentiviruses. A caprine fetal synovial membrane cell line (F-$CFSM_1$) was used to propagate USA-CAEV and the persistent French ovine lentivirus. All cell lines were grown in Eagle minimal essential medium supplemented with 2% newborn calf serum, 8% lamb serum, and antibiotics as previously described (10). Before infection of the cells, the growth medium was removed and replaced by Eagle minimal essential medium containing only 2% lamb serum. To isolate lentiviruses, leukocytes or explants of solid tissues (lung, choroid plexus) obtained from healthy or ill sheep were cocultivated with the ovine lentivirus-negative cell line F-OCP_1. Leukocytes obtained from heparinized sheep blood and mononuclear cells were purified by centrifugation in Ficoll-Hypaque gradients as previously described (2). Solid tissue explants were minced with scissors before cocultivation.

Radiolabeling of cells, protein immunoprecipitation, and gel electrophoresis. Lentivirus-infected cells were grown in methionine-free Eagle minimal essential medium for 1 h. When the cytopathic effects were abundant, the cells were then labeled with 250 μCi of [^{35}S]methionine (600 Ci/mmol; Radiochemical Centre) per ml and incubated for 24 h at 37°C. Viral proteins were extracted from cells, immunoprecipitated with various lentivirus-specific sera, and analyzed in sodium dodecyl sulfate–6 to 18% polyacrylamide gels as previously described (35).

Antisera. To characterize the protein content of the new ovine lentiviruses, we used three monospecific sera raised against p30, p16, and gp135 of the Icelandic strain K1514 of visna virus and previously characterized (35). A goat anti-CAEV serum was obtained from a CAEV-infected goat.

Unintegrated viral DNA preparation. Unintegrated viral DNA was selectively extracted from infected cells by a modification of the Hirt method (16). Cell monolayers were washed twice in phosphate-buffered saline and then treated for 10 min at 37°C with a buffer containing 100 mM Tris-hydrochloride (pH 8.1), 1 M NaCl, 10 mM EDTA, and 0.2% sodium dodecyl sulfate. After 16 h at 4°C, the cell extracts were centrifuged at 15,000 × *g* for 30 min. The Hirt supernatant was treated with 100 μg of proteinase K per ml for 24 h at 37°C, and nucleic acids were extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated by 2.5 volumes of ethanol. For some cases, RNAs were eliminated from the DNA preparations by RNase A treatment (20 μg/ml).

Restriction enzyme digestion and Southern analysis. Various amounts of Hirt supernatant DNAs extracted from lentivirus-infected cells were digested with restriction enzymes obtained from Bethesda Research Laboratories under the conditions suggested by the manufacturer. Digested DNAs were run in 0.8% horizontal agarose gels containing 40 mM Tris-hydrochloride (pH 8.2), 5 mM sodium acetate, and 1 mM EDTA, stained with ethidium bromide, and transferred to nitrocellulose (30) with 20× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate).

Virion purification and hybridization probe preparation. Virions of ovine and caprine persistent lentiviruses were purified from extracellular fluids of infected cells, and polyadenylic acid-containing genomic 70S RNAs were purified on oligodeoxythymidylic acid-cellulose columns as previously described (34). ^{32}P-labeled cDNA specific for persistent lentiviruses was obtained by reverse transcription of heated polyadenylic acid-containing virion 70S RNA with the avian myeloblastosis virus reverse transcriptase in the presence of random fragmented calf thymus DNA as previously described (31).

Preparation of visna virus recombinant lambda phage DNA and synthesis of nick-translated hybridization probe. The Icelandic K1514 visna virus recombinant clone was a generous gift of A. T. Haase, University of California, San Francisco (submitted for publication). A partially digested *Sst*I DNA fragment of 8.75 kilobase pairs (kbp) was cloned into a lambda charon 10 vector (9). This fragment contained an internal *Sst*I site which was located at 0.55-kbp from the 5′ *Sst*I site. With the distal *Sst*I sites located in the long

TABLE 1. Isolation of two types of maedi-visna viruses from sheep with progressive pneumonia

