Wild-Type Equine Infectious Anemia Virus Replicates In Vivo Predominantly in Tissue Macrophages, Not in Peripheral Blood Monocytes

DEBRA CLABOUGH SELLON, STEPHANIE T. PERRY, LEROY COGGINS,
AND FREDERICK J. FULLER*

*Department of Microbiology, Pathology, and Parasitology, North Carolina State University College of
Veterinary Medicine, 4700 Hillsborough Street, Raleigh, North Carolina 27606*

Received 1 April 1992/Accepted 2 July 1992

In situ hybridization of tissues from two horses infected with the wild-type Wyoming strain of equine infectious anemia virus (EIAV) identified the liver, spleen, lymph nodes, kidney, lung, and adrenal gland as the primary host tissue sites for viral transcription during acute infection. Combined immunohistochemistry, with a monoclonal antibody recognizing a cytoplasmic antigen of equine mononuclear phagocytes, and in situ hybridization for viral RNA identified most infected cells as mature tissue macrophages. In contrast, in situ hybridization of adherent peripheral blood mononuclear cells collected from horses on various days during the first 2 weeks postinfection with the Wyoming strain of EIAV failed to detect any viral RNA in these cells. For the two horses described here, serum reverse transcriptase activity correlated directly with the degree of replication detected in tissue macrophages on the day of sacrifice. These results suggest that unlike other lentivirus infections in which mature tissue macrophages accumulate cytoplasmic viral RNA to a high level but fail to produce infectious virions, mature tissue macrophages are the likely primary source of the high titer of viremia present during acute infection with EIAV. No significant posttranscriptional block of viral replication in tissue macrophages appears to occur with EIAV.

Equine infectious anemia virus (EIAV) is a lentivirus that shares considerable homology with human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) (5, 17, 18, 31, 37, 43). Unlike infection with most other lentiviruses, infection of horses with EIAV frequently results in acute febrile illness within 1 to 2 weeks and is followed by a syndrome of recrudescing fever, weight loss, ventral edema, and anemia (20). Each episode of viremia and fever is associated with the emergence of a novel antigenic variant of the virus (32, 37, 40) and a high titer of viremia (20). As with lentivirus infections in other species, once infected with EIAV, a horse remains infected for life (20). Although EIAV has been recognized as an important cause of disease in horses since the early 1900s (45), many steps in EIAV pathogenesis have not been clearly elucidated. Following infection with a virulent wild-type strain of EIAV, most horses become febrile and thrombocytopenic within 7 to 14 days (7, 20). This febrile episode, like those that follow, is associated with a high titer of viremia in which virus is predominantly cell free (20, 24, 25). Plasma viremia subsides coincident with resolution of clinical signs. In asymptomatic animals, only a very low titer of viremia is present and infectivity is cell associated (20, 24, 25). The cellular site of viral replication during acute infection, and hence the source of the high titer of viremia, is not known. Although widely theorized to replicate in vivo predominantly in cells of monocyte/macrophage lineage (20, 26, 27), this has never been proven. With the technique of in situ hybridization, we have more specifically localized the cellular and tissue sites of viral replication during acute infection with wild-type EIAV.

Previous attempts to identify the tissues and cells infected by EIAV have implicated the monocyte/macrophage as the

principal host cell in vivo (12, 20, 26, 27, 39). This is supported by in vitro growth of the virus in adherent peripheral blood mononuclear cells (4, 12). Direct immunofluorescence analysis of tissues from acutely infected horses revealed viral antigen in cells of the liver, spleen, lymph nodes, kidney, lung, heart, brain, stomach, bone marrow, intestine, adrenal, thymus, and blood mononuclear cells (27). These cells were postulated to be macrophages on the basis of their morphology and tissue localization. However, sera used as primary antibody for fluorescence assays were of equine origin. Since antiviral antibody can be detected in infected horse serum by Western immunoblot as early as 10 days postinfection (7), binding by exogenous equine antisera in the direct immunofluorescence test may have been partially blocked, making it difficult to estimate relative quantities of virus present. The presence of viral antigen may reflect antigen-antibody complex deposition in tissues and not active viral replication (2, 28).

