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Tropism of Sheep Lentiviruses for Monocytes: Susceptibility to Infection and Virus Gene Expression Increase during Maturation of Monocytes to Macrophages

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Visna lentiviruses have a natural tropism for cells of the macrophage lineage of sheep and goats, but virus replication in these cells in vivo is restricted so that only small quantities of virus are produced. One restricting factor suggested in previous studies is that virus replication is dependent on the maturity of the cells: the more mature the cell, the less restrictive the replication of the virus. Since monocytes in peripheral blood are precursors of macrophages, we investigated the effect of cell maturation on virus replication under limited control conditions in vitro by inoculating blood leukocytes with virus and retarding the maturation of monocytes to macrophages during cultivation in serum-free medium. Using enzyme markers that identified the cells in their resting monocytic stage (peroxidase) and mature macrophage stage (acid phosphatase) along with quantitative in situ hybridization and immunocytochemistry with viral reagents to trace the efficiency of virus replication, we correlated virus replication with cell maturation. Only a few monocytes were susceptible to infection, and virus replication did not extend beyond a low level of transcription of viral RNA. In the acid phosphatase-positive, maturing macrophage, susceptibility of the cells to infection was increased and virus replication was greatly amplified to the level of translation of viral polypeptides. However, virus maturation was delayed by 3 days until further cell maturation had occurred. Thus, the entire life cycle of the virus, from its attachment to the target cell to its maturation in the cell, was dependent on the level of maturation/differentiation of the monocytic cell.

Lentiviruses are horizontally transmitted nononcogenic retroviruses (13) that cause persistent infections in their natural hosts and gradual onset of progressive disease characterized by inflammation, degeneration, or immunosuppression. These diseases are exemplified by visna-maedi and caprine arthritis-encephalitis virus infection of sheep and goats (16), infectious anemia in horses (1), and AIDS (acquired immune deficiency syndrome) in humans (21), respectively. Although the AIDS virus has not been completely characterized, the ungulate lentiviruses replicate productively and lytically in cell culture without requirement for cell division, a critical factor for replication of oncogenic retroviruses. In vivo, the viruses have tropism for cells of the immune system, and in this environment virus replication is persistent and continuous at a minimally productive, "slow" rate (4, 7, 11).

The ruminant lentiviruses (visna-maedi and caprine arthritis-encephalitis viruses) have a high tropism for cells of the macrophage lineage and replicate productively in macrophages cultivated from the peripheral blood and lungs of sheep and goats (19). In vivo the viruses maintain their tropism for this cell type, but replication is subject to several types of restriction. First, only relatively few cells are infected. Among mature macrophages, infection was confined to cells in specific tissues such as lung and spleen but not the liver (18). Similarly, among blood leukocytes of persistently infected animals, not only was the infection confined to monocytes, but also, only a small number of these cells were infected (17). A second form of restriction is the reduced ability of immature macrophages to support replication. This was particularly evident in the bone marrow of persistently infected sheep, where precursors of macrophages carried only a few copies of viral RNA in contrast to thousands of copies of viral genome in mature tissue macrophages (11). A further type of restriction occurred in the infected mature macrophage, in which the virus life cycle was aborted at a stage between transcription of viral RNA and maturation of virions (11). Recently, this has been correlated with effects of an interferon that is induced during interaction between the infected macrophage and T lymphocyte (17a). Restriction of virus replication in vivo is thus closely associated with the biology of the macrophage cell type, although the mechanisms associated with these types of restriction of replication are poorly understood.

Since monocytes in the peripheral blood are precursors of tissue macrophages and readily mature into macrophages when cultivated in vitro (2, 3), we inoculated cultures of peripheral blood leukocytes (PBL) with virus to determine whether all or only a few monocytes in the blood are susceptible to infection and whether delay in the maturation of monocytes to macrophages would also retard virus replication.

MATERIALS AND METHODS

Virus. Lentivirus VMA-5, a field strain of visna-maedi virus, was isolated in Idaho from a sheep with inflammatory lesions in the central nervous system, lungs, and joints typical

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of those seen in visna-maedi- and lentivirus-induced arthritis (20).