Sheep no.[a]	Explant origin[b]	Latent period before cpe appearance (weeks)[c]	Type and denomination of virus isolate[d]	
			Highly lytic (type I)	Persistent (type II)
Sheep with pneumonia				
564-79	Blood leukocytes	5	F-OMVV-1(I)	ND
1982-81	Lung	5	F-OMVV-2(I)	—
1343-1	Lung	5	F-OMVV-3(I)	—
1343-2	Lung	5	F-OMVV-4(I)	—
105-82	Lung	14	F-OMVV-5(I)	—
2150-81[e]	Lung	18	—	F-OMVV-1(II)
1389-81	Lung	—	—	—
1390-81	Lung	—	—	—
Healthy sheep				
1200-80	Lung	—	—	—
F-OCP$_1$	Choroid plexus	—	—	—

[a] Lentiviruses were tentatively isolated from eight sheep with clinical signs of progressive pneumonia and with enzyme-linked immunosorbent assay lentivirus-positive sera. As a control, an experiment was also performed with a healthy sheep (1200-80), which was enzyme-linked immunosorbent assay lentivirus negative. F-OCP$_1$ represents a cell culture generated from the choroid plexus of an enzyme-linked immunosorbent assay lentivirus-negative healthy sheep; this ovine cell line was never induced to produce lentiviruses during our virus isolation experiments.

[b] To isolate lentiviruses, lung explants or blood leukocytes from healthy sheep or from sheep with progressive pneumonia were seeded on confluent F-OCP$_1$ cells and cocultivated for several 2-week interval passages until cytopathic effect appeared.

[c] cpe, Cytopathic effect; —, no cytopathic effect determined.

[d] ND, Not done; —, no isolate determined.

[e] The coculture 2150-81 was virus negative for 12 weeks and was frozen for 18 weeks. After thawing, cytopathic effect appeared 6 weeks later.

terminal repeat of the linear 9.25-kbp proviral DNA, all of the sequences of visna virus were represented in the recombinant clone. K1514 visna virus recombinant phage particles and phage DNA were prepared as described by Maniatis et al. (19).

^{32}P-labeled K1514 visna virus-specific probe was obtained by nick translation of K1514 visna virus recombinant phage DNA by the method described by Rigby et al. (25).

Hybridization conditions. Southern blots were prehybridized for 4 to 15 h at 68°C in buffer A containing 6× SSC and 1× Denhardt solution (8) and then hybridized for 48 to 72 h at 68°C in buffer A plus 0.2% sodium dodecyl sulfate, 200 μg of denatured calf thymus DNA per ml, and 5×10^6 to 5×10^7 cpm of heat-denatured [^{32}P]cDNA. Filters were washed six times for 7 min in 2× SSC–0.1% sodium dodecyl sulfate at 68°C and then for 10 min in 0.1× SSC at room temperature, dried, and exposed to Kodak X-OMAT R (or S) film in the presence of intensifying screens (Dupont Lightning Plus) at −70°C.

RESULTS

Isolation of lentiviruses from French sheep with naturally occurring progressive pneumonia which are either highly lytic or persistent in ovine fibroblast cultures. To isolate lentiviruses, blood leukocytes or dispersed lung cells were taken from French sheep with lentivirus-specific antibodies determined by enzyme-linked immunosorbent assay and with clinical signs of interstitial pneumonia (27, 36). They were cocultivated with F-OCP$_1$. This indicator cell line was obtained from a lentivirus-negative healthy sheep and was not induced to produce lentiviruses during the entire period of virus isolations in the laboratory. The presence of lentiviruses was subsequently detected by the appearance of cytopathic effects typical of lentiviruses, i.e., formation of fused giant cells and retractile dying cells (Fig. 1A and B). The results of this research are shown in Table 1. Six lentiviruses were isolated from six of the eight sheep with pneumonia.