Southern blot analysis of DNA extracted from tissues of an acutely infected horse implied a similar tissue distribution of virus (39). The highest concentration of viral DNA was found in the liver, lymph nodes, bone marrow, and spleen, with lower levels in the kidney, choroid plexus, and peripheral blood leukocytes. The presence of viral DNA in a tissue implies that infected cells are present but does not indicate transcription of viral templates. Most of the EIAV DNA detected was not integrated into host DNA but existed in both linear and circular unintegrated forms (39). Whether these unintegrated forms serve as efficient templates for mRNA transcription is uncertain. Also, by extracting DNA from tissue, no conclusions as to cell tropism can be made. We have used in situ hybridization to test the hypothesis that the predominant cell types infected with EIAV during acute infection in vivo are cells of the monocyte/macrophage lineage.

* Corresponding author.

MATERIALS AND METHODS

Animals and virus. The wild-type Wyoming virus strain 158 was obtained from L. O. Mott (21) and propagated in horses. Serum was obtained from an infected horse during the peak febrile response, and the titer of this stock (hereafter referred to as Wyoming wild-type serum) was determined to be approximately 10^6 infectious doses per ml (as determined by horse inoculation assay). Two crossbred horses (E390 and E516) tested negative for serum antibody to EIAV by agar gel immunodiffusion (9) and by Western immunoblot (7) and negative for EIAV proviral DNA in peripheral blood mononuclear cells by the polymerase chain reaction (48). These horses were inoculated intravenously with 1 ml of Wyoming wild-type serum as previously described (7, 48). Complete blood count and temperature were monitored daily, and the horses were sacrificed when their temperatures reached at least 41°C . This was on postinfection day (PID) 11 for horse E390 and PID 10 for horse E516. The Malmquist strain of EIAV was derived from the wild-type Wyoming strain by adaptation of the wild-type virus in equine dermis fibroblast cells (35).

Preparation of adherent mononuclear cells. Heparinized venous blood samples were obtained from horse E390 prior to infection and on PID 4, 7, 9, 10, and 11. Similar samples were obtained from three other horses infected with Wyoming wild-type serum. Blood was mixed with colloidal particles (Sepracell) and centrifuged at $1,200 \times g$. The mononuclear cell fraction was collected, washed twice with phosphate-buffered saline (PBS), and plated in 75 cm^2 flasks in RPMI media supplemented with 10% fetal bovine serum. Cells were allowed to adhere for approximately 2 h at 37°C . Nonadherent cells were removed, and adherent cells were washed twice with PBS. Adherent cells were collected by gentle scraping and centrifuged onto poly-L-lysine-coated slides (1) with a cytocentrifuge (Shandon). One slide from each collection was immediately stained with a Giemsa-type stain (DifQuik) to assess cellular morphology. The remaining slides were fixed for 10 min in 4% paraformaldehyde, washed in PBS, and dehydrated through graded ethanol. Slides were stored at -70°C until used.

Tissue preparation. Immediately after the horses were sacrificed, representative tissue samples were collected from each horse and fixed overnight in fresh 4% paraformaldehyde. The next day, tissues were removed to 70% ethanol until paraffin embedded with routine procedures. Paraffin blocks were cut into 3- μm sections, and the sections were mounted on glass slides coated with aminopropyltriethoxysilane. Liver, spleen, kidney, lung, bone marrow, thymus, and lymph node samples were collected from horse E516. Liver, spleen, kidney, lung, bone marrow, thyroid, lymph node, adrenal, cerebrum, cerebellum, medulla, skeletal muscle, and cardiac muscle samples were collected from horse E390.

Probe preparation. An 825-bp *KpnI*-*Bgl*II fragment from the *gag* gene of a cell culture-adapted proviral clone of EIAV was cloned into the *KpnI*-*Bam*HI site of the transcription vector pGEM-4Z (Promega). The probe was prepared as previously described (7) with independent in vitro transcription reactions of *Apa*I-linearized plasmid with SP6 and T7 polymerases (Promega) (1), with α - ^{35}S -UTP. The percent incorporation of α - ^{35}S -UTP into the final probe was typically about 70 to 80% (specific activity, approximately 10^6 cpm/ μg). The SP6 polymerase reaction resulted in an RNA probe complementary to the viral message and 280 bases in length. Transcription with the T7 polymerase produced a message sense probe 545 bases in length (Fig. 1). Probe activity was

pEIA-5'=pGEM3Z(2,743bp) + EIAV1015-1840(825 bp)

FIG. 1. Map of transcription vector pEIA-5'. Plasmid is linearized with the restriction enzyme *Apa*I. Transcription with SP6 polymerase results in an RNA probe complementary to the viral message and 280 bases in length. Transcription with T7 polymerase produces a message sense probe 545 bases in length.

quantitated by counting the radioactivity of 1- μl samples in scintillation fluid with an automated beta counter (Pharmacia LKB).