Cell cultures. (i) Simian virus 40-transformed macrophages. Alveolar macrophages were obtained from a Corriedale lamb by broncho-alveolar lavage, and the cells were inoculated in culture with simian virus 40 at high multiplicity. Proliferating colonies of macrophages obtained 2 weeks later were propagated in Dulbecco minimal essential medium (DMEM) plus 20% heated (56°C for 30 min) lamb serum and maintained in DMEM plus 2% lamb serum. These macrophages are trypsin dissociable and grow into density-dependent monolayers. In addition to simian virus 40 T antigen, the cells have nonspecific esterases and surface Fc receptors by which they phagocytize antibody-coated materials (10). Inoculation of VMA-5 virus into these cells at a multiplicity of 1 resulted in productive replication of the virus to a titer of 5×10^5 50% tissue culture infective doses per ml when assayed in goat synovial membrane cultures (see below). The macrophage cultures degenerated approximately 2 weeks after inoculation with the virus, some of the cells showing multinucleated giant cell formation.

(ii) GSM cultures. Goat synovial membrane (GSM) cells were derived from early subcultures of explants of synovial membranes from a newborn colostrum-deprived goat. VMA-5 virus replicated to titers of 5×10^6 50% tissue culture infective doses per ml in these cultures, which developed multinucleated giant cell formation within 3 days after inoculation at a multiplicity of 1. Infectivity determinations in this study were performed by inoculation of 10-fold dilutions of test suspensions into these cultures, which were seeded in microtiter plates (15).

(iii) PBL. Fifty milliliters of heparinized blood obtained from sheep was diluted 1:3 in Ca^{2+}-Mg^{2+}-free Hanks balanced salt solution and centrifuged at $1,500 \times g$ for 10 min. The buffy coat cells at the top of the pellet were removed in Hanks balanced salt solution and treated with 0.84% ammonium choloride in 0.14 M NaCl to lyse the erythrocytes. The leukocytes were then washed twice in Hanks balanced salt solution and once in DMEM and suspended in DMEM at a concentration of 10^6 cells per ml (17). Samples (10 ml) of these cell suspensions were added to Teflon bottles (Cole-Parmer, Chicago, Ill.) and cultivated in a CO_2 incubator at 37°C. Different samples of these suspensions were supplemented with lamb serum at different concentrations varying from none to 5% serum. Virus was added to some of these cell suspensions at a multiplicity of 2 and incubated at 37°C for 6 h. The cells were then removed from the Teflon bottles in an excess of DMEM, washed twice by centrifugation in DMEM, resuspended in 10 ml of DMEM with the appropriate amount of serum, and cultivated in the Teflon bottles at 37°C as described above. In some experiments, concanavalin A (ConA; Sigma Chemical Co., St. Louis, Mo.) was added to cultured cell suspensions at a concentration of 10 μg/ml, a concentration which is known to induce lymphocyte blastogenesis in sheep cultures (12).

Assay for infectivity. Supernatant fluids from the cultured leukocyte suspensions were clarified by centrifugation and stored at 4°C until assayed for infectivity in GSM cells seeded in microtiter plates. Ten-fold dilutions of the fluids were added to replicates of four cultures in 96-well microtiter plates, and endpoint results (determined by cell fusion) were read 2 weeks after inoculation as previously described (15).

Cytochemical enzyme assays. At various intervals after culture and infection, the cells were diluted 10-fold in DMEM plus 10% lamb serum and sedimented in a Shandon Cytospin-II cytocentrifuge onto glass slides precoated with poly-D-lysine. The preparations were air dried and processed for the following enzyme assays.

(i) Nonspecific esterases. Nonspecific esterases are a marker for monocytes and macrophages (14). These enzymes were measured by the use of naphthyl butyrate as a substrate. The slides were then counterstained with hematoxylin, dehydrated in alcohols, and mounted.

(ii) Peroxidase. Peroxidase is a marker for monocytes and is not present in macrophages (2, 22). The air-dried preparations were incubated with 3,3′-diaminobenzidine tetrahydrochloride (Polyscience Inc., Warrington, Pa.), 0.5 mg/ml in phosphate-buffered saline with 0.01% hydrogen peroxide. The slides were counterstained as described above.

(iii) Acid phosphatase. Acid phosphatase is a marker for macrophages and is not detectable histochemically in monocytes (3). The enzyme was demonstrated in macrophages by using the diazo coupling method with naphthol as the phosphoric acid sodium salt (Sigma) (5) as a substrate.