As a negative control, no lentiviruses were isolated from a lung explant from one healthy sheep cocultivated with F-OCP$_1$. The presence of lentivirus was detected 5 weeks after the beginning of explantation for sheep 564-79, 1982-81, 1343-1 and 1343-2, but 14 and 18 weeks were necessary to observe the activation of lentiviruses from sheep 105-82 and 2150-81, respectively. Five isolates from sheep 564-79, 1982-81, 1343-1, 1343-2, and 105-82 were able to completely lyse infected ovine fibroblasts as previously described for the Icelandic strains of visna virus (15, 33). In contrast, the ovine lentivirus isolated from sheep 2150-81 failed to induce complete lysis of the cells but established a persistent infection of the cell cultures, as was the case for USA-CAEV in CSFM cells (20) and for new ovine PPV recently isolated in the United States (22, 23). Those distinct biological properties appeared to be conserved by the new lentivirus isolates after cloning by endpoint dilution and passaging in ovine or caprine fibroblast cultures. Clonal isolates of the new, highly lytic, and persistent lentiviruses were designated, respectively, ovine maedi-visna viruses (OMVV) type I and type II [OMVV(I) and OMVV(II)]. The names of maedi and visna were chosen to refer to the first two slow virus diseases observed in the Icelandic sheep and not to indicate precisely the pathogenic potential of the new lentivirus isolates.

Table 1 shows that only one or the other type of virus was isolated from individual animals. These data suggest that each naturally infected sheep may contain only one type of virus. However, the possibility that some sheep may contain more than one virus was not excluded, but one type, particularly that which is highly lytic in fibroblast cultures, may have been preferentially isolated.

High degree of relatedness in protein content of all OMVV(I) to that of the prototype strains of maedi-visna viruses. To determine the relatedness between all highly lytic viruses, including the prototype strains of maedi-visna viruses, analysis of viral proteins synthesized in cells infected by those various viruses was performed as previously described (35). Briefly, infected cells were labeled with [^{35}S]methionine, and the extracted viral proteins were immunoprecipitated by three monospecific sera raised against the two

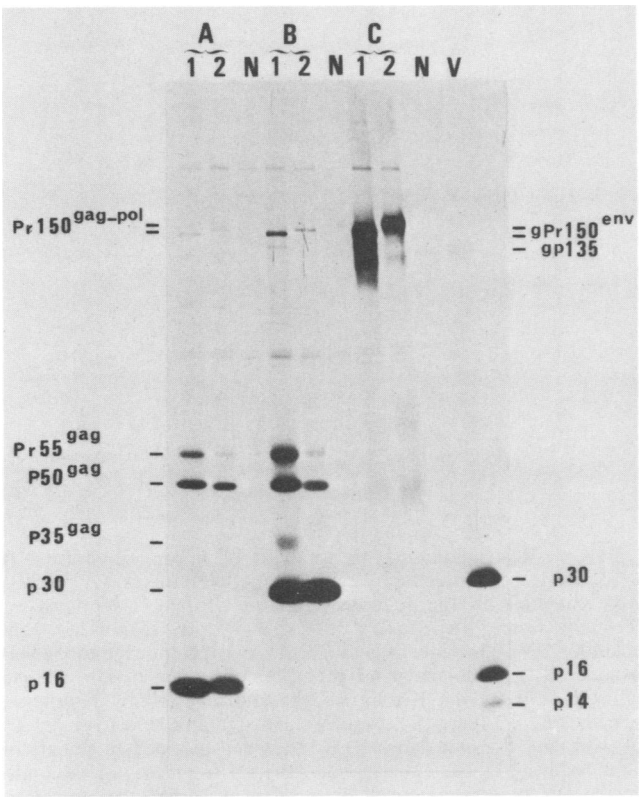

FIG. 2. Intracellular protein expression of type I ovine lentiviruses. Ovine fibroblasts infected by various highly lytic viruses were labeled for 5 h with [^{35}S]methionine. Labeled proteins were extracted from the cells and immunoprecipitated by three monospecific sera raised against two major internal proteins, p16 (A) and p30 (B) and the major envelope glycoprotein gp135 (C). N, Proteins nonspecifically retained by normal serum. Two highly lytic viruses were compared for their protein expression in F-OCP$_1$ fibroblasts: Icelandic visna virus strain K796 (lanes 1) and F-OMVV-1(I) (lanes 2). V, [^{35}S]methionine-labeled proteins of Icelandic visna virus.

internal proteins (p30 and p16) and the major envelope glycoprotein (gp135) of the Icelandic strain K1514 of visna virus. As previously described (35), OCP fibroblasts infected by the Icelandic strain K1514 of visna virus contain not only the three corresponding mature structural proteins but also their precursors (Pr55^{gag}, Pr150$^{gag\text{-}pol}$, and gPr150^{env}).