In situ hybridization. In situ hybridization of cytospin samples and tissue samples was performed as previously described (1, 19), with slight modifications (7). After rehydration through ethanol, cytospin samples were denatured with 0.2 M HCl at room temperature and $2\times$ SSC at 70°C ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Cells were refixed briefly in fresh 4% paraformaldehyde and rinsed with PBS. Nonspecific binding was blocked with 0.01 M *N*-ethylmaleimide–0.01 M iodoacetamide in PBS, followed by 0.25% acetic anhydride in 0.1 M triethanolamine buffer. After dehydration through graded ethanol, each slide was hybridized with 50,000 cpm of the appropriate probe in 5 μl of hybridization mix under a siliconized coverslip. Slides were hybridized in duplicate with SP6 and T7 probes. Slides were immersed overnight in mineral oil at 45°C . Control samples included equine dermal cells infected with a cell-adapted strain of EIAV, uninfected equine dermal cells or Vero cells, and peripheral blood mononuclear cells from an uninfected, seronegative horse.

Tissue samples were deparaffinized with xylene, rehydrated through ethanol, and treated as described above, except for the addition of a denaturing step with 1 μg of proteinase K per ml in 20 mM CaCl₂–20 mM Tris-HCl. Slides were hybridized in duplicate with SP6 and T7 probes. Tissues from an uninfected, seronegative horse were hybridized as a negative control.

After hybridization, slides were rinsed in chloroform twice to remove residual mineral oil, and coverslips were floated off in $2\times$ SSC. Washing and RNase treatment to remove nonspecifically bound probe were performed as described previously (1). The high-stringency wash conditions (70°C in $0.1\times$ SSC for 10 min) help to reduce the background and are optimal only for RNA-RNA hybrids (19). It has been previously reported (19) that digestion of sections with RNase prior to hybridization with riboprobes is not a good control for probe specificity since, despite a vigorous post-RNase washing step, sufficient RNase remains tissue associated to subsequently digest the probe. Even DNA targets are undetected by specific riboprobes after prior tissue digestion by

RNase. Therefore, our best controls were uninfected versus infected tissues and the T7 probe (which could detect the minus strand of the DNA provirus) and SP6 probe (detects RNA transcripts) comparisons. Slides were dehydrated through graded ethanol and dipped in Kodak NTB-2 emulsion. They were stored in a light-tight slide box at 4°C for 4 to 6 days. After development with Kodak developer and fixer, the slides were rinsed, dried, and routinely stained with DifQuik (cytospin slides) or hematoxylin and eosin (tissue sections).

Combined immunohistochemistry and in situ hybridization. Tissue sections were deparaffinized and rehydrated as described above. Tissue macrophages were localized by using a mouse monoclonal antibody (1.646) recognizing a cytoplasmic antigen of equine mononuclear phagocytes (6). Antibody binding to tissue macrophages was detected with a biotin-labeled second antibody and a streptavidin-peroxidase conjugate, with a commercial kit (Histostain SP; Zymed Laboratories). 3,3'-Diaminobenzidine was substituted as an enzyme substrate in the final step because it is insoluble in ethanol. Diethylpyrocarbonate was added to all the steps that involved serum-containing reagents to inhibit ribonuclease activity. After incubation with 3,3'-diaminobenzidine, slides were washed in deionized, distilled water and pre-treated for in situ hybridization as described above.

