Analysis of Ovine Lentivirus Infectivity and Replication by Using a Focal Immunoassay and an Antigen-Capture Enzyme-Linked Immunosorbent Assay

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A focal immunoassay and an antigen-capture enzyme-linked immunosorbent assay (antigen-capture ELISA) were developed to quantify infectious ovine lentivirus (OvLV) and OvLV capsid protein (CA) (p27), respectively. The in vitro kinetics of replication and cytopathogenicity of distinct biological clones of OvLV (rapid/high and slow/low phenotypic variants) were assessed. Both viruses were detected by focal immunoassay within 48 h postinfection, 2 days before syncytia were observed in goat synovial membrane cells infected with rapid/high OvLV and 4 days before they appeared in cultures infected with slow/low OvLV. CA was first detected by antigen-capture ELISA in supernatants of cells infected with rapid/high OvLV 4 days postinfection, and it reached a plateau by 10 days, 4 days after peak syncytium formation. In contrast, in cultures infected at the same multiplicity of infection with slow/low OvLV, CA was detected 8 days postinfection, and the titer gradually increased over the following 12 days while the number of syncytia gradually decreased. Peripheral blood mononuclear cells (PBMC) from seropositive sheep treated with phorbol 12-myristate 13-acetate (PMA) generally expressed CA earlier and at higher levels than PBMC treated with either phytohemagglutinin or concanavalin A. Serum CA levels above 3 ng/ml were found in 58% (18 of 31) of seropositive sheep. However, there was no correlation between PMA-induced CA expression and levels of antigenemia. Viral heterogeneity may account for variations both in CA expression in cultures of PBMC and in antigenemia, humoral immune response, and viral pathogenicity in infected animals.

Ovine lentivirus (OvLV) infection of sheep has been proposed as an animal model for the study of certain aspects of the pathogenesis of human immunodeficiency virus (HIV) infection (16, 21, 24). Like HIV, isolates of OvLV have been reported to vary in their replication kinetics and cytopathogenicity (15, 26). Focal immunoassays (FIA) (4, 25) and antigen-capture enzyme-linked immunosorbent assays (cELISA) (10, 17) developed for the detection and quantification of HIV type 1 (HIV-1) offer several advantages over other methods of virus detection. The FIA can detect foci of cell-free virus or virus-infected cells in monolayers of permissive cells. In this assay, a single infectious unit leads to a discrete response; therefore, a linear relationship between the number of virally induced cytopathic effects and the first order of virus concentration exists. Unlike cELISA and reverse transcriptase assays, the FIA provides a direct quantitative measurement of infectious virus particles per unit in the inoculum. Detection of one infected cell in 10⁶ uninfected cells by FIA demonstrates that the sensitivity of the FIA nearly equals that of the polymerase chain reaction (25). The cELISA is extremely sensitive, detecting nanogram to picogram amounts of viral antigen, requires small sample volumes, does not require radioactive reagents, and is convenient for large-scale testing (11). The cELISA can be used to detect and quantify lentivirus capsid protein (CA) in sera and supernatants from cell cultures.

We describe here the development and application of two

immunoassays, FIA and cELISA, for the quantification and characterization of OvLVs in cell monolayers, cell culture supernatants, and sera. The FIA and cELISA were used to evaluate the in vitro growth kinetics of two plaque-purified, phenotypically distinct OvLV strains. In addition, the cELISA was used to study OvLV expression in cultured peripheral blood mononuclear cells (PBMC) treated with phorbol 12-myristate 13-acetate (PMA), concanavalin A (ConA), or phytohemagglutinin (PHA) and for the detection of CA in sera. Comparisons among the presence of antibodies, the presence of antigenemia, and the magnitude of mitogen-induced virus production in PBMC cultures in vitro were then made.

MATERIALS AND METHODS

Animal sources. Adult female sheep at least 2 years old and naturally exposed to OvLV came from flocks in Idaho and Colorado. Seventy-four percent (23 of 31) were seropositive for OvLV by agar gel immunodiffusion, and most had clinical signs of OvLV infection such as emaciation, dyspnea, polyarthritis, and mastitis. Serum and PBMC were collected by jugular venipuncture.