Figure 2 shows the results of a comparative study of viral polypeptides of one French type I virus [F-OMVV-1(I); lanes 2] with those of the Icelandic strain K796 of visna virus (lanes 1). Both highly lytic viruses express in OCP fibroblasts viral polypeptides which have similar sizes, namely, four major *gag*-related polypeptides (p16, p30, Pr55^{gag}, and Pr150$^{gag\text{-}pol}$) and two *env*-related polypeptides (gp135 and gPr150^{env}) as previously described for the Icelandic strain K1514 of visna virus (35). A similar result was obtained for the American PPV, strain Kennedy (20), and for the four other F-OMVV(I). Nevertheless, the large precursors of some strains of type I viruses could be distinguished by slight differences of electrophoretic mobilities for Pr150$^{gag\text{-}pol}$ and gPr150^{env} of F-OMVV-1(I) relative to visna virus strain K796 (Fig. 2).

We concluded that all of the highly lytic viruses isolated from distinct geographic areas such as Iceland, France, and the United States have a high degree of relatedness among antigens in their major structural polypeptides and constitute a homogenous group of viruses (presently designated type I).

Distinct protein contents of highly lytic and persistent viruses. A similar approach of protein analysis in polyacrylamide gels was performed to compare the protein content of the new virus, which persistently infects OCP fibroblasts [F-OMVV-1(II)], with that of type I viruses and the goat persistent virus, USA-CAEV. Protein biosynthesis of type I viruses was studied in F-OCP$_1$ cells. Type II virus was studied in F-OCP$_1$ cells or in F-CFSM$_1$. That of USA-CAEV was analyzed in this last caprine cell line. Figure 3 shows the results of immunoprecipitation of [^{35}S]methionine-labeled proteins of both types of viruses with the three sera raised against Icelandic visna virus p30, p16, and gp135. When proteins of cells infected by type I Icelandic visna virus (lane 1A), F-OMVV-4(I) (lane 3A and B), USA-CAEV (lane 2A), and F-OMVV-1(II) (lane 4A) were immunoprecipitated by visna virus anti-p30 serum, p30-related antigens (in the form of a mature p30 and precursor intermediates slightly different in size, Pr50^{gag} and Pr55^{gag}) were recognized in all of the cases, indicating that all of the lentiviruses share p30 antigen determinants. In contrast, when the same cell extracts were immunoprecipitated by visna virus anti-p16 serum, p16-related antigens (in the form of mature p16 and a precursor Pr55^{gag}) were observed for Icelandic visna virus (lane 1B) and F-OMVV-4(I) (lane 3A and B) but not for USA-CAEV