RESULTS

Peripheral blood mononuclear cells. In situ hybridization of peripheral blood mononuclear cells collected at PID 4, 7, 9, 10, and 11 revealed no significant accumulation of viral RNA. Equine dermal cells infected in vitro with the Malmquist strain of EIAV were strongly positive for viral RNA by in situ hybridization. Despite the fact that nearly 100% of the cells were infected (data not shown), the amount of viral RNA detected in each cell was widely variable. Negative-control slides had only a low, nonspecific background level of silver grains (Fig. 2). Adherent peripheral blood mononuclear cells obtained from viremic horses were identified as predominantly mononuclear phagocytes by nonspecific esterase staining and by fluorescent-antibody staining with antibody 1.646 (6). Viral RNA was not detectable in these cells with in situ hybridization despite the fact that buffy-coat cells from the same horses were positive for viral DNA by the polymerase chain reaction (7, 48; unpublished data).

Table 1 shows the results that we obtained by comparing the range and mean number of silver grains overlying adherent peripheral mononuclear cells hybridized with the SP6 and T7 RNA probes. These data demonstrated that no significant difference ($P = 0.05$, pooled variance t test) between silver grain numbers could be detected in this population of cells. We were also unable to detect viral RNA in buffy-coat cells by using these same procedures, suggesting that viral RNA is not detectable in nonadherent cells as well (data not shown).

Paraffin-embedded tissue samples. The strongest positive hybridization signals were detected in the liver, spleen, and lymph node. Strong positive signals were also detected in the lung, kidney, and adrenal gland. Only rare positive cells were detected in neuronal, endocrine, and reproductive tissues and cardiac muscle. No positive cells were found in skeletal muscle. In the liver, most positive cells had elongated nuclei and were located adjacent to sinusoidal spaces (Fig. 3a). Obvious hepatocytes and endothelial cells lining blood vessels expressed no viral RNA. In the spleen, posi-

FIG. 2. In situ hybridization with EIAV-specific RNA probes. Equine dermis cells were infected in vitro with the Malmquist strain of EIAV and hybridized with the radiolabeled SP6 (A) or T7 (B) RNA probe. Adherent peripheral blood mononuclear cells from a horse infected with EIAV were collected on PID 10 and hybridized with the SP6 RNA probe (C). No viral message was detected. Uninfected equine dermal cells hybridized with the SP6 RNA probe were similar in appearance to the cells in panel B (data not shown).

tive cells were predominantly in the reticular tissue between lymph nodules (Fig. 3b). In lymph nodes, positive cells were most frequently seen in the subcapsular sinus, the reticular area between lymph nodules, and occasionally in germinal

TABLE 1. Range and mean number of silver grains overlying adherent peripheral blood mononuclear cells hybridized with the SP6 and T7 EIAV probes^a

Horse	No. of silver grains with RNA probe:			
	SP6		T7	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
E390	3.1 $\pm$ 3.5	0–21	3.6 $\pm$ 4.0	0–22
E4313	3.6 $\pm$ 3.3	0–19	3.3 $\pm$ 3.1	0–18

^a Slides prepared from peripheral blood collected from horse E390 on PID 11 and on PID 10 for E4313 were hybridized with the SP6 and T7 probes. Under oil immersion, 50 cells with typical monocyte morphology were identified on each slide, and the number of silver grains overlying each cell was recorded. By using a pooled variance *t* test (42), there was no significant difference between the mean number of silver grains overlying cells hybridized with the SP6 or T7 probe (*P* = 0.05).

centers. Distribution appeared to exclude areas that should be composed entirely of lymphocytes (Fig. 3c). In the lung, positive cells lined the alveoli. These positive cells were usually single and not found in clusters (Fig. 3d). Epithelial cells lining upper airways and endothelial cells of blood vessels expressed no viral RNA. Almost all renal glomeruli contained at least one positive cell (Fig. 3e). Rarely, cells in the interstitial area between tubules were positive, but the tubular epithelium was consistently negative. The adrenal gland expressed a surprisingly large number of positive cells, predominantly in the supporting cells of the zona glomerulosa (Fig. 3f). In other tissues, only rare positive cells were seen. These usually were cells in supporting tissue and not in specialized cells involved in the primary organ function (i.e., obvious neurons, glandular cells, and epithelia, etc., were consistently negative).