Cells. Ovine cornea (OC₄) cells and goat synovial membrane (GSM) cells grown from tissue explants of OvLV-free newborn animals were verified to be lentivirus free by polymerase chain reaction amplification of the OvLV long terminal repeat (2, 6) and mycoplasma free by DNA fluorochrome staining of cultures (2, 3). The cells were grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum (Irvine Scientific, Santa Ana, Calif.) and 10 mg of L-glutamine, 10,000 U of sodium penicillin G, 10 mg

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of streptomycin sulfate, and 25 µg of amphotericin B per ml (GIBCO, Grand Island, N.Y.).

Virus. Lairmore et al. (15) isolated and characterized OvLV strains 85/34 and 84/28. Strain 85/34 induced rapid fusion and lysis (rapid/high phenotypic variant) in monolayer cultures of GSM cells. In contrast, infection of GSM cells with OvLV strain 84/28 at the same multiplicity of infection (MOI) resulted in persistent infection and fewer syncytia (slow/low phenotypic variant). To quantify total OvLV proteins, a standard curve was constructed by using optical density readings from the reaction of bicinchoninic acid (27) with sucrose density gradient-purified OvLV 85/34 (19). The CA was estimated as 40% of the total protein value.

MAb. Production and characterization of monoclonal antibodies (MAb) that recognize four distinct epitopes on OvLV structural polypeptides were described previously (19). Ammonium sulfate precipitates of ascites were dialyzed against 100 mM NaHCO₃ (pH 8). The MAb was then diluted to a concentration of 1 mg/ml, biotinylated with aminohexanoyl-biotin *N*-hydroxysuccinimide (Zymed Laboratories, Inc., South San Francisco, Calif.) at a ratio of 10:1 (wt/wt) for 1 h, dialyzed against TEN buffer (50 mM Tris, 1 mM EDTA, 150 mM NaCl [pH 7.4]), and then stored at -20°C.

cELISA. MAb which recognized distinct epitopes of the OvLV CA were used for cELISA (19). Different pairs of MAbs were tested for their effectiveness in capturing CA and in detecting captured CA. Capture MAb were screened at five concentrations, and OvLV CA was detected with biotinylated MAb diluted 1:200. Optimal conditions were determined by titration of the MAb with different coating buffers at various incubation times and temperatures. The coating buffers tested were TEN buffer, 100 mM NaHCO₃ (pH 8.5), 10 mM sodium borate, and phosphate-buffered saline (PBS) (1.8 mM NaH₂PO₄ dihydrate, 8.4 mM Na₂HPO₄, 150 mM NaCl [pH 7.2]). Dilutions of the biotinylated MAb and purified OvLV were evaluated at 4, 25, and 37°C after 0.5, 1, 2, 4, and 6 h of incubation.

In the standardized assay, MAb 5A was used at a concentration of 2 µg/ml in TEN buffer for 16 h at 37°C to coat flat-bottom 96-well ELISA plates (Corning Glass Works, Corning, N.Y.). The plates were blocked with 200 µl of BLOTTO buffer (2% nonfat dried milk, 1% Triton X-100, 50 mM Tris hydrochloride [pH 8.5], 0.05% Tween 20, 10 mM EDTA) (1) for 1 h at 37°C. Use of BLOTTO buffer as the blocking and dilution buffer in all ELISA protocols resulted in the lowest background value without reduction of sensitivity. Following removal of the blocking agent, 50 µl of cell culture supernatant or virus diluted in BLOTTO buffer was added. After the addition of biotinylated MAb 2F diluted 1:400 in BLOTTO buffer, the plates were incubated for 4 h at 37°C. Following rinsing five times with 0.05% Tween 20 in PBS, 50 µl of streptavidin-conjugated horseradish peroxidase (Zymed Laboratories, Inc.) diluted 1:2,000 in BLOTTO buffer was added for 30 min at 37°C. Bound peroxidase was detected by rinsing as described above and adding 100 µl of chromogen-substrate to each well for 10 min at room temperature in the dark. The chromogen, 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma Chemical Co., St. Louis, Mo.), was prepared as a stock solution (100 mg of TMB in 10 ml of dimethyl sulfoxide) and stored in the dark at room temperature. The chromogen-substrate solution consisted of 7.5 µl of 30% H₂O₂, 50 ml of 0.1 M citrate-acetate buffer (pH 6.0), and 0.5 ml of TMB stock. Addition of 50 µl of 1 N H₂SO₄ stopped the reaction. The optical densities of the test wells were measured at 450 nm (MR600 Dynatech Plate Reader).