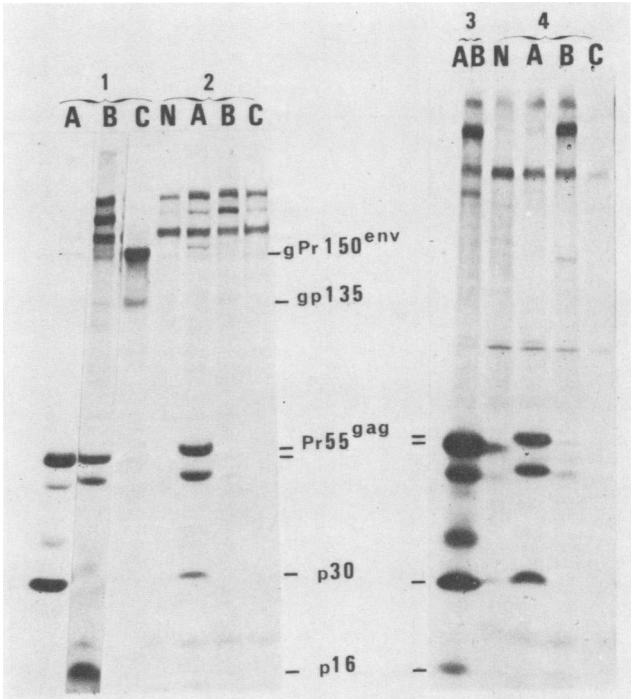

FIG. 3. Intracellular protein expression of type II ovine lentivirus in comparison with those of type I ovine lentivirus and CAEV. F-OCP$_1$ fibroblasts were infected by both types of ovine lentiviruses, namely the Icelandic strain K1514 of visna virus (panel 1), F-OMVV-4(I) (panel 3), and F-OMVV-1(II) (panel 4). As a control, protein expression of USA-CAEV was analyzed in F-CFSM$_1$ cells which were chronically infected by USA-CAEV (panel 2). Infected cells were labeled for 16 h with [^{35}S]methionine, and cell lysates were immunoprecipitated with three monospecific sera to three major proteins of visna virus strain K1514, namely, p30 (lanes A), p16 (lanes B), p30 and p16 (lane AB), and gp135 (lanes C); nonspecific precipitations of proteins by normal sheep serum (lanes N).

(lane 2B) and F-OMVV-1(II) (lane 4B). This last result allowed us to conclude that, with the antisera used here, p30 tends to have lentivirus-specific determinants and p16 might have type I lentivirus-specific determinants.

Investigation of antigens related to Icelandic visna virus gp135 was also performed in cells infected by OMVV(I), OMVV(II), and CAEV. Icelandic visna virus gp135-related antigens were efficiently detected only in cells infected by Icelandic visna virus in the form of a precursor $gPr150^{env}$ and a mature gp135 (Fig. 3, lanes 1C, 2C, and 4C).

To more directly define the protein content of the persistent viruses in comparison with that of the highly lytic viruses, we immunoprecipitated their intracellular proteins with an anti-whole virus serum from a CAEV-infected goat (Fig. 4). This serum detected p30 proteins of the three lentiviruses F-OMVV-4(I) (lane A1), F-OMVV-1(II) (lane A2), and USA-CAEV (lane A3) but also detected smaller proteins p18 and p14.5 in caprine cells infected by USA-CAEV (lane A3) and F-OMVV-1(II) (lane E). Those two proteins (p18 and p14.5) were also present in ovine cells infected by F-OMVV-1(II) (lane A2), but the corresponding bands were too faint to be adequately reproduced in the figure. Those two small proteins which are contained in mature virions of USA-CAEV and F-OMVV-1(II) and not in OMVV(I) viral particles (data not shown) (7) are specific to persistent viruses. Immunoprecipitation of cell extracts by anti-CAEV goat serum which also contains antibodies against the envelope glycoprotein of CAEV confirmed the very low level of gp135-related antigens, such as $gPr150^{env}$ of type I viruses (lane A1), in cells infected by USA-CAEV and F-OMVV-1(II) (lanes A2 and A3). Nevertheless, CAEV-infected goat serum precipitated mature envelope gp135 glycoproteins of both persistent lentiviruses (Fig. 4, lanes A2 and A3) just as visna virus anti-gp135 serum precipitated gp135 of type I lentivirus (lane B1). Mature gp135, which are present in very low amounts in both highly lytic and persistent virus-infected cells, were found in the viral particles of all of the lentiviruses analyzed thus far (data not shown). To summarize our present data, highly lytic and persistent lentiviruses can be clearly distinguished by their content in small proteins: p16 and p14 for type I virus and p18 and p14.5 for type II and CAEV viruses.