In situ hybridization of bone marrow and thymus sections was difficult to interpret because of a nonspecific binding of SP6 and T7 probes to tissue eosinophils. This binding could not be blocked by high concentrations of proteinase K solution (up to 75 μ g/ml) or by prehybridization with a nonradioactive S-UTP-containing solution. The high level of background that resulted from this nonspecific binding made it difficult to recognize specific binding to other cells. Bone marrow megakaryocytes were easily distinguishable however, and no viral transcription in these cells was demonstrated despite a profound thrombocytopenia in both horses on the day of sacrifice (7).

Combined immunohistochemistry and in situ hybridization. Liver, spleen, lymph node, and lung sections were tested by combined immunohistochemistry and in situ hybridization. Probe hybridization was decreased when the tissue sections were stained by immunoperoxidase prior to in situ hybridization. This effect was markedly lessened when serum-containing reagents used in the immunoperoxidase procedure were pretreated with diethylpyrocarbonate to inhibit ribonuclease activity. The majority of positive cells was identified by immunohistochemical staining with monoclonal antibody 1.646 as mononuclear phagocytes (Fig. 4). Not all cells positive by in situ hybridization were positive by immunoperoxidase staining, and not all cells positive by immunoperoxidase staining were simultaneously positive by in situ hybridization.

DISCUSSION

To better define the cellular and tissue locations of viral transcription during the acute stages of EIAV infection, we

used the highly sensitive technique of in situ hybridization to detect viral RNA. The distribution of viral RNA was very similar to that reported for viral DNA (39) and antigen (27), with a few exceptions. The most notable exception was the absence of detectable viral RNA in peripheral blood mononuclear cells. Both antigen and viral DNA have been identified in these cells in previous studies (34, 39), and viral DNA was detected by the polymerase chain reaction in peripheral blood mononuclear cells of the horses described here (data not shown).

The spleen, liver, and lymph nodes showed the most abundant viral activity detected (Fig. 3). In these tissues, most infected cells were identified as tissue macrophages (Fig. 4). Infected cells that did not stain with antibody 1.646 may be macrophages that do not express enough of the cytoplasmic antigen that the antibody recognizes to be identified in this assay system. Alternatively, they may represent a less-abundant secondary cell type(s) that supports viral replication in vivo. Other lentiviruses, including visna virus of sheep, caprine arthritis-encephalitis virus, and HIV-1, replicate in vivo and in vitro in cells of monocyte/macrophage lineage (14–16, 29, 33). Visna virus replication occurs almost exclusively in macrophages of the lung, lymph nodes, and spleen. Infected macrophage precursors are seen in the bone marrow (15) and may be a reservoir of latently infected cells in sheep. HIV-1 infects tissue macrophages of the central nervous system (11, 23, 44, 46), lung (8, 38), and lymph nodes (3) in vivo and human Kupffer cells in vitro (41). Although human bone marrow macrophage progenitor cells may be susceptible to infection with HIV-1 (13, 22), infected cells, if they exist at all in vivo, are present only very infrequently (10, 30, 47).

If the viral RNA detected by in situ hybridization in this study is viral message and not viral genome, evidence for which is discussed below, then the conclusion may be that EIAV replicates primarily, if not exclusively, in differentiated tissue macrophages and not in peripheral blood monocytes. This is not surprising, in light of similar evidence concerning visna virus of sheep (15, 16) and HIV-1 of humans (22, 36). Undifferentiated monocytes are permissive for these viruses, but viral replication appears restricted. When monocyte differentiation is induced by an interferon activity, visna virus gene expression is increased (16). Similarly, when the human monocytic cell line U937 is infected with HIV-1 and induced to differentiate by phorbol ester, high levels of viral RNA accumulate in the cell cytoplasm (36). Differentiation between viral genomic RNA and mRNA transcripts is difficult by using in situ hybridization. Therefore, silver grains overlying cells of monocyte/macrophage lineage may result from phagocytosed virus rather than from active infection and viral mRNA transcription. Several facts argue against this theory: (i) distribution of viral DNA (39) and antigen (27) in infected tissues closely parallels the distribution of viral RNA described in this study, implying active infection; (ii) in peripheral blood mononuclear cells, no viral RNA was detected despite the presence of viral cDNA; and (iii) tissues in which only immune complex deposition would be expected (e.g., erythrocytes in the splenic pulp) were routinely negative by in situ hybridization.