A positive reaction was defined as twice the optical density value of a TEN buffer control.

To evaluate the specificity of the cELISA, clarified supernatants from tissue cultures infected with OvLV strain 85/34, 84/28, or 1514 (visna virus) or the closely related caprine arthritis-encephalitis virus were used as positive controls. Supernatants from tissue cultures infected with unrelated viruses included equine infectious anemia virus, bovine immunodeficiency virus, bovine leukemia virus, feline leukemia virus, bovine viral diarrhea virus, and parainfluenza virus type 3.

FIA. Ovine cornea or GSM cells were seeded at a density of 2×10^4 per chamber in eight-chamber plastic tissue culture slides (Nunc Inc., Naperville, Ill.). Plaque-purified OvLV strain 85/34 or 84/28 was added at MOIs of 0.01, 0.1, and 1. Cell culture supernatants were collected daily in duplicate for 20 days and stored at -20°C for analysis by the cELISA. The chamber slides with infected monolayers were fixed in 100% cold acetone at 4°C for 10 min. Endogenous peroxidase was neutralized with 1% aqueous H₂O₂ for 30 min at room temperature. The slides were rinsed three times for 5 min in Coplin jars containing TNE (10 mM Tris HCl [pH 7.5], 150 mM NaCl, 2 mM EDTA) and then blocked with 10% normal horse serum and 10% bovine calf serum (HyClone Laboratories, Logan, Utah) for 30 min. MAb 2F (specific for OvLV CA) was applied at 2 µg/ml in TNE containing 10% normal horse serum for 1 h at room temperature. The slides were rinsed as described above and incubated with biotinylated rabbit anti-mouse immunoglobulin G and avidin-biotin-peroxidase complex according to Vector ABC kit protocol (ABC Vectastain; Vector Laboratories, Burlingame, Calif.). Rinsed slides were then reacted with diaminobenzidine substrate (70 mg of 3,3'-diaminobenzidine free base in 150 ml of Tris buffer [120 ml of 7.9-g/liter Trizma HCl and 30 ml of 6.1-g/liter Trizma base] and 0.05 ml of 30% H₂O₂). The slides were incubated for 5 min, thoroughly rinsed in double-distilled water, and counterstained with Harris hematoxylin for 1 to 2 min. The number of immunoperoxidase-stained cells or foci of cells per 10 10× microscopic fields was determined.

Syncytium induction assay (SIA). Monolayer chamber slide cultures of GSM or OC₄ cells were infected with OvLV strain 85/34 or 84/28 by using the same MOI and other conditions described for FIA. The monolayers were fixed for 10 min in 100% methanol at room temperature and stained for 2 h with Giemsa (EM Diagnostic Systems, Inc., Gibbstown, N.J.). The number of syncytia (giant cells containing more than three nuclei per cell) per 10 10× microscopic fields was determined. Slides were collected daily for 20 days.

Detection of OvLV-specific antibodies in sheep serum. Serum antibody to OvLV was detected by using an agar gel immunodiffusion assay (Veterinary Diagnostic Technology, Inc., Wheatridge, Colo.).

Isolation and culture of PBMC. Leukocytes from venous blood of 31 sheep were separated according to the method of Cole et al. (5). PBMC were rinsed four times with Hanks balanced salt solution before being resuspended in RPMI medium (supplemented with 10% bovine calf serum, 1× nonessential amino acids, 5×10^{-6} M 2-mercaptoethanol, 2 mM L-glutamine, and 50 µg of gentamicin [Sigma Chemical Co.] per ml) at a concentration of 5×10^6 PBMC per ml. Five milliliters of the cell suspension was added to 25-cm² tissue culture flasks (Corning Glass Works), and PMA (1 ng/ml), ConA (5 µg/ml), or PHA (5 µg/ml) (Sigma Chemical Co.) was added immediately. Adherent, nonadherent, and total

FIG. 1. FIA for OvLV. Monolayers of GSM cells were infected with a highly cytolytic (rapid/high) strain (85/34) of OvLV at an MOI of 0.01. By 4 days postinfection, there were several well-delineated immunoperoxidase-stained foci of OvLV-infected cells (a); however, at this time no syncytia were observed with Giemsa stain (b). By 8 days postinfection, there were numerous syncytia and foci of viral infection. Bar = 20 μ m.

PBMC were tested. Total cells were incubated for 2 h before nonadherent cells were removed and transferred to a new flask. Medium was replaced in the adherent flask, and the appropriate stimulating agent was added to all flasks. A 200- μ l aliquot of supernatant was removed from each flask on odd-numbered days and stored at -20°C until assayed for OvLV antigen by the cELISA.

RESULTS

Optimization and specificity of cELISA. Of the 15 capture and biotinylated MAb pairs tested, only 3 pairs (5C and 1B, 5A and 1B, and 5A and 2F) were suitable for use in cELISA. The selection criteria included sensitivity, stability of conjugate, low background signal, and capacity to bind OvLV antigen. The 5A-2F MAb pair most efficiently captured OvLV antigen with the lowest background levels and was thus used to optimize the conditions of the cELISA. A capture antibody concentration of 2 μ g per well was chosen as the working concentration. The highest signal at all concentrations of capture MAb and virus and the lowest background level occurred with the use of TEN buffer. When sheep sera were tested by cELISA, borate coating buffer had the lowest background level (data not shown).

A dilution of 1:400 of the biotinylated MAb 2F (MAb 2F-B) detected 3.2 ng of OvLV CA per ml with an absorbance of 0.191 ± 0.021 (mean background values were 0.071 ± 0.004). The optimal incubation time and temperature were determined by comparing three temperatures at five incubation times (data not shown). The longer incubation times resulted in higher absorbance values at all virus concentrations. A 4-h incubation time was chosen for convenience, since there was little increase in signal with additional incubation time. Incubation at 37°C resulted in optimal sensitivity.

Supernatants from cell cultures infected with ovine or caprine lentiviruses, other retroviruses, and nonretroviruses of ovine origin were tested by cELISA to determine the specificity of the assay. Bovine leukosis virus, bovine immunodeficiency-like virus, equine infectious anemia virus, feline leukemia virus, parainfluenza virus type 3, and bovine viral diarrhea virus used as antigens gave absorbance readings of <0.037 and thus were not detected by the cELISA.

OvLV strains 85/34, 84/28, and 1514 (visna virus) gave readings of 2.0, 1.84 ± 0.045 , and 0.480 ± 0.016 , respectively. The closely related goat lentivirus (caprine arthritis-encephalitis virus) gave a value of 0.806 ± 0.003 .

Characteristics of phenotypically distinct OvLV strains in cell culture monolayers. Two phenotypically distinct strains of OvLV were compared by FIA and SIA in terms of their *in vitro* kinetics of replication and cytopathogenicity and they were compared by cELISA in terms of CA release into cell culture supernatants. OvLV CA was observed in both individual and multinucleated cells by 2 days postinfection (Fig. 1a), and virus phenotype did not greatly influence the kinetics of infection as measured by FIA (Fig. 2). Cell culture monolayers inoculated with strain 85/34 at an MOI of 0.01 became positive by FIA 24 h earlier than cells infected with strain 84/28 at the same MOI. There was no significant difference in the number of foci of infection between 2 and 6 days postinfection regardless of OvLV strain. However, secondary spread of virus was evident by 8 days postinfection (Fig. 1b). By 12 days postinfection, OvLV CA was present throughout the entire monolayer with either virus strain and at all MOIs. Similar levels of infection and viral kinetics were observed in both GSM and OC₄ monolayers (OC₄ data not shown).

Strain 85/34 produced syncytia more rapidly than did 84/28, as determined by the SIA (Fig. 2). Syncytia were first detected in monolayers of GSM cells infected with strain 85/34 at the higher MOI (0.1 or 1.0) 4 days postinfection (Fig. 2) and were detected in cells infected at an MOI of 0.01 6 days postinfection. However, multinucleated cells were not easily discernible until 8 days postinfection (Fig. 2). Syncytia did not appear until 5 days postinfection with the higher MOI in monolayers infected with OvLV 84/28, and formation peaked within 6 days of infecting cultures with an MOI of 1.0 of either strain 85/34 or strain 84/28.

The initial detection of CA in cell culture supernatants by cELISA correlated with syncytium induction. CA was detected in supernatants of cultures infected with strain 85/34 at an MOI of 0.01 by 4 days postinfection (Fig. 3). The CA titer reached a plateau 10 days postinfection, 4 days after peak syncytium formation. The culmination of CA production by strain 85/34 preceded monolayer lysis and infection

FIG. 2. Comparison between the FIA (top panels) and the SIA (bottom panels) in GSM cell cultures infected with a persistent (slow/low) strain (84/28) or a highly cytolitic (rapid/high) strain (85/34) of OvLV at three different MOIs. The last datum point for each curve indicates the last time point before complete infection of the monolayer. Values are means and standard errors of the means of triplicate determinations.

of the entire monolayer as measured by FIA. In contrast, CA from cultures infected with OvLV strain 84/28 at an MOI of 0.01 was first detected 8 days postinfection, and the titers gradually increased over the following 12 days. The CA titers in these cultures increased over time even though the entire monolayer demonstrated infection by FIA at 12 days postinfection and the number of syncytia per 10 10× microscopic fields had decreased between 12 and 20 days postinfection.

Assay of OvLV capsid antigen in supernatants from mitogen- or PMA-treated PBMC. PBMC from 31 sheep were treated with PMA, PHA, or ConA. Specific antibodies to OvLV, as determined by agar gel immunodiffusion, were found in 74% of the sera. CA released into the culture supernatants was detected by cELISA and was first evident in culture supernatants from seven animals 9 days after initiation of the cultures. Thirty percent (6 of 20) of the PBMC cultures treated with PMA tested positive for OvLV

CA. In comparison, 16% (5 of 31) of the cultures treated with PHA and only 11% (3 of 28) of the cultures treated with ConA tested positive. The PMA-treated cultures expressed antigen earlier and at higher levels than cultures treated with one of the mitogens ConA and PHA. CA was detected in the sera of 58% (18 of 31) of the sheep, all of which were seropositive by agar gel immunodiffusion for OvLV. CA was not detected in serum from seronegative sheep.

Adherent, nonadherent, and total PBMC of four seropositive and two seronegative sheep were compared for expression of CA over 17 days. Control cells from each population of PBMC that received no PMA or mitogen were included to determine baseline levels of viral antigen expression. No CA was detected in supernatants from the seronegative sheep. Untreated total and adherent PBMC populations of two sheep (no. 22 and 670) had high levels of CA expression by day 17 after culture (Fig. 4). There was no OvLV CA expression in treated or untreated nonadherent PBMC populations of any of the sheep tested (data not shown). CA expression in both total and adherent PBMC populations of two sheep (no. 570 and 670) was stimulated to a much greater extent by PMA than by ConA or PHA. This was true also in the adherent cell populations of the other two sheep (no. 22 and 538). Adherent cells treated with PMA expressed OvLV CA at a more rapid rate and at higher levels than did adherent cells treated with either of the two mitogens. PMA was the only reagent that consistently induced a substantial level of OvLV antigen expression in cultured PBMC. Only PBMC of sheep 22 and sheep 538 and adherent cells of sheep 670 responded to stimulation by the mitogens ConA and PHA. The sera from sheep 22, 670, and 538 had detectable levels of CA (25.5, 29.5, and 13 ng/ml, respectively), whereas serum from sheep 570 was negative for antigen.

FIG. 3. The kinetics of CA released into the supernatants of GSM cells infected with OvLV strain 84/28 (●) or strain 85/34 (■) (MOI, 0.01) as determined by cELISA. Values are means and standard errors of the means of triplicate determinations. O.D., optical density.

DISCUSSION

The FIA and cELISA enabled comparisons between two phenotypically distinct strains of OvLV and quantification of CA expression of wild-type OvLV. Since the FIA and cELISA do not rely on syncytium formation or lysis of

FIG. 4. OvLV expression, as assessed by cELISA, in supernatants of PBMC populations of four OvLV seropositive sheep (no. 22, 670, 570, and 538). PBMC were treated for 17 days with ConA, PHA, and PMA. CA was detected in sera from sheep 22, 670, and 538. Values are means and standard errors of the means of triplicate determinations. O.D., optical density.

infected indicator cells for detection of virus, they are not as influenced by virus phenotype as assays that depend on these criteria. In addition, immunologic detection eliminates false-positive scoring of nonspecific cell fusions. Such fusions may occur in stressed or highly passaged cells or in cultures that are latently infected with another syncytium forming agent (4). The FIA was a more rapid, sensitive, and objective method for quantifying OvLV infectivity than was the SIA. GSM and OC₄ cells infected with strain 85/34 or strain 84/28 at an MOI of at least 0.1 could be identified by FIA as early as 48 h postinfection. Complete lysis of the monolayer by strain 85/34 correlated with maximal concentrations of CA in cell culture supernatants. Increasing concentrations of CA in supernatants of cells infected with strain 84/28 suggested that the virus continued to replicate, even though the number of syncytia had diminished. Reduction in the number of syncytia with time likely resulted from lysis of fused cells or fusion of syncytia.

There was little difference in the kinetics of virus replication between the two OvLV strains; however, there were distinct differences in the kinetics of CA released into the supernatant and in the magnitude and occurrence of cytopathic effects. Although these viruses apparently infect similar numbers of cells, they replicate to different titers and induce different degrees of severity of cytopathology in vitro and disease in vivo (16). It is possible that the slow/low strain 84/28 may spread through direct cell-cell contact without always inducing cell fusion, a pattern similar to that reported for herpesvirus (18). The virus may also spread by budding directly into endosomes that are then transferred to other cells (28). Genomic differences between these two virus

strains are currently being explored, and these studies may elucidate the basis for phenotypic differences.

Both GSM and OC₄ cells supported replication of culture-adapted as well as low-passage wild-type OvLV strains (wild-type OvLV data not shown). Chesebro and Wehrly (4) used FIA to detect wild-type HIV-infected lymphocytes with high efficiency compared with detection of cell-free wild-type virions. They postulated that infected cells delivered multiple virions upon contact with the cell monolayer and that high concentrations of released virus in the region of contact increased the probability of initiating a successful local infection. However, it was also observed that certain cell lines could be efficiently infected by cell-free HIV and certain culture-adapted strains had a higher affinity for particular targets than did most wild-type strains. In the present study, cell culture-adapted cell-free virions, when inoculated at an MOI between 0.01 and 1.0, efficiently infected host cells (GSM and OC₄). A recent study used FIA to quantify infectious wild-type OvLV in alveolar macrophages (2). It was shown that as many as 1 in 200 alveolar macrophages was infected with OvLV, but detection and quantification of virus were highly dependent on the severity of pulmonary disease. PBMC obtained from patients seropositive for HIV-1 often expressed detectable viral antigen within 4 to 30 days of culture (10, 17, 30); however, detection of viral antigen was again highly dependent on the severity of disease.

The cELISA detected as little as 3.2 ng of CA of either ovine or caprine lentivirus per ml in supernatants or sera. The MAb used in the cELISA did not recognize antigens from other lentiviruses, oncornaviruses, or other viruses that commonly infect sheep. The use of cELISA and immunoblotting allows monitoring of the changing concentrations of OvLV antigen and antibody in sera and can be used to monitor the progression of clinical disease (2, 13). Detection of OvLV antigen in antibody-negative serum may represent recent or occult infection. Cutlip et al. (7) found that experimentally infected animals required 6 months to 1 year to develop a humoral response to OvLV infection. In the present study, the failure to detect CA in sera from 16% of the seropositive sheep may be due to immune-complex formation which may interfere with the binding of the MAb in the cELISA. Alternatively, viral antigen levels may have been below detection limits for the cELISA during the time of sampling.

We compared production of OvLV CA in PBMC cultures treated with PMA, ConA, and PHA. PMA stimulated OvLV CA expression in cultured PBMC more effectively than did ConA or PHA, on the basis of concentrations of CA in supernatants and the time from treatment to detection. One recognized action of PMA is to induce differentiation and maturation of monocytes to macrophages (20, 29). Peripheral blood monocytes, tissue macrophages, and bone marrow-derived promonocytes serve as important reservoirs for both ruminant and human lentiviruses and allow the spread of the virus to other tissues (22). OvLV replication in monocytes is highly restricted in the host (8). When monocytes are cultured, they quickly differentiate into macrophages and become more susceptible to OvLV infection. Consequently, there is an acceleration of virus replication and synthesis of viral proteins in mature cells. Interferon induced from the interaction of T lymphocytes with infected macrophages can retard lentivirus replication in mature macrophages (8, 14). The time required for monocytes to differentiate into macrophages along with possible inhibitory effects of an OvLV-induced interferon and possibly other

cytokines may explain the delay in OvLV antigen expression in our PBMC cultures, especially in mitogen-treated cultures.

In the present study, viral antigen was not detected in any of the nonadherent cultures of PBMC or in PBMC from seronegative sheep. Others also have failed to demonstrate OvLV in nonadherent PBMC (8, 22). Only PMA induced consistently detectable concentrations of OvLV antigen in cultured PBMC. The PBMC from sheep 22 and 538 released CA into the supernatant without drug stimulation; this may have resulted from a higher virus load in these cells compared with that of PBMC from sheep 570 and 670, neither of which spontaneously released CA. Sera from sheep 22 and 538 had higher levels of CA than sera from sheep 570 and 670. The level of antigenemia detected in these sheep may indicate different stages of OvLV disease progression (2). In HIV-1 infection, an increase in antigenemia and loss of antibodies to CA was found to correlate with the progression of disease from a subclinical stage to the development of AIDS or AIDS-related complex (9, 12, 23). Ho et al. (12) used cELISA to determine the titers of HIV-1 in plasma and in end point dilution PBMC cultures. Dilutions of PBMC or plasma were incubated with PHA-treated HIV-1-negative PBMC and monitored for p27 antigen production for up to 28 days. Patients with asymptomatic infection, AIDS-related complex, and AIDS had mean titers of 30, 3,200, and 3,500 tissue culture infective doses per ml, respectively. These results suggested an association between the amount of virus released by PBMC and different stages of disease progression.

The present study demonstrates the applicability of two highly sensitive immunoassays for detection, quantification, and characterization of laboratory-adapted and wild-type OvLV. The results suggest that viral heterogeneity may account for the observed variations in CA expression in treated cultures of PBMC and in levels of antigenemia. Viral heterogeneity may also affect the humoral immune response and viral pathogenicity, as previously reported.

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