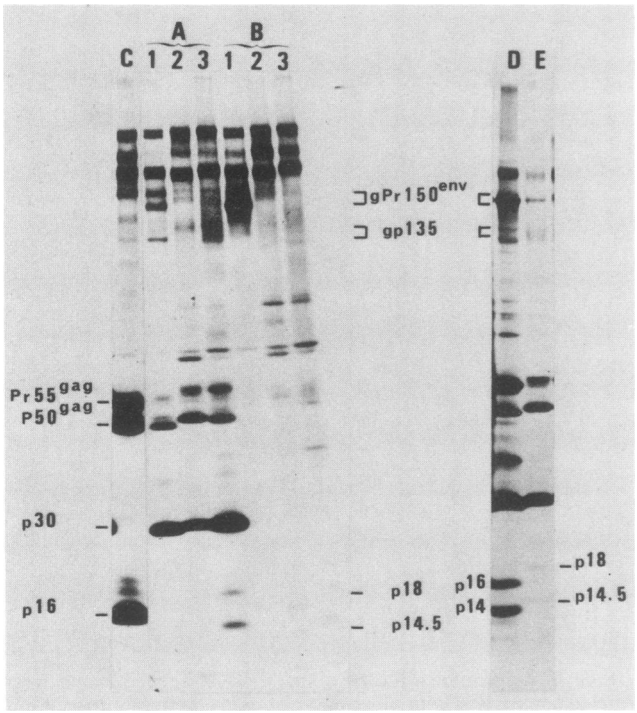

FIG. 4. Protein immunoprecipitation of both types of ovine lentiviruses and of CAEV by an anti-CAEV goat serum. F-OCP_1 or F-$CFSM_1$ cells infected by various lentiviruses were labeled for 16 h with [^{35}S]methionine. Intracellular viral proteins of F-OMVV-1(I) in ovine cells (lanes 1), or OMVV(II) in ovine (lanes 2) or caprine cells (panel E), and of CAEV in caprine cells (lanes 3) were analyzed by immunoprecipitation with an anti-CAEV goat serum (panel A) and a monospecific serum to Icelandic visna virus gp135 (panel B). Panels C and D represent, respectively, lysate proteins of visna virus-infected ovine cells immunoprecipitated by visna virus anti-p16 serum and anti-whole visna virus serum.

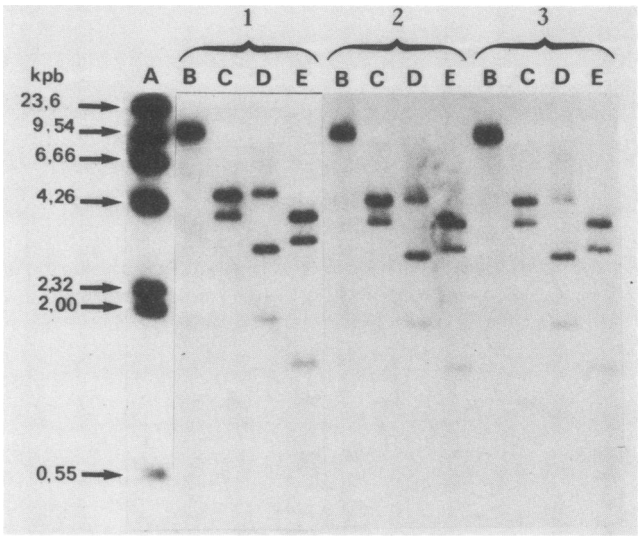

FIG. 5. Restriction enzyme analysis of linear proviral DNA produced in cells infected by type I ovine lentiviruses. Ovine cells were infected by the following isolates of type I lentiviruses: Icelandic visna virus strain K1514 (panel 1), F-OMVV-2(I) from coculture 1982-81 (panel 2), and F-OMVV-4(I) from coculture 1343-2 (panel 3). Low-molecular-weight DNAs were extracted from those infected cells by the Hirt procedure and digested by restriction enzymes *Eco*RI (lanes C), *Bam*HI (lanes D), and *Hin*dIII (lanes E) or were not digested (lanes B). (A) *Hin*dIII-digested fragments of lambda phage DNA. The enzyme-resistant fragments or the undigested DNAs were analyzed on a 1% agarose gel, transferred to nitrocellulose, and hybridized with [^{32}P]cDNA specific for Icelandic visna virus strain K1514.