Together, this evidence suggests that viral RNA detected by in situ hybridization could be the result of active viral transcription. However, we cannot definitively rule out the possibility that some in situ-positive cells contain multiple copies of input viral RNA from infecting virus particles. High levels of viral RNA transcription in a cell do not

FIG. 3. In situ hybridization of paraffin-embedded tissue sections collected on PID 10 from a horse infected with EIAV. Hybridization with the EIAV-specific SP6 RNA probe revealed that the greatest amount of viral mRNA was present in the liver (A), spleen (B), and lymph nodes (C). The lung (D), kidney (E), and adrenal gland (F) tissues also contained viral message. When tissue sections from an infected horse were hybridized with the T7 RNA probe or when tissue sections from an uninfected horse were hybridized with the SP6 RNA probe, only nonspecific background silver grains were detected (data not shown).

necessarily equate to production of infectious virion particles. In situ hybridization studies and infectious particle assays during visna virus infection of sheep show that only a very low number of infectious particles are produced in vivo, despite high levels of virus transcription (15, 16). Similarly, U937 cells infected with HIV-1 and induced to differentiate accumulate high levels of cytoplasmic viral RNA but fail to release infectious particles (36). Such a posttranscriptional

block does not appear to exist in horses acutely infected with EIAV. The high titer of infectivity (6×10^6 to 8×10^6 /ml) of blood from an acutely infected, febrile horse (20) implies that active transcription results in the production of large numbers of infectious virions. The serum reverse transcriptase activity of the two horses used in this study increased with increasing temperature (7). This increase in reverse transcriptase activity correlated with the level of hybridization

FIG. 4. Combined immunohistochemistry and in situ hybridization of tissue sections collected on PID 10 from a horse infected with EIAV. Sections were stained with monoclonal antibody 1.646 recognizing a cytoplasmic antigen of equine mononuclear phagocytes and hybridized with the EIAV-specific SP6 RNA probe. Liver (A), spleen (B), lymph node (C), and lung (D) macrophages expressed viral mRNA. Specific hybridization was not detected when tissue sections were hybridized with the T7 RNA probe (data not shown).

signal identified in the tissue macrophages of each horse. Horse E516 had very dense silver grains overlying tissue macrophages, and a peak serum reverse transcriptase activity of 6,164 cpm on the day of sacrifice. Conversely, horse E390 had many fewer silver grains overlying hepatic and splenic macrophages, and a peak reverse transcriptase activity on the day of sacrifice of only 267 cpm (7).

During acute infection with EIAV, the virus replicates to high titer in mature tissue macrophages, primarily in the liver, spleen, and lymph nodes. A lesser degree of viral replication occurs in macrophages of the kidney, lung, and adrenal gland. Only rare cells can be demonstrated to produce viral RNA in neuronal tissues, thyroid gland, female reproductive organs, or muscle. No viral mRNA can be demonstrated in circulating monocytes of acutely infected horses, despite the presence of a strong positive polymerase chain reaction signal for viral cDNA in these cells (48). Although circulating monocytes are susceptible to infection in vivo, we suggest that detectable levels of viral transcription do not occur until cells differentiate into mature tissue macrophages. Unlike other lentivirus infections in which mature tissue macrophages accumulate cytoplasmic viral RNA to a high level but fail to produce infectious virions, mature tissue macrophages are the primary source of the high titer of cell-free viremia present during acute infection with EIAV. If other cell types are concurrently infected,

their number is small, and they do not appear to contribute markedly to virion production during acute infection.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant 1K11-AI00963 (D.C.S.), grant R01-AI24904 from the National Institutes of Health, and grant 91-37204-6732 from the Department of Agriculture.

We thank Michael Jones for excellent technical assistance in these studies and Barbara Sherry for a critical reading of the manuscript. We extend special thanks to Allison Jilbert for useful discussions concerning the in situ hybridization procedures.

REFERENCES

1. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.). 1989. Current protocols in molecular biology. John Wiley and Sons, New York.
2. Banks, K. L., J. B. Henson, and T. C. McGuire. 1972. Immunologically mediated glomerulitis of horses. I. Pathogenesis in persistent infection by equine infectious anemia virus. *Lab. Invest.* 26:701-707.
3. Baroni, C. D., F. Pezzella, M. Pezzella, B. Macchi, D. Vitolo, S. Uccini, and L. P. Ruco. 1988. Expression of HIV in lymph node cells of LAS patients. *Immunohistology, in situ hybridization, and identification of target cells.* *Am. J. Pathol.* 133:498-506.
4. Carpenter, S., and B. Chesebro. 1989. Change in host cell

- tropism associated with *in vitro* replication of equine infectious anemia virus. *J. Virol.* **63**:2903-2915.
5. Casey, J. M., Y. Kim, P. R. Andersen, K. F. Watson, J. L. Fox, and S. G. Devare. 1985. Human T-cell lymphotropic virus type III: immunologic characterization and primary structure analysis of the major internal protein, p24. *J. Virol.* **55**:417-423.
 6. Clabough, D. L., J. Cullen, L. Whetter, D. Gebhard, L. Coggins, and F. J. Fuller. Production and characterization of a monoclonal antibody recognizing a cytoplasmic antigen of equine mononuclear phagocytes. Submitted for publication.
 7. Clabough, D. L., D. Gebhard, M. T. Flaherty, L. E. Whetter, S. T. Perry, L. Coggins, and F. J. Fuller. 1991. Immune-mediated thrombocytopenia in horses infected with equine infectious anemia virus. *J. Virol.* **65**:6242-6251.
 8. Clarke, J. R., V. Krishnan, J. Bennett, D. Mitchell, and D. J. Jeffries. 1990. Detection of HIV-1 in human lung macrophages using the polymerase chain reaction. *AIDS* **4**:1133-1136.
 9. Coggins, L., N. L. Norcross, and S. R. Nusbaum. 1972. Diagnosis of equine infectious anemia by immunodiffusion test. *Am. J. Vet. Res.* **33**:11-18.
 10. Davis, B. R., D. H. Schwartz, J. C. Marx, C. E. Johnson, J. M. Berry, J. Lyding, T. C. Merigan, and A. Zander. 1991. Absent or rare human immunodeficiency virus infection of bone marrow stem/progenitor cells *in vivo*. *J. Virol.* **65**:1985-1990.
 11. Eilbott, D. J., N. Peress, H. Burger, D. LaNeve, J. Orenstein, H. E. Gendelman, R. Seidman, and B. Weiser. 1989. Human immunodeficiency virus type 1 in spinal cords of acquired immunodeficiency syndrome patients with myelopathy: expression and replication in macrophages. *Proc. Natl. Acad. Sci. USA* **86**:3337-3341.
 12. Evans, K. S., S. L. Carpenter, and M. Sevoian. 1984. Detection of equine infectious anemia virus in horse leukocyte cultures derived from horses in various stages of equine infectious anemia viral infection. *Am. J. Vet. Res.* **45**:20-25.
 13. Folks, T. M., S. W. Kessler, J. M. Orenstein, J. S. Justement, E. S. Jaffe, and A. S. Fauci. 1988. Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow. *Science* **242**:919.
 14. Gartner, S., P. Markovits, D. M. Markovits, M. H. Kaplan, R. C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**:215-219.
 15. Gendelman, H. E., O. Narayan, S. Molineaux, and J. E. Clements. 1985. Slow, persistent replication of lentiviruses: role of tissue macrophages and macrophage precursors in bone marrow. *Proc. Natl. Acad. Sci. USA* **82**:7086-7090.
 16. Gendelman, H. E., O. Narayan, S. Kennedy-Stoskopf, P. G. E. Kennedy, Z. Ghotbi, J. E. Clements, J. Stanley, and G. Pezeshkpour. 1986. Tropism of sheep lentiviruses for monocytes: susceptibility to infection and virus gene expression increase during maturation of monocytes to macrophages. *J. Virol.* **58**:67-74.