In vitro labeling with [^{3}H]thymidine. On days 2 and 5 after culture, different lots of PBL were pulsed with [^{3}H]thymidine (specific activity, 5 mCi/ml; Becton Dickinson Co., Rutherford, N.J.) and processed for autoradiography 24 h later. The cells were cytocentrifuged, air dried, fixed in periodate-lysine-paraformaldehyde-glutaraldehyde, dipped in autoradiographic emulsion, and exposed for 3 days as previously described (10). Autoradiograms were then developed, fixed, and stained with hematoxylin. Accumulation of silver grains over the cell nuclei was indicative of DNA synthesis in the cells.

Immunocytochemistry. Cytocentrifuged preparations were fixed in periodate-lysine-paraformaldehyde-glutaraldehyde (8) for 10 min, rinsed in phosphate-buffered saline, and stained immunocytochemically. Two antibodies were used in these studies: (i) a mouse monoclonal antibody to the glycoprotein antigen (gp135) of visna virus (J. Stanley and J. E. Clements, unpublished data), and (ii) a rabbit antiserum to sheep alveolar macrophages as described previously (10). In PBL cultures, antibodies in the latter antiserum bound exclusively to the monocyte-macrophage cell type (10). Primary antibodies were applied to the cytocentrifuged preparations for 30 min. After washing, biotinylated antiglobulins (Vector Laboratories, Burlingame, Calif.) were applied at a concentration of 1:100 for 30 min, followed by a phosphate-buffered saline wash and application of avidin-biotinylated horseradish peroxidase complex for 30 min. The label was developed with 3,3′-diaminobenzidine tetrahydrochloride, and specimens were dehydrated in graded ethanols and either counterstained or processed for in situ hybridization (9).

In situ hybridization. The techniques for in situ hybridization and in situ hybridization combined with immunocytochemistry have been described previously (9, 14a). Briefly, cloned DNA of visna virus and the nucleocapsid gene of measles virus were separated from pBR322 and radiolabeled in vitro by nick translation (14a) using [^{35}S]dATP and [^{35}S]dCTP (Amersham Corp., Arlington Heights, Ill.). The concentration of DNase used for nick translation was adjusted to produce probes approximately 70 base pairs in length. The probes had specific activities of approximately 4×10^8 to 6×10^8 cpm/μg. For combined immunocytochemical and in situ hybridization techniques, the slides were first treated immunocytochemically and then, after incubation with the chromagen, washed several times in phosphate-buffered saline and processed directly for in situ hybridization.

Cytocentrifuged preparations were fixed in periodate-lysine-paraformaldehyde-glutaraldehyde, treated with acid, heat, and proteinase K (1 μg/ml), and acetylated before in situ hybridization. For detection of viral RNA in the preparations, probe at a concentration of 0.2 μg/ml was added to the slides and hybridized for 50 h at 22°C. After hybridization the slides were washed, autoradiographed, and exposed for 1, 5, and 10 days.

The sensitivity of the in situ hybridization procedure for detecting virus-specific RNA was determined by comparing the average number of grains per cell detected by in situ hybridization technique with the number of copies of viral RNA per cell determined by dot-blot hybridization (11). Using the amount of viral RNA accumulated in the cells 24 h after infection, we determined that the in situ hybridization procedure was capable of detecting approximately 10 copies of viral RNA per grain above background after 10 days of autoradiographic exposure.

Electron microscopy. Cell suspensions intended for examination by electron microscopy were centrifuged at 1,000 × *g* for 10 min, and the cell pellets were fixed for 48 h in phosphate-buffered Karnovsky fixative. The pellets were then postfixed in 1% buffered osmium tetroxide, dehydrated in graded alcohols, cleaned in propylene-oxide, and embedded in araldite. Sections 1 μm thick were cut and stained with 1% toluidine blue, and blocks were then selected for ultrastructural analysis. Thin sections were cut and stained with uranyl acetate and lead citrate and examined in a Zeiss 9 electron microscope. Monocytes and maturing macrophages were examined at different stages of development. Five days after cultivation of infected PBL in 5% serum, approximately 150 macrophages were identified and examined ultrastructurally for evidence of virus replication.

RESULTS

Monocyte-macrophage markers in PBL cultures. Approximately 3% of PBL suspensions were identified morphologically as monocytes by Wright-Giemsa staining. These cells were also specifically stained with markers for nonspecific esterases and the antimacrophage immunoglobulin. The enzyme and antigen markers were retained in the monocytes after they had differentiated into macrophages and were therefore used in fresh as well as aging cultures for identification of monocyte-macrophage cell populations.

The peroxidase and acid phosphatase enzyme contents in cells varied with the age of the cultures and the amount of serum in the culture medium. Thus, examination of fresh PBL cultures showed that all monocytes and polymorphonuclear neutrophils had the peroxidase enzyme (Fig. 1), but this enzyme disappeared from monocytes after 24 h when PBL were cultivated in medium with 1% serum or more (Fig. 2). Loss of the enzyme was delayed 48 to 72 h when the cells were cultivated in medium with no serum. The neutrophils degenerated within 1 day after cultivation in this medium.

Examination of fresh PBL for the presence of cells containing the lysosomal enzyme acid phosphatase showed that the enzyme was not detectable. However, after 24 h of cultivation in medium with serum, all the monocytes developed large amounts of this enzyme. All macrophages had this enzyme, and staining intensity increased in aging cultures. Reaction of preparations of fresh and aging PBL with markers for the macrophage antigen and the enzymes (peroxidase and acid phosphatase, respectively) thus showed that there was an inverse relationship among the monocytes between the presence of peroxidase and the detection of acid phosphatase. In serum-free medium, the switch from peroxidase to detectable levels of acid phosphatase occurred 48 to 72 h after initiation of the culture, and in medium with serum this switch occurred within 24 h. Thus, the synthesis of acid phosphatase may have been an early indicator of monocyte maturation, and this process could be delayed by exclusion of serum from the medium. These results are in complete agreement with earlier studies on monocyte maturation (2, 3).

Morphologically, monocytes began to mature into macrophages soon after they were placed in culture, and this occurred irrespective of whether they grew as adherent macrophages in cell cultures dishes or in suspension in Teflon bottles. Examination of 1-μm-thick sections of monocytes fixed shortly after suspension in cell culture medium showed that they had large, centrally placed nuclei surrounded by a thin layer of cytoplasm. While the cells remained basically unchanged during overnight cultivation in serum-free medium, those cultured in serum for the same period changed rapidly in culture. Nuclei assumed eccentric positions in the cell, and cytoplasm increased in volume. Multiple villous processes appeared on cell surfaces, and mitochondria, Golgi apparatus, and lysosomes became prominent. By day 5 the macrophages had increased five- to sixfold and multiple phagocytic vesicles had appeared. Beyond 5 days, phagocytosis of cell debris was evident, and many of the cells had fused to become multinucleated cells.

Infection in PBL. In preliminary experiments, we tested the specificity of the double immunocytochemical-in situ hybridization procedure in cytocentrifuged preparations of infected transformed macrophages. All cells were labeled immunocytochemically with the antimacrophage antibodies, and most had silver grains, indicating hybridization with ^{35}S-labeled viral DNA. Neither of the two labels was found in control uninfected GSM cell preparations. Infected transformed macrophages were then mixed with normal GSM cells and processed for double labeling. Confinement of the double label to the infected macrophages gave assurance of the specificity of the system (data not shown).

Using the double-label procedure on cytocentrifuged preparations of PBL inoculated 2 days previously with virus at a multiplicity of infection of 2, we then showed that monocytes had become infected. Only cells bearing the macrophage marker had grains (Fig. 3). An occasional neutrophil had a few copies of viral RNA, but grain counts did not increase with time, and these cells did not have viral antigens. Viral RNA in the neutrophils was therefore attributed to phagocytosis of viral particles and not to virus replication.

Lack of localization of viral RNA in cells other than those bearing the macrophage marker suggested that lymphocytes did not become infected. We therefore modified culture conditions to enhance growth of lymphocytes in attempts to cause infection in these cells. Although lentiviruses do not require dividing cells for infection, PBL were nevertheless cultivated in RPMI medium plus 10% fetal bovine serum and treated with ConA at a concentration of 10 μg/ml to stimulate division of T lymphocytes. Prior studies have shown that this dose of ConA is highly mitogenic for sheep T cells (12). Virus and ConA were added simultaneously in one culture, and in another, virus was added 24 h after addition of ConA. In both cases, examination of the cells 24 h after virus inoculation showed many cells in mitosis. However, there was no change in virus tropism; i.e., all cells with viral RNA had the macrophage marker.

Serum dependence of virus replication in monocyte-macrophages. Since there was only a brief period (approxi-

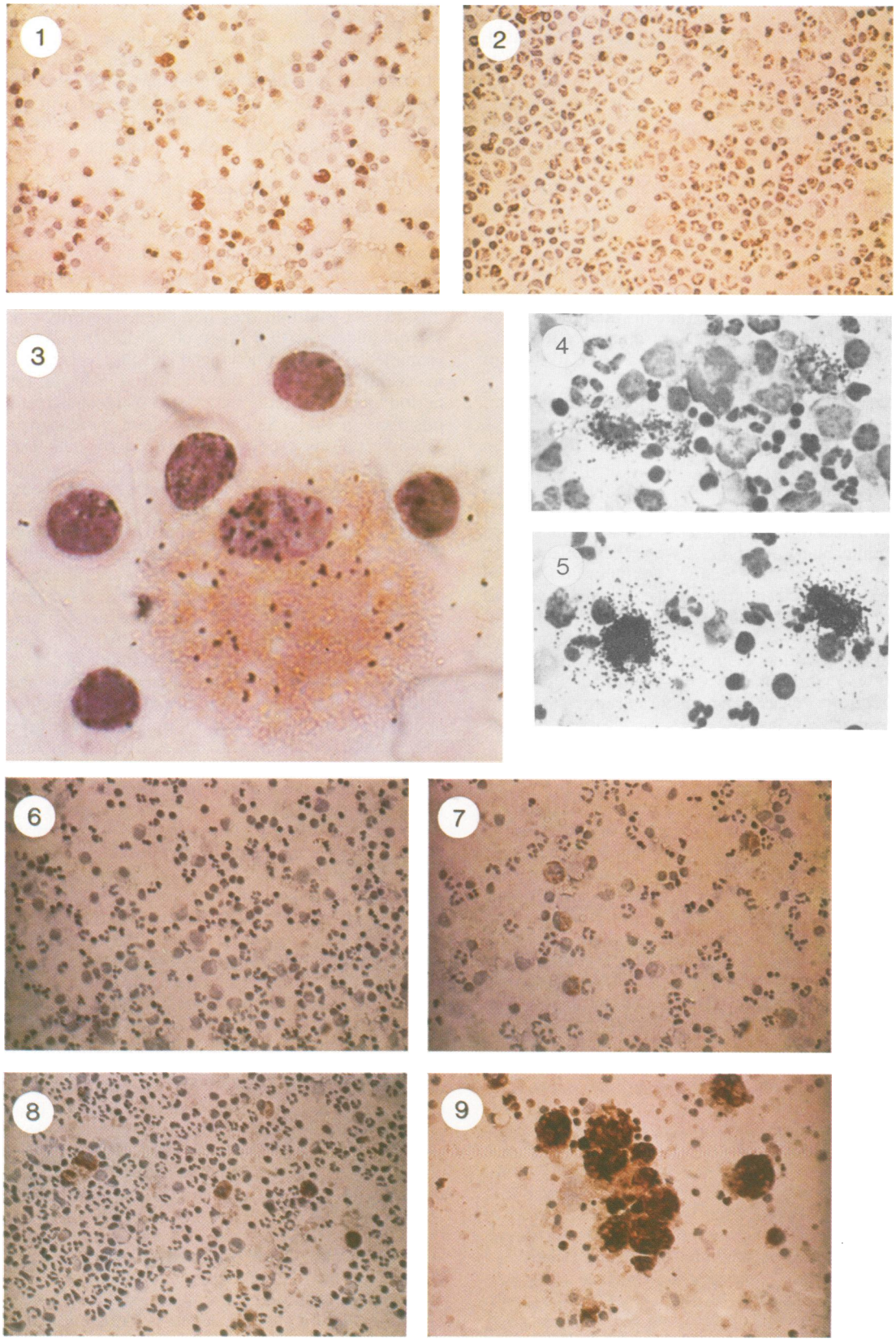
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TABLE 1. Visna virus replication in differentiating monocytes

Time (h) post-infection	Treatment of PBL	% Macrophages with visna viral RNA	Copies of viral RNA per cell	% Macrophages with visna viral antigen
0	Fresh	0	0	0
24	No serum	7	50–350	0
	5% Serum	50	500–1,000	50
120	No serum	83	>10,000	83
	5% Serum	87	>10,000	87

TABLE 2. Indices of monocyte differentiation as a function of serum concentration

Time (h) post-infection	Treatment of PBL	Monocytes as % of total PBL[a]	Enzyme[b]			Cell viability (%)
			Peroxidase (% positive)	Acid phosphatase		
				% positive	Intensity	
0	Fresh	3	3	0	–	100
24	No serum	3	2	3	+	98
	5% Serum	3	0	3	++	98
120	No serum	12	0	12	++	30
	5% Serum	8	0	8	+++	90

[a] Number determined by nonspecific esterase and by immunoperoxidase using the antimacrophage antibodies.

[b] The acid phosphatase and peroxidase assays were standard cytochemical tests on fixed cells as stated in the text. +, ++, +++, Relative intensity of staining reaction.

mately 24 h) in which monocytes could be prevented from maturing into macrophages, we inoculated PBL cultures in medium containing 1, 2, and 5% lamb serum (monocyte maturation-promoting medium) and in medium containing no serum (monocyte maturation-retarding medium) and examined them 24 h later to determine the extent of virus replication. The in situ hybridization procedure showed vast differences in the percentage of infected cells among the groups and also in the extent of transcription of viral RNA. Only 7% of monocytes cultured in serum-free medium had viral RNA, and the RNA copy numbers ranged from 50 to 350 genomes per cell (Table 1). In contrast, 50% of the monocytes grown in 5% lamb serum had grains, and here the number of copies of viral RNA per cell ranged from 500 to 1,000 (Table 1) (Fig. 5). A further difference between the two groups was seen in the extent of translation of the viral RNA in infected monocytes. No viral antigens were detected in infected monocytes in serum-free medium (Table 1) (Fig. 6), whereas nearly all of the cells in the serum groups had viral antigen (Fig. 7 and 8).

Differences between the groups of infected cells were less apparent when cultures were examined at 5 days after inoculation. All macrophages had acid phosphatase, although cells cultivated in medium with serum stained more densely. Comparison of the serum-free and 5% serum groups showed that there was an apparent increase in macrophage numbers, from 3% to 12% in the serum-free group and from 3% to 8% in the serum group (Table 2). The increased density of macrophages was due in part to selective "die-off" of the other cell populations. Thus, whereas neutrophils comprised more than 50% of the cells at the beginning of the experiment, none of these cells were found at 5 days. Although large numbers of lymphocytes must have degenerated, numbers were not tabulated. There was a significant loss in macrophage populations, especially among those in serum-free medium. However, to test whether the relative increase in macrophages may have been due in part to division of the cells, cultures were pulsed with [^{3}H]thymidine on days 2 and 5 and processed for autoradiography on days 3 and 6, respectively. Silver grains, indicating DNA synthesis, were detected in approximately 5% of the macrophages. However, no mitotic figures were seen among these cells.

More than 80% of macrophages had viral RNA and had synthesized viral antigens 5 days after inoculation (Table 1, Fig. 9). We examined approximately 150 of these macrophages ultrastructurally in the hope of identifying the sites of virus maturation. Surprisingly, no virions were found in thin sections of any of the morphologically intact macrophages. However, a few degenerating macrophages had small numbers of virions budding from cell membranes and numerous intracytoplasmic particles (Fig. 10 and 11). Thus, despite the high levels of transcription and translation of viral RNA in the macrophages, there was only a limited amount of virus assembly at 5 days.

Daily assay of infectivity in supernatant fluids from the cultures showed negligible amounts during the first 5 days of infection (Fig. 12). This confirmed the lack of virus morphogenesis in the ultrastructural study described above. However, virus production increased steadily during the next 5 days, accompanied by dense staining of the macrophages with antiviral antibody, and was followed eventually by cell degeneration. Thus, virus maturation in the macrophages was retarded for at least 4 days after virus polypeptides had been synthesized.

DISCUSSION

We used cytochemical and immunocytochemical markers, together with in situ hybridization and infectivity assay

FIG. 1–9. PBL from a normal sheep were inoculated with visna virus at a multiplicity of 2 and cultured at 37°C for various intervals in DMEM.

FIG. 1. PBL cultivated for 24 h in serum-free medium. Peroxidase stain (red) shows several cells with the enzyme. Magnification, ×250.

FIG. 2. PBL cultivated for 24 h in medium plus 5% serum. Peroxidase stain shows lack of enzyme in these cell preparations. Magnification, ×250.

FIG. 3. PBL were cultivated for 5 days in medium plus 5% serum. The preparation was reacted first for immunocytochemical labeling of cells with the antimacrophage antibodies (red) and later processed for in situ hybridization using ^{35}S-labeled viral DNA and autoradiography (black dots). Note localization of the two labels in a single cell. Magnification, ×1,000.

FIG. 4 and 5. In situ hybridization of ^{35}S-labeled cells cultured for 24 h in serum-free medium or 5% lamb serum. Note the much larger number of grains in cells grown in 5% serum (Fig. 5) than in cells grown in medium with no serum (Fig. 4). Magnification, ×500.

FIG. 6, 7, and 8. PBL cultivated for 24 h in serum-free medium (Fig. 6), medium plus 1% serum (Fig. 7), or medium plus 5% serum (Fig. 8) were reacted immunocytochemically with monoclonal antibodies to visna virus glycoprotein gp135. Note the lack of staining in Fig. 6, slight staining in Fig. 7 (red), and higher intensity of staining in Fig. 8. Magnification, ×250.

FIG. 9. PBL cultivated for 5 days in medium plus 5% serum. Preparation was reacted immunocytochemically with monoclonal antibodies to gp135 of visna virus. Note intensity (red) of stain in large round cells.

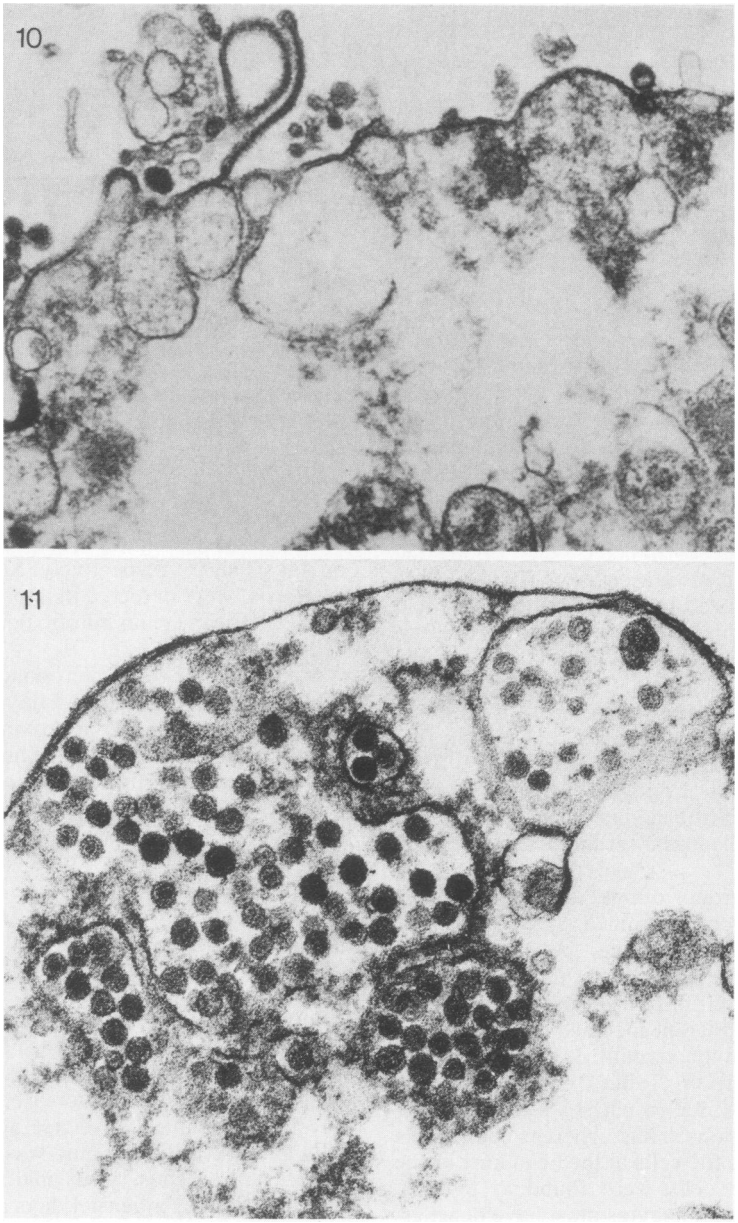

FIG. 10 and 11. PBL cultivated for 5 days in medium plus 5% serum. Electron micrographs show small numbers of budding virions on the surface of a degenerating cell (Fig. 10) and a large number of virions in the intracytoplasmic area (Fig. 11). Magnification, ×57,000.

techniques, to study replication of visna virus in cultured PBL from sheep. The experiments showed that only cells of the monocyte-macrophage lineage supported virus replication. Previous studies had shown that neither inoculation of virus into fresh PBL nor cultivation of PBL from naturally infected animals resulted in productive virus replication before monocytes had matured into macrophages (17; unpublished data). In contrast, different strains of visna viruses have been propagated routinely in cultures of PBL macrophages from sheep (19). In this study we show that the susceptibility of the cells to infection as well as the magnitude and rate of virus RNA transcription and protein synthesis were closely linked to the rate of maturation of monocytes to macrophages, both in turn depending on the concentration of serum in the culture medium. In serum-free medium, monocyte maturation was delayed by 24 h, and infection of the cells in this inactive state apparently resulted in latent infection. Increase in viral transcription and initiation of translation occurred only when monocytes began to mature in culture. The evidence for this maturational process was loss of peroxidase and increased synthesis of acid phosphatase. Thus, restriction of maturation of the infected cell limited replication of the virus.

In addition to latency of the virus in the resting monocytes, there was apparently a later phase of restriction of replication in the maturing macrophages after 5 days of culture. These cells had acid phosphatase and also large numbers of copies of viral RNA and virus proteins, yet there was minimal evidence of virus morphogenesis or infectivity at this stage of the infection. This is in clear contrast to

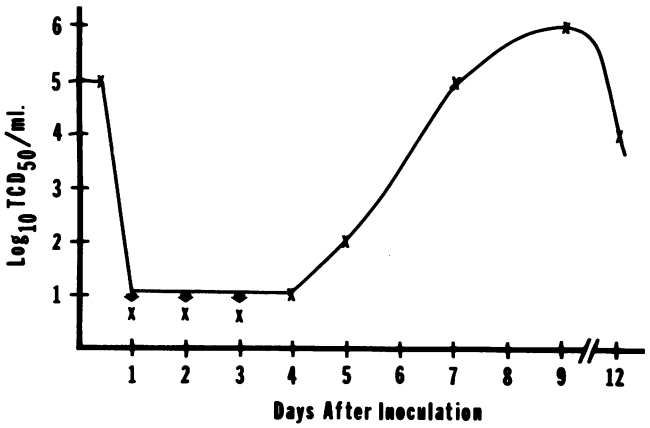

FIG. 12. PBL cultivated in medium plus 5% serum and supernatant fluids, examined daily for infectivity. Arrows indicate that no infectivity was detected on days 1, 2, and 3 after inoculation.

studies of infection in sheep fibroblasts and mature macrophages (7- to 10-day-old macrophages) in which virus replication can be readily seen at 5 days after inoculation (6, 19). In the present study, restriction of virus replication in the 5-day cultured PBL was paralleled in the infection in mature macrophages in tissues, where minimal quantities of virions were produced despite large numbers of copies of viral RNA (11). The mechanism for restriction in this phase of virus replication may be mediated by a recently discovered interferon produced by lymphocytes. This interferon was produced during interaction between lymphocytes and visna virus-infected macrophages, and it restricted replication of the virus in cultured macrophages (14a).

The confinement of infection to a limited number of resting monocytes could be explained in part by a lack of receptors for the virus. This is supported by recent findings that visna virus infection is dependent on virus binding to specific receptors (S. Kennedy-Stoskopf, submitted for publication). The lack of infection in lymphocytes may be due to the absence of receptors for the virus. This deficiency could not be overcome by culture conditions which favored cell division. In contrast, monocytes were susceptible to infection, though this was clearly dependent on culture conditions. This suggests that only few resting monocytes had receptors for virus and that the number of these receptors increased upon maturation of the cells. This would explain the data that 7 and 50% of resting and maturing monocytes, respectively, and 80% of the macrophages were susceptible to infection. In the infected animal, the sharp variation in susceptibility of tissue macrophages as seen between susceptible pulmonary macrophages and resistant Kupffer cells may in fact be due to the absence of receptors in the latter cells.

This study emphasizes the narrow cellular host range of lentiviruses in their natural hosts. The tropism of the ruminant virus for certain macrophage populations is matched by the tropism of equine infectious anemia virus for other macrophages, including Kupffer cells of the liver (4), and apparently that of AIDS retrovirus for lymphocytes bearing the T4 marker (7). In the cell culture system described in this report, all restrictions of virus replication were removed during cultivation of the cells, and cell maturation was the sole determining factor associated with amplified virus replication. Since normal cell maturation processes undoubtedly occur in subclinically infected hosts in whom virus replication is restricted, factors in addition to maturation of target cells must be involved in lentivirus restriction in vivo.

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