Distinct nucleic acid contents of highly lytic and persistent viruses. To confirm the relatedness between OMVV-1(II) and USA-CAEV and their differences relative to type I viruses, we compared their unintegrated proviral DNA molecules present in infected cells. For this study, unintegrated proviral DNA from cells infected by various lentiviruses was extracted by the Hirt method, digested by restriction enzymes, and electrophoresed in agarose gel. After the transfer of DNA fragments onto a nitrocellulose filter, viral DNA was detected by hybridization with [^{32}P]cDNA representative of lentivirus genomes.

In an early experiment, we compared the structure and organization of the highly lytic viruses newly isolated from French sheep [F-OMVV-1 to F-OMVV-5(I)] with the well-known structure of the Icelandic visna virus strain K1514 linear DNA by using a [^{32}P]cDNA representative of the genome of visna virus K1514 (3, 14). Figure 5 shows that

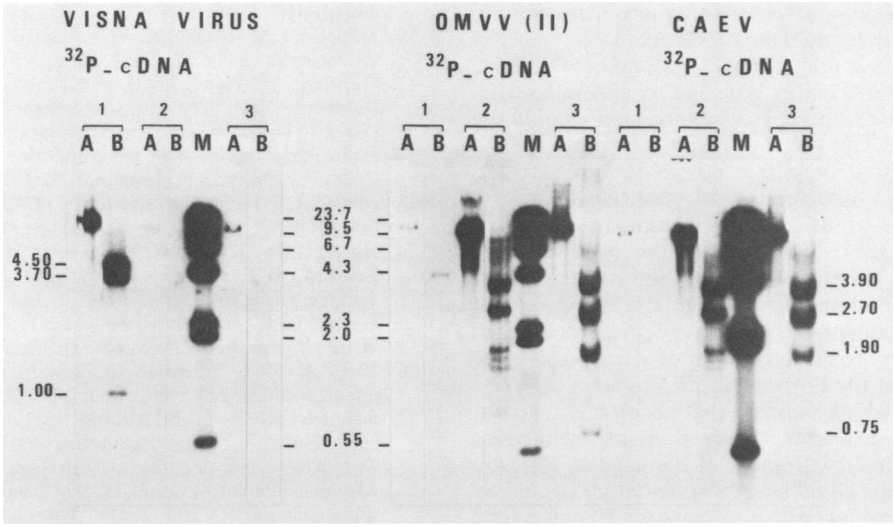

FIG. 6. Proviral DNA analysis of three lentiviruses, visna virus, OMVV(II), and USA-CAEV. F-OCP$_1$ cells were infected by the Icelandic strain K1514 of visna virus (panels 1), and F-CFSM$_1$ cells were infected by F-OMVV-1(II) (panels 2) and USA-CAEV (panels 3). Unintegrated proviral DNAs were extracted from the infected cells by the Hirt procedure and digested by *Eco*RI (lanes B) or were not digested (lanes A). The digested and undigested DNAs were analyzed on 1% agarose gel, transferred to nitrocellulose, and hybridized independently with three [^{32}P]cDNA specific for Icelandic strain K1514 of visna virus, F-OMVV-1(II), and USA-CAEV. (M) *Hin*dIII-digested fragments of lambda phage DNA. Sizes (kbp) of the DNA fragments are indicated on the sides of the panels.

ovine cells infected by either the prototype strain K1514 or two new lytic viruses [F-OMVV-2 and F-OMVV-4(I)] contain high amounts of linear 9.25-kbp DNA and that the DNA fragments resistant to three enzymes tested (*Eco*RI, *Bam*HI, *Hin*dIII) were similar in size and efficiently hybridized with visna virus K1514-specific cDNA. Because similar results were obtained for the three other type I viruses, we concluded that DNA sequences are relatively well preserved in the French as well as the Icelandic lytic viruses.