 17. Gonda, M. A., M. J. Braun, J. E. Clements, J. M. Pyper, F. Wong-Staal, R. C. Gallo, and R. V. Gilden. Human T-cell lymphotropic virus type III shares sequence homology with a family of pathogenic lentiviruses. *Proc. Natl. Acad. Sci. USA* **83**:4007-4011.
 18. Gonda, M. A., H. P. Charman, J. L. Walker, and L. Coggins. 1978. Scanning and electron microscopic study of equine infectious anemia virus. *Am. J. Vet. Res.* **39**:731-740.
 19. Gowans, E. J., A. R. Jilbert, and C. J. Burrell. 1989. Detection of specific DNA and RNA sequences in tissues and cells by *in situ* hybridization, p. 139-158. *In* R. H. Symons (ed.), *Nucleic acid probes*. CRC Press, Inc., Boca Raton, Fla.
 20. Issel, C. J., and L. Coggins. 1979. Equine infectious anemia: current knowledge. *J. Am. Vet. Med. Assoc.* **174**:727-733.
 21. Kemeny, L. J., L. O. Mott, and J. E. Pearson. 1971. Titration of equine infectious anemia virus. Effect of dosage on incubation time and clinical signs. *Cornell Vet.* **61**:6878-6895.
 22. Kitano, K., C. C. Abboud, D. H. Ryan, S. G. Quan, G. C. Baldwin, and D. W. Golde. 1991. Macrophage-active colony-stimulating factors enhance human immunodeficiency virus type 1 infection in bone marrow stem cells. *Blood* **77**:1699.
 23. Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* **233**:1089-1093.
 24. Kono, Y. 1969. Viremia and immunological responses in horses infected with equine infectious anemia virus. *Natl. Inst. Anim. Health Q. (Tokyo)* **9**:1-9.
 25. Kono, Y. 1973. Recurrences of equine infectious anemia, p. 175-186. *In* Proceedings of the 3rd International Conference on Equine Infectious Diseases. Karger, Basel.
 26. McConnell, M. B., M. Katada, S. McConnell, and R. Moore. 1977. Demonstration of equine infectious anemia virus in primary leukocyte cultures by electron microscopy. *Am. J. Vet. Res.* **38**:2067-2069.
 27. McGuire, T. C., T. B. Crawford, and J. B. Henson. 1971. Immunofluorescent localization of equine infectious anemia virus in tissue. *Am. J. Pathol.* **62**:283-292.
 28. McGuire, T. C., T. B. Crawford, and J. B. Henson. 1972. Equine infectious anemia: detection of infectious virus-antibody complexes in the serum. *Immunol. Commun.* **1**:545-551.
 29. Meltzer, M. S., D. S. Skillman, P. J. Gomas, D. C. Kalter, and H. E. Gendelman. 1990. Role of mononuclear phagocytes in the pathogenesis of human immunodeficiency virus infection. *Annu. Rev. Immunol.* **8**:169-174.
 30. Molina, J. M., S. T. Scadden, M. Sakaguchi, B. Fuller, A. Woon, and J. E. Groopman. 1990. Lack of evidence for infection of or effect on growth of hematopoietic progenitor cells after *in vivo* or *in vitro* exposure to human immunodeficiency virus. *Blood* **76**:2476.
 31. Montagnier, L., C. Daugey, C. Axler, S. Chamaret, J. Gruet, M. T. Nugeyre, F. Rey, F. Barre-Sinoussi, and J. C. Chermann. 1984. A new type of retrovirus isolated from patients presented with lymphadenopathy and acquired immune deficiency syndrome: structural and antigenic relatedness with equine infectious anemia virus. *Ann. Virol.* **135E**:119-134.
 32. Montelaro, R. C., B. Parekh, A. Orrego, and C. J. Issel. 1984. Antigenic variation during persistent infection by equine infectious anemia virus, a retrovirus. *J. Biol. Chem.* **259**:10539-10544.
 33. Narayan, O., S. Kennedy-Stoskopf, D. Sheffer, D. E. Griffin, and J. E. Clements. 1983. Activation of caprine arthritis-encephalitis virus expression during maturation of monocytes to macrophages. *Infect. Immun.* **41**:67-73.
 34. O'Rourke, K. I., M. L. Besola, and T. C. McGuire. 1991. Proviral sequences detected by polymerase chain reaction in peripheral blood cells of horses with equine infectious anemia lentivirus. *Arch. Virol.* **117**:109-119.
 35. Orrego, A., C. J. Issel, R. C. Montelaro, and W