VIROLOGY 222, 21–30 (1996)
ARTICLE NO. 0394

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Received February 14, 1996; accepted May 17, 1996

Caprine arthritis–encephalitis virus (CAEV) is a natural lentivirus pathogen of goats. CAEV, like all members of the ovine/caprine lentivirus family, has an *in vivo* tropism for cells of the monocyte/macrophage cell lineage and activation of viral gene expression is observed only following differentiation of monocytes to macrophages. In addition to cells of the monocyte/macrophage lineage, CAEV and the closely related maedi visna virus of sheep (MVV) can also replicate productively in fibroepithelial cells derived from synovial membrane of goats (GSM). However, these viruses varied greatly in their ability to replicate in fibroblasts. We studied the biological and biochemical properties of CAEV and maedi-visna virus (MVV) of sheep following inoculation into the three ovine/caprine cell types. Our data showed no substantial differences in virus titers, viral protein biosynthesis, or processing of the viral proteins between CAEV and MVV following inoculation into primary macrophages and GSM cells. However, unlike MVV, CAEV failed to replicate productively in ovine fibroblasts (sheep choroid plexus cells). This correlated with a specific but abnormal proteolytic cleavage of the envelope glycoprotein of the virus. This abnormal proteolytic cleavage represents a novel type of host cell restriction of lentivirus replication.

INTRODUCTION

Maedi-visna virus (MVV) of sheep and caprine arthritis–encephalitis virus (CAEV) of goats are molecularly cloned prototypic lentiviruses that are closely related antigenically and have extensive relatedness in the gag-pol region of their genomes (Pyper *et al.,* 1986). The complete nucleotide sequence of CAEV (strain CO) and different strains of MVV genomes have been determined (Andresson *et al.,* 1993; Sargan *et al.,* 1991; Saltarelli *et al.,* 1990; Querat *et al.,* 1990; Braun *et al.,* 1987; Sonigo *et al.,* 1985). Comparison of the amino acid sequence of CAEV and MVV showed 74.5 to 77.5% relatedness in the *gag-pol* genes, but only 60 to 63% in the *env* gene (Saltarelli *et al.,* 1990) whereas comparison of the *env* gene of two isolates of MVV (SA-OMVV and VLV) or CAEV (strains CO and 63) revealed at least 80% relatedness, respectively (Saltarelli *et al.,* 1990; Knowles *et al.,* 1991; Querat *et al.,* 1990)

In nature, MVV and CAEV cause persistent lifelong infections in their respective hosts (Sigurdsson, 1954; Sigurdsson *et al.,* 1957; Crawford *et al.* 1980a; Narayan *et al.,* 1980). Infected animals develop slowly progressive diseases (paralysis, dyspnea, arthritis, and mastitis) affecting specific organ systems after periods of subclinical infection lasting months to years (Sigurdsson, 1954; Adams *et al.* 1980; Crawford *et al.,* 1980b; Oliver *et al.,* 1981; Narayan and Cork, 1985). Recent studies have shown that cells of the monocyte/macrophage lineage are the major host cell for infection and differentiation of monocytes to macrophages induced virus replication (Klevjer Anderson *et al.,* 1984; Gorrell *et al.,* 1992; Gendelman *et al.,* 1986; Narayan *et al.,* 1982, 1983, 1985; Gendelman *et al.* 1985). However, it is unknown whether this is the only cell type that is productively infected by the virus *in vivo.* In subclinically infected animals, virus replication in cells is restricted to a minimal level of expression in all tissues, and relatively few cells are infected. In contrast, in animals with disease, this indolent infection erupts into a highly productive phase in local macrophage populations in the affected tissues (Petursson *et al.,* 1976; Haase *et al.,* 1977; Narayan *et al.,* 1977). Recent studies using *in situ* PCR technique on histological sections of tissues from MVV-infected sheep revealed a much higher number of cells containing the proviral genome than cells expressing the viral RNA detected by *in situ* hybridization (Peluso *et al.,* 1985; Haase *et al.,* 1990; Staskus *et al.,* 1991). These data suggested that a restrictive type of replication occurs in certain cell types but the nature of these infections is unknown since under cell culture conditions, the picture is dominated by viruses that replicate productively.

Historically, lentiviruses were cultivated first from Icelandic sheep with maedi visna disease (Sigurdsson, 1954). Prior to identification of macrophages as the pri-

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0042-6822/96 $18.00

mary host cell for the virus, MVV was propagated in monolayers of fibroblasts derived from the sheep choroid plexus (SCP) because visna lesions invariably involved inflammation in this tissue. Subsequent isolations of MVV, from sheep with maedi, the pneumonic phase of the disease, was also performed in fibroblasts (Querat *et al.,* 1984; Sargan *et al.,* 1991). Another lentivirus was later isolated from goats with synovitis/arthritis and paralysis in the United States (Narayan *et al.,* 1980; Crawford *et al.,* 1980a). Since the most characteristic syndrome of goats infected with CAEV was arthritis, cells derived from the synovial membrane of goats (GSM), a fibro-epithelial cell type, were used to propagate this virus (Narayan *et al.,* 1980; Crawford *et al.,* 1980a). The ovine MVV and CAEV are closely related antigenically and have extensive relatedness in the *gag-pol* region of their genomes (Pyper *et al.,* 1986).

Both MVV and CAEV replicate productively in primary macrophage and GSM cultures. However, whereas MVV replicates productively and lytically in fibroblasts, CAEV caused only a nonproductive, noncytopathic infection in these cells (Narayan *et al.,* 1980). We have examined this phenomenon as a possible model for restrictive virus replication in tissues. We present data that CAEV caused infection in fibroblasts but the virus life cycle was terminated prior to assembly of infectious virus particles. Our results indicate that the CAEV genome remained intact in inoculated fibroblasts and the virus genome could be rescued by co-cultivation with permissive cells. We show that aberrant proteolytic processing of the *env* glycoprotein and reduced synthesis of gag protein were responsible for the inability of CAEV to replicate in fibroblasts. This type of abortive replication from a persistent viral genome represents a model of a restrictive type of virus replication ongoing in tissues of subclinically infected animals. This phenomenon is shared by some macaques infected with simian immunodeficiency virus (SIVmac) and possibly humans infected with HIV.

MATERIALS AND METHODS

Viruses and cells

The CO strain of CAEV, obtained originally from an encephalitic goat (Narayan *et al.,* 1980) and the CAEV-63 strain, isolated from an arthritic goat (Crawford *et al.* 1980a), were used in this study. Viruses were propagated in monolayers of GSM cultures. Icelandic maedi-visna virus, strain MVV-1514 (Petursson *et al.,* 1976), was propagated in fibroblast cultures. Macrophage cultures were derived from freshly drawn heparinized blood from two donor sheep maintained in the AAALAC approved facility at KUMC. Mononuclear cells (peripheral blood mononuclear cells, PBMC) were isolated by centrifugation through gradients of Ficoll–Hypaque (Narayan *et al.,* 1983). Approximately 10^8 cells were suspended in macrophage differentiation medium (MDM) which consisted of RPMI medium supplemented with 2 m*M* glutamine, 0.05 m*M* 2-mercaptoethanol, 0.05 mg/ml gentamicin, 10 m*M* HEPES buffer, pH 7.3, and 20% heated (56°, 30 min) lamb serum (GIBCO). Cultures were incubated in Teflon bottles at 37° for 2 weeks with medium change after 1 week. During this period, monocytes differentiated into macrophages and upon seeding into tissue cultures dishes, macrophages became adherent and spread into typical adherent, esterase-positive phagocytic cells. Monolayers of macrophages were cultured for 3–5 days, and nonadherent cells were removed by rinsing the culture with serum-free medium prior to virus inoculation at a multiplicity of less than one. Inoculated cultures were incubated in MDM containing 5% lamb serum.

GSM and fibroblast cultures were obtained from a colostrum-deprived newborn goat and lamb, respectively (Narayan *et al.,* 1980). The cells were propagated as monolayer cultures in medium consisting of minimum essential medium (MEM), supplemented with 10% heat inactivated fetal bovine serum (FBS) (GIBCO). Prior to inoculation, cultures were rinsed with MEM, and inoculated cultures were replenished with maintenance medium consisting of MEM plus 2% heat-inactivated lamb serum.

Assay of virus infectivity

For quantal assay of cytopathic effects (CPE), CAEV and MVV were titrated in cultures of primary blood-derived macrophages, GSM, and SCP. GSM and SCP fibroblasts were seeded in microtiter plates and cells in four wells were inoculated with 10-fold dilutions of virus in maintenance medium. Ten days later, cultures were fixed in formalin (10%), stained with Giemsa, and examined for the presence of CPE by phase microscopy. Two-week-old macrophages in Teflon bottles were distributed into polycarbonate tubes containing 1×10^5 cells per milliliter and cells in two tubes were inoculated with dilutions of virus as described above. Macrophages remained nonadherent in these tubes. Seven days later, cells from each tube were plated into one well of a 24-well culture plate and 1 day later, after macrophages had become adherent, GSM cells were added to each well. Fusion of GSM cells indicated presence of infected macrophages. Three days post co-culture cells were formalin fixed and Giemsa stained, and then CPE were scored.

Preparation of cells for ultrastructural studies

Five days after inoculation of each of the two viruses into cultures of GSM cells and fibroblasts, cells were rinsed three times with MEM without serum and fixed overnight at 4° with 2% glutaraldehyde solution in phosphate-buffered saline (PBS, pH 7.4). Fixed cells were washed twice in PBS and scraped from the surface of

the dish, and the cell suspension was pelleted by centrifugation in a microfuge tube. Cell pellets were postfixed in a solution containing 1% osmium tetroxide, dehydrated in a series of graded ethanols, and embedded in LR-White resin. Sections were stained with uranyl acetate and lead nitrate and examined using a Joel 100Cx electron microscope.