In contrast, when a similar experiment was done with OMVV-1(II) and CAEV linear DNAs undigested or digested with *Eco*RI, visna virus recombinant lambda phage [^{32}P]DNA did not reveal the pattern of *Eco*RI-resistant fragments observed by type I viruses (4.5, 3.7, and 1.0 kbp) but weakly detected novel *Eco*RI fragments generated from the full-length linear DNAs of OMVV(II) and CAEV (Fig. 6). When these same linear DNAs prepared from F-CFSM$_1$ cells infected by F-OMVV-1(II) or USA-CAEV were hybridized to OMVV(II) or CAEV-specific [^{32}P]cDNAs, novel *Eco*RI fragments, previously weakly revealed by a type I virus-specific cDNA, were then detected much more efficiently by OMVV(II) as well as CAEV probes (Fig. 6, lanes 2B and 3B). Reciprocally, those two probes could poorly hybridize with several visna virus DNA fragments (Fig. 6, lanes 1A and 1B), suggesting some homologous sequences between type I and II viruses.

F-OMVV-1(II) and CAEV linear DNAs were ca. 9.25 kbp long and were digested by *Eco*RI into four DNA fragments (3.9, 2.7, 1.9, and 0.75 kbp). Those preliminary data allowed us to conclude that F-OMVV-1(II) and USA-CAEV are highly related and quite distinct from type I lentiviruses.

DISCUSSION

In the present work, we analyzed field isolates of ovine lentiviruses from French sheep with naturally occurring progressive pneumonia. Their biological and biochemical properties were compared with those of highly lytic ovine (such as Icelandic visna virus) and persistent caprine (CAEV) lentiviruses. One of them was able to establish chronic infections of ovine or caprine fibroblasts, as described for CAEV (18, 20) and American ovine field isolates (22). However, we have shown that five other isolates possess lytic properties as strong as those of the Icelandic strains of visna virus. The natural presence in the ovine species of two types of lentiviruses, either highly lytic or persistent, suggests that highly lytic viruses are not viruses that were originally persistent and subsequently adapted to tissue cultures, as published by Narayan et al. (20, 22), but are, rather, natural viruses genetically distinct from persistent viruses.

The present analysis of the protein content of both types of viruses demonstrated that highly lytic viruses [OMVV(I)] are closely related in terms of structure to the prototype strains of Icelandic visna virus (K796 and K1514) and American PPV (strain Kennedy), whereas the persistent virus [OMVV(II)] is very similar to the American strain of CAEV. As previously shown in serological studies of visna virus and CAEV (7, 20, 26), type I and type II ovine lentiviruses share common antigenic determinants in their major internal protein, p30, and major envelope glycoprotein, gp135. In contrast, smaller internal proteins [p16 and p14 for OMVV(I); p18 and p14.5 for OMVV(II) and CAEV)] do not seem to share major antigenic determinants. For example, with the use of a monospecific serum raised p16 of OMVV(I), we have observed that the major antigenic determinants of the small internal protein p16 were only type I lentivirus specific.

Nucleic acid hybridization on Southern blots in stringent conditions confirmed the results of protein analysis. OMVV(I) DNA proviruses are very similar to those of visna virus K1514, whereas nucleic acid sequences of OMVV(II) and CAEV, which are homologous all along their genome, are poorly recognized by a type I probe. Others have detected 20 to 30% sequence homology between ovine highly lytic viruses and caprine persistent viruses (11, 26), but further blot hybridization experiments in less stringent

conditions will be necessary to assess where in their viral genomes the sequence homology is located.

In addition to the clear differences in protein contents and nucleic acid sequences of highly lytic and persistent lentiviruses, differences in *env*-precursor expression should be pointed out. Whereas OMVV(I)-infected cells contain high amounts of polypeptide precursor to gp135 ($gpPr150^{env}$), OMVV(II)- and CAEV-infected cells do not. Nevertheless, mature gp135 seems to be present in similar, very low amounts in cells infected by both lytic and persistent viruses. Further experiments will be necessary to determine whether differences of protein content or level of expression of particular viral components represent specific biochemical features implicated in the establishment of virus persistence or irreversible lysis of the host cells.

Our present data, which indicate that sheep can be naturally infected by a lentivirus which is highly related to CAEV, are in accordance with those of Banks et al. (1), who found that young lambs could be experimentally infected by CAEV and could develop arthritis lesions. Experimental infections of sheep and goats with the new persistent ovine lentivirus will be necessary to determine its virulence and pathogenic potential.

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