Immunoprecipitation of viral proteins

Cultured GSM, fibroblast, or macrophage monolayers in 35-mm culture dishes were inoculated with virus at a multiplicity of infection (m.o.i.) of 0.1 and maintained at 37° for 3–5 days. Infected cells were then rinsed twice and incubated for 2 hr in MEM medium without methionine and cysteine and radiolabeled with 100 μCi/ml of [^{35}S]methionine/cysteine (ICN, Costa Mesa, CA) for 16 to 18 hr. Virus-specific proteins released into the culture medium, or present in the cells, were immunoprecipitated using either a serum obtained in our laboratory from a goat (G62) that had received several injections of CAEV and visna virus, or monoclonal antibodies against gp135 (mAb F7-299) and gp41 (mAb CAE92A1) (McGuire *et al.,* 1992). Cell debris was removed from culture medium by centrifugation in a microfuge (5 min, 12000 rpm) and the clarified fluid made 1× with respect to RIPA buffer (50 m*M* Tris–HCl, pH 7.5, 50 m*M* NaCl, 0.5% deoxycholate, 0.2% SDS, and 10 m*M* EDTA). The cell monolayers were lysed in 1× RIPA buffer, and nuclei and cell debris were removed by centrifugation in a microfuge (5 min, 12,000 rpm). Clarified cell culture medium and cell lysates were incubated and rotated overnight at 4° in presence of the G62 serum (10 μl) and Sepharose–protein A or mAb F7-299 or mAb-92A1 and Sepharose–protein G. The beads were washed four times with 1× RIPA buffer and resuspended in sample reducing buffer (Laemmli *et al.,* 1970) and boiled for 5 min. Immunoprecipitated proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Following electrophoresis, the gels were fixed for 30 min in a solution containing 10% acetic acid and 30% methanol and then immersed in Amplify solution (Amersham) for 30 min. The gels were dried and protein bands visualized using standard autoradiographic techniques. The CAE92A1 monoclonal antibody specific to the transmembrane gp41 did not result in immunoprecipitation of bands corresponding to gp41 or the precursor Pr-gp170^{env} from CAEV-infected GSM and SCP cells. This monoclonal antibody was produced against the transmembrane of CAEV strain 63, which diverged from the CO strain about 15% in the env; thus the failure of precipitation of CAEV-specific proteins with this monoclonal antibody could result from the absence of a specific epitope in CAEV strain CO used in this study.

Infected cell assay by PCR (PCR–ICA)

PCR–ICA was used to determine the number of fibroblasts infected with CAEV as previously described (Joag *et al.,* 1994). Cell monolayers were dissociated with trypsin and resuspended in MEM at 2×10^7 cells per milliliter concentration. Successive 10-fold dilutions of the cells, 2×10^6 to 2×10^2/ml, were lysed for PCR–ICA, then two rounds of PCR amplification were used to detect the genome of CAEV in the gag sequences. Primers GEX5: 5′-GAAGTGTTGCTGCGAGAGGTGTTG-3′ and GEX3: 5′-TGCCTGATCCATGTTAGCTTGTGC-3′ which are complementary to bases 393 to 416 and 1268 to 1291 of the CAEV leader-gag sequences (Saltarelli *et al.,* 1990), respectively. Oligonucleotide primers ES30: 5′-TCATGTTTGAGACCTTCAACACCCCAG-3′ and ES32: 5′-CCAGGGGAAGGCTGGAAGAGTGCC-3′, specific to the fourth exon of the human β-actin gene were used as an internal standard for cell number. The first round of PCR was performed according the following conditions: initial DNA denaturation at 94° for 3 min followed by 35 cycles of denaturation at 92° for 1 min, annealing at 56° for 1.5 min, and primer extension at 70° for 3 min. To increase the sensitivity of the DNA amplification, 5 μl of the PCR product was used as a template for a second round of amplification using the same conditions except the primers. Primers GIN5: 5′-GATAGAGACATGGCGAGGCAAGT-3′ and GIN3: 5′-GAGGCCATGCTGCATTGCTACTGT-3′ which are complementary to bases 524 to 546 and 1013 to 1036 of CAEV gag gene, respectively and ES31: 5′-CCCCAGCCATGTACGTTGCTATCC-3′ and ES33: 5′-GCCTCAGGGCAGCGGAACCGCTCA-3′ specific to β-actin gene were used for the nested PCR. Following the 35 cycles of amplification, 10 μl of each sample was removed and loaded in 1.5% agarose gel. After electrophoresis, bands of DNA were visualized by staining with ethidium bromide. Positive signals for samples on the gel were correlated with the dilutions and the percentage of CAEV-infected fibroblasts was determined.

RESULTS

Cytopathicity of CAEV and MVV in inoculated cultures of macrophages, GSM, and fibroblasts

To determine the effect of virus replication in each of the three cell types, CAEV and MVV were inoculated in macrophage, GSM, and fibroblast cultures. Three to 5 days following the inoculation of GSM cells with MVV, the cells underwent multinucleated giant cell (MGC) formation and in the next 3 to 5 days underwent lysis. The virus had similar effects in fibroblasts although the lytic effect was more apparent. Primary sheep macrophages inoculated with MVV mainly developed MGC with slow progressive cell lysis after 2 weeks (Fig. 1).

GSM inoculated with CAEV developed focal MGC for-

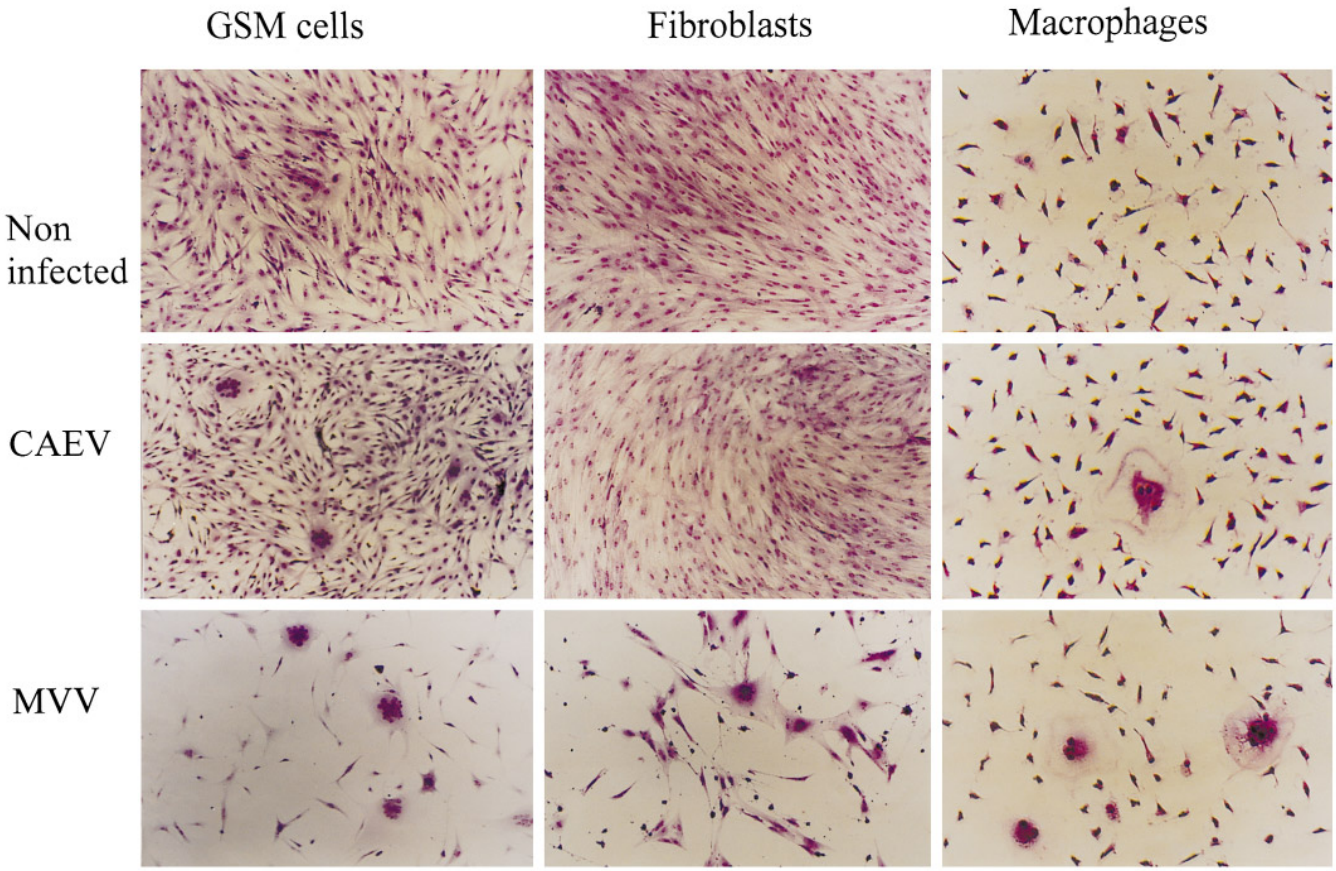

FIG. 1. Cytopathic effects caused by CAEV and MVV in different cell types. Noninfected cells and cells infected with CAEV or MVV were formalin-fixed and stained with Giemsa as described under Materials and Methods. Cytopathic effect was identified as multinucleated giant cells obtained after fusion with infected cells. Original magnifications, ×250.

mation that persisted even after the cells had been dissociated with trypsin and subcultured. In contrast, no CPE developed in fibroblast cultures inoculated with this virus. CPE was not observed even when the inoculated fibroblasts were passaged and subcultured for a period exceeding 3 weeks. Primary sheep macrophage cultures infected with CAEV resulted in enlarged cells that were not lysed 2 weeks postinoculation (Fig. 1).

These results indicated that, unlike MVV, CAEV did not replicate or cause CPE in fibroblasts.

CAEV does not replicate productively in fibroblasts but virus can be rescued by co-cultivation

To examine whether CAEV caused a defective replication in fibroblasts, we attempted to rescue virus by co-cultivation with the permissive GSM cells. CAEV or MVV was inoculated into macrophage, GSM, or SCP cell cultures at a multiplicity of infection of one (m.o.i. = 1) as shown in Table 1. After 1 week in culture macrophages and GSM cells released 10^5 to 10^6 syncytium-forming units (SFU) per milliliter of CAEV into the culture medium as determined by virus quantitation in GSM cultures. In contrast, after 1 week in culture no virus with syncytium-forming activity was released into the culture medium of fibroblast cultures inoculated with CAEV. Three successive cell passages, using trypsin to dissociate the cul-

TABLE 1

Virus Titration of CAEV and MVV-1514 in GSM, Fibroblasts, and Macrophage Cells

	Cell type		
Virus	GSM	Fibroblasts	Macrophages
CAEV	10^6	Negative[a]	10^6
MVV-1514	10^7	10^7	10^5

Note. Macrophages, GSM cells, and fibroblasts were inoculated at a m.o.i. of 1 and culture medium was harvested at 1 week postinoculation. Various dilutions were used to inoculate fresh GSM cells. Infectious syncytium-forming units were determined as reported in the text.

[a] CAEV-infected fibroblasts were passaged three times and maintained for 3 weeks in culture, after which the culture medium was harvested and tested for infectious virus. No infectious virus capable of inducing syncytium forming in GSM cells was detected. Titers are in $TCID_{50}$/ml.

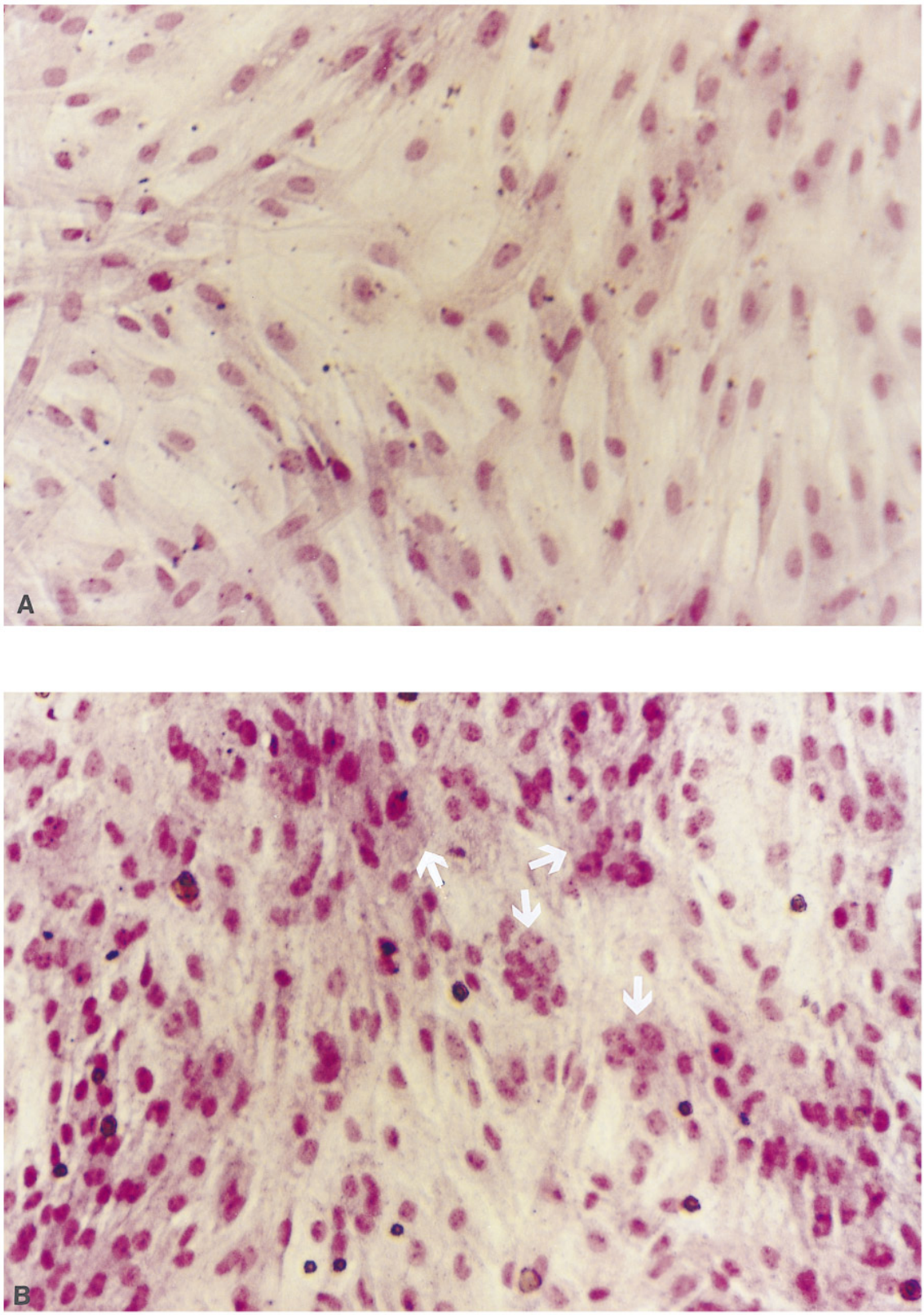

FIG. 2. Rescue of CAEV virus from fibroblasts following co-cultivation with GSM cells. Fibroblast cultures were inoculated at a m.o.i. of 0.1 and passaged at 1 week postinoculation. In one culture of CAEV-infected fibroblasts, GSM cells were added and the co-culture was observed every hour for development of CPE, then formalin-fixed and stained with Giemsa after 10 hr post-co-culture (B). Arrows indicate the localizations of some of the massive fusions. CAEV-infected fibroblasts which were not cocultivated were used as control (A).

tures, and subcultivations of these cells failed to result in virus production in the culture medium (as assayed in GSM cultures).

However, addition of GSM cells to these SCP fibroblast cultures resulted in multiple foci of fusion of the indicator GSM cells within 10 hr (Fig. 2B). Further, the rapid development of fusion suggested that a functional gp41 was expressed on the surface of fibroblasts. Culture supernatant from the co-culture was harvested at 48 hr, clarified by centrifugation, and inoculated into GSM cultures. This resulted in MGC formation seen typically in CAEV-infected GSM cells (data not shown).

These results indicated that the intact CAEV genome was present in the fibroblasts and was rescuable by the GSM cells.

PCR–ICA on DNA of CAEV-infected fibroblasts

To quantitate the number of fibroblasts and GSM cells harboring the CAEV genome following inoculation of the cells with CAEV, we performed the PCR–ICA technique using oligonucleotide primers that specifically amplify a fragment from *gag* of CAEV. GSM cells and fibroblasts were inoculated with CAEV at a m.o.i. of 0.1 and at 5 days after inoculation, PCR–ICA was performed on dilutions containing 10,000, 1000, 100, 10, 1, and 0.1 cells as previously described (Joag *et al.,* 1994). Our results indicated that samples containing a single CAEV-infected GSM cell had a positive PCR signal with primers specific to CAEV and those specific to β-actin gene (Fig. 3A), demonstrating the sensitivity of the technique for CAEV-infected cells. Examination of CAEV-inoculated fibroblasts by this procedure showed that dilutions containing 1 or 0.1 cell lacked the 512-bp band specific to CAEV but samples containing a minimum of 10 cells were positive (Fig. 3B). PCR amplification using oligonucleotide primers specific to the cellular β-actin gene showed a positive signal in dilutions containing at least one cell but not in higher dilutions (Fig. 3B). The PCR results demonstrated that inoculation of fibroblasts at a m.o.i. of 0.1 resulted in infection in 10% of the cells examined at 5 days postinoculation. Examination of CAEV-infected fibroblasts by PCR-ICA at 3 weeks postinoculation showed that 10% of the cells were positive, suggesting a persistent infection that did not spread throughout the culture.

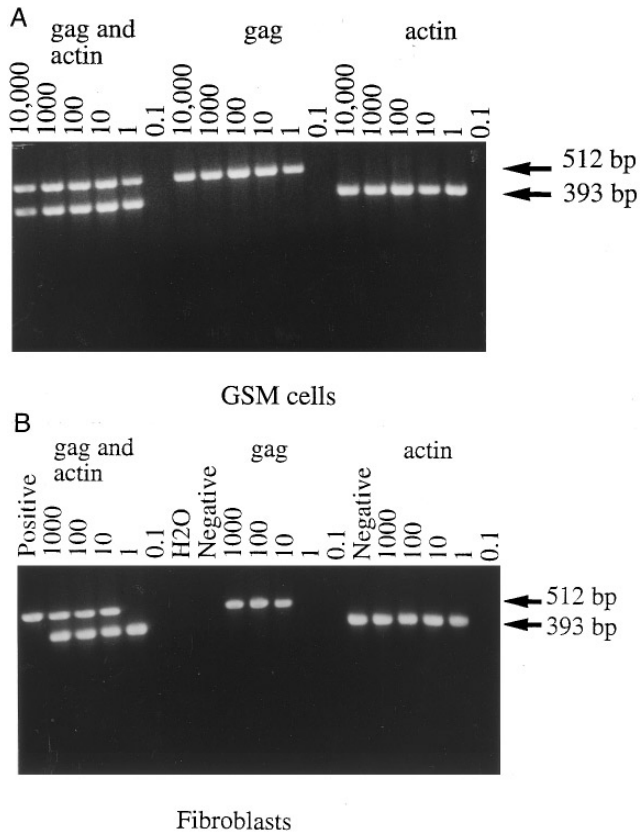

FIG. 3. Infected cell assay of CAEV-infected fibroblasts and GSM cells determined by PCR–ICA. Fibroblasts and GSM cells were infected with CAEV (MOI 0.1) and at 5 days postinoculation various dilutions of cells were used to prepare lysates for PCR. Oligonucleotide primers and conditions for PCR are described in the text. PCR products were separated in 1.5% agarose gels and bands visualized by staining with ethidium bromide. (A) PCR products using oligonucleotide primers to amplify both the 512-bp CAEV gag and 393-bp β-actin fragments (gag and actin), or the 512-bp CAEV gag fragment only (gag) or the 393-bp β-actin fragment only (actin) on lysates from indicated numbers of CAEV-infected GSM cells. (B) PCR products using the same oligonucleotide primers to amplify the 512-bp CAEV gag and 393-bp β-actin fragments from lysates from indicated numbers of CAEV-infected fibroblasts. Lysates prepared from CAEV-infected GSM cells (positive) were used with the CAEV gag primers as positive control, and lysates from uninfected fibroblasts (negative) were used with CAEV gag and β-actin specific oligonucleotide primers as negative controls, respectively. The sample with no DNA (H_2O) was used for the specificity of the PCR products.

Ultrastructure analyses of GSM and fibroblasts infected with CAEV

Because inoculation of fibroblast cultures with CAEV did not result in CPE but co-cultivation of the CAEV-infected fibroblasts with GSM cells resulted in the rescue of CAEV, we examined CAEV-infected fibroblasts by electron microscopy for the possible presence of virus particles in different stages of assembly and release. Examination of numerous preparations of these cells failed to reveal viral particles budding into cytoplasmic vacuoles or at the cell surface. In contrast, examination of GSM cells infected with CAEV revealed numerous virus particles assembling at the cell surface or released from infected cells (data not shown).

Immunoprecipitation studies reveal differences in the processing of viral proteins in different cell types

To determine if viral protein biosynthesis was similar in the three cell types infected with CAEV or MVV, immu-

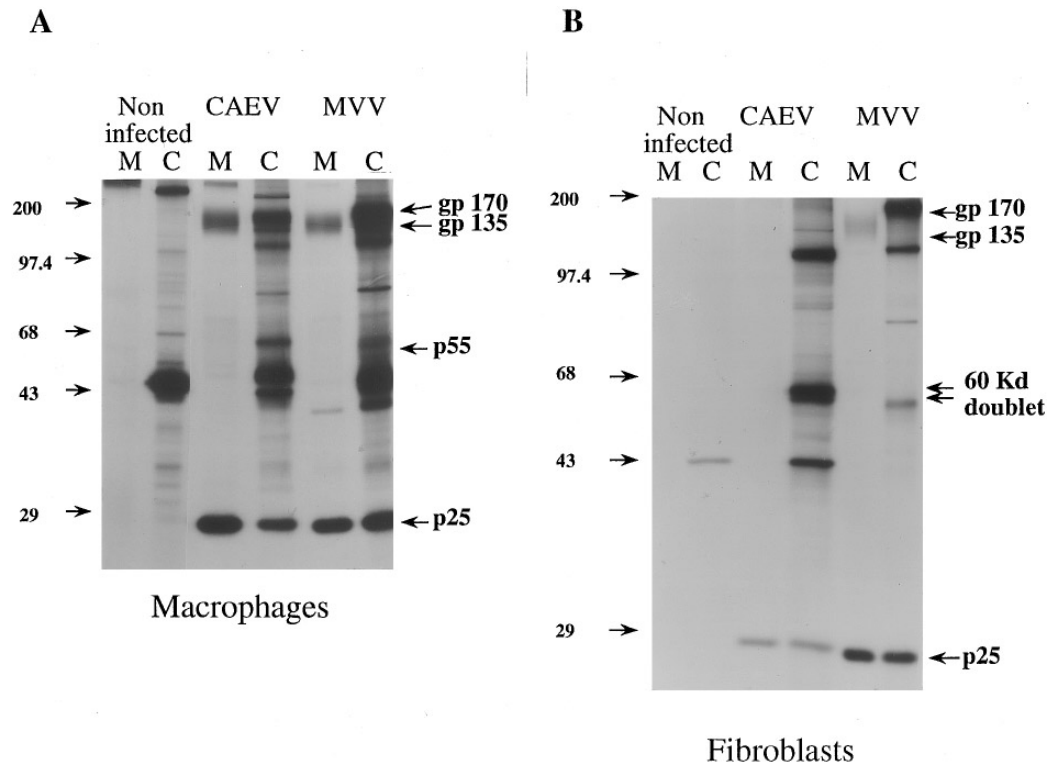

FIG. 4. Immunoprecipitation of virus-specific proteins from sheep macrophages and fibroblasts infected with CAEV and MVV. Sheep macrophages and fibroblasts were inoculated with CAEV or MVV as described under Materials and Methods. At 5 days postinfection, proteins were radiolabeled overnight by addition of 100 μCi of [^{35}S]methionine/cysteine in the culture medium of infected cells. The culture medium and cell lysates were prepared and immunoprecipitated using the hyperimmune (G62) goat serum and protein A–Sepharose. Proteins from each sample were separated using SDS–PAGE (8.5% gel), and then visualized using standard autographic techniques. (A) Immunoprecipitation from culture medium (M) and cell lysate (C) from noninfected, CAEV-infected, and MVV-infected macrophages (MVV). (B) Immunoprecipitation from culture medium (M) and cell lysate (C) from noninfected fibroblasts, fibroblasts infected with CAEV (CAEV), and fibroblasts infected with MVV (MVV). Molecular mass (in kilodaltons) of prestained markers is shown on the left. Positions of the major viral *gag* and *env* proteins and their precursors as well as the 60-kDa doublet are indicated to the right of each gel.

noprecipitation of virus proteins from culture medium and cell lysates was performed using the G62 serum. As shown in Fig. 4A, CAEV- and MVV-infected macrophages revealed no substantial difference in protein patterns or in the intensity of the viral proteins. The virus-specific proteins released into the culture medium were the capsid gag protein (p25-CA) and the surface envelope glycoprotein (gp135-SU). Immunoprecipitation of virus proteins from cell lysates revealed the envelope glycoprotein precursor (Pr-gp170^{env}) and surface glycoprotein (gp135-SU) as well as the gag protein precursor (Pr-55^{gag}) and capsid gag protein (p25-CA) (Fig. 4a). The protein bands corresponding to the precursors (Pr-55^{gag} and Pr-gp170^{env}) had been identified following a pulse–chase experiment (data not shown). The precursor proteins of gag and env (Pr-55gag and Pr-gp170env) and their cleavage products (p25-CA and gp135-SU) were absent from the culture medium and the lysates prepared from noninfected macrophages used as negative controls (Fig. 4a). These data showed that the viral proteins of both viruses were synthesized and processed correctly in the infected macrophage cultures.

Immunoprecipitation of virus proteins from MVV-infected fibroblasts (Fig. 4b) showed similar patterns to those obtained with MVV- and CAEV-infected macrophage cultures. In contrast to MVV-infected fibroblasts, only small amounts of gag p25-CA and the glycoprotein precursor Pr-gp170^{env} were immunoprecipitated from CAEV-infected fibroblasts, and gp135-SU was not detectable in either the culture medium or the cell lysates (Fig. 4b). However, a novel protein doublet with an apparent M_r of 60,000 was immunoprecipitated from the cell lysates of CAEV-infected fibroblasts. This doublet was not detected in either the culture medium or the lysates from noninfected fibroblasts or in fibroblasts productively infected with MVV (Fig. 4b). The faint band comigrating with the second band of the 60-kDa doublet in the cell lysate prepared from MVV-infected fibroblasts corresponded to Pr-55^{gag} (Fig. 4b).

The origin of the doublet (M_r 60,000) immunoprecipitated from CAEV-infected fibroblasts was investigated using the hyperimmune goat serum G62 and monoclonal antibody (mAb) F7-299 directed against gp135-SU. As shown in Fig. 5, the doublet was immunoprecip-

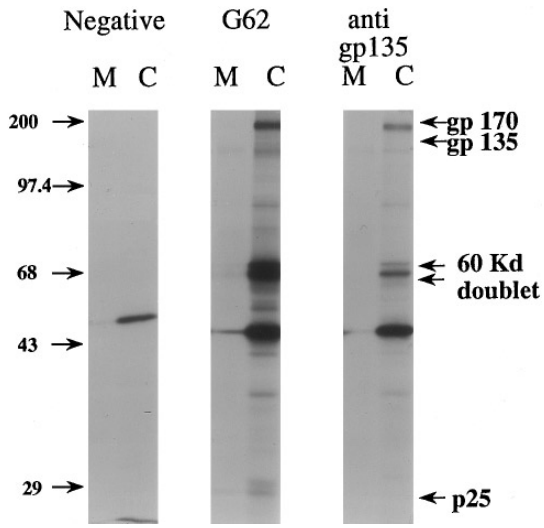

FIG. 5. The "60-kDa doublet" is derived from the env glycoprotein. Fibroblasts were infected with CAEV as described under Materials and Methods. At 5 days postinfection, proteins were radiolabeled overnight with 200 μCi of [^{35}S]methionine/cysteine and immunoprecipitated from culture medium and cell lysates using the goat serum described above or monoclonal antibodies directed against CAEV gp135-SU (F7-299). Protein samples were separated using SDS–PAGE (8.5% gel) and visualized using standard autoradiographic techniques. M, culture medium; C, cell lysate. Negative, serum from an uninfected goat. G62, hyperimmune serum from goat 62 used to immunoprecipitate virus proteins from the culture medium (M) and cell lysate (C) from CAEV-infected fibroblasts. Anti-gp135-SU, monoclonal antibody directed against gp135-SU used to immunoprecipitate virus proteins from the culture medium (M) and cell lysates (C) from CAEV-infected fibroblasts. Molecular mass (in kilodaltons) of prestained markers is shown on the left. Positions of the characteristic viral bands are indicated on the right.

itated from lysates of CAEV-infected fibroblasts using the polyclonal G62 serum (Fig. 5). In contrast, use of the mAb F7-299 anti-gp135-SU showed that whereas the lower band of the doublet was efficiently immunoprecipitated (Fig. 5), the intensity of the immunoprecipitated upper band was only slight. Thus, the improperly processed gp135-SU was still recognizable by mAb F7-299, albeit poorly. The results with mAb F7-299 suggest that the env glycoprotein is specifically improperly cleaved in fibroblasts into two protein species with an apparent M_r of 60,000.

DISCUSSION

In this study we have demonstrated a novel type of host cell restriction of lentivirus replication during persistent infection. Our results suggested that abnormal processing of viral proteins in CAEV-infected fibroblasts was responsible for the restriction of replication of this virus in this cell type. The surface env glycoprotein was specifically cleaved into two protein fragments migrating as a doublet with a M_r of 60,000. Since the viral genome was capable of initiating productive virus replication in GSM cells and macrophages, the restriction seen here was caused by the fibroblasts. The ability of CAEV to infect fibroblast cultures and to maintain an infectious genome that could be detected by PCR after 5 days and 3 weeks postinoculation and rescued by co-cultivation with the permissive GSM cells illustrated a type of the semipermissive replication of the virus not seen before. The early events of the viral life cycle (entry into the cell, reverse transcription, and proviral gene expression) must have progressed normally since the viral genome was detected by PCR in 10% of the cells inoculated at m.o.i. 0.1, and the infectivity of this genome was demonstrable by rescue of infectious virus by co-cultivation with permissive GSM cells. The finding that the number of infected cells did not increase between the Day 5 and the Day 21 sampling times indicated a type of persistent infection that failed to spread to neighboring cells.

Based on studies with other lentiviruses, we can assume that the cleavage of the gp135-SU occurred either in the Golgi complex or in the trans Golgi compartment during transport to the cell membrane via vesicles. Whether the new additional specific cleavage of the env glycoprotein that generated the 60-kDa doublet in the CAEV-infected fibroblasts occurred also in the Golgi or trans Golgi compartment or at the cell surface is unknown. The fact that co-cultivation of CAEV-infected fibroblasts with the GSM cells resulted in a rapid fusion of GSM cells within a few hours after co-cultivation suggested that the transmembrane glycoprotein gp41 was expressed on the surface of the infected fibroblasts. The abnormal posttranslational cleavage of the precursor envelope glycoprotein of CAEV in infected fibroblasts illustrated a novel mechanism by which the host cell restricted the replication of this virus. A similar mechanism could be involved during persistent nonproductive infection in animals in which infected precursor cells may lack enzymes necessary for functional cleavage of the viral glycoproteins. Such enzymes may not become functional until infected precursors become mature and/or activated, as suggested from previous studies on mononuclear cells obtained from blood of infected animals (Gendelman *et al.,* 1986, 1985, Narayan, 1983, Narayan *et al.,* 1982, 1983).

The inability of CAEV to complete its viral life cycle in cultures of fibroblasts, while surprising, is not unique, since this phenomenon has been demonstrated in cultures infected with primate lentiviruses HIV and SIV. Molecularly cloned SIVmac239, which replicated productively in macaque CD4$^+$ T cells, replicated inefficiently in macrophages with the failure of cell to cell virus spread being attributed to the inefficient cleavage of the viral precursor proteins (Stephens *et al.,* 1995). Similar studies on a lymphocyte-tropic strain of HIV-1 have suggested that the restriction is at the level of transcription in infected macrophages (Huang *et al.,* 1993, Schmidtmayer-

ova *et al.,* 1992). Restriction of virus replication was also observed after comparison of the frequency of cells that have the viral DNA genome detected by an *in situ* PCR technique and that of cells which express the viral RNA detected by an *in situ* hybridization technique in tissues of MVV-infected sheep and HIV-1-infected patients (Embretson *et al.,* 1993a, 1993b; Haase, 1994, Staskus *et al.,* 1991). The main conclusion of these studies was that more cells have the proviral genome than express viral RNA. Thus, following integration of viral DNA in host cells, the completion of the virus life cycle could be interrupted at many stages, depending on the virus and the cell. The studies reported in this paper identify one type of restriction not reported previously. This type of restriction could explain in part the latency of the clinical disease and in part the persistence of immunity since viral proteins incorrectly processed for assembly into virus particles could still be functional in inducing and maintaining immune responses to the virus.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Grant NS-12127 from the National Institutes of Health. We thank the Philippe Foundation for providing partial financial support of Y.C., Dr. Cheevers and Dr. McGuire for providing monoclonal antibodies, and Jean Pemberton for typing the manuscript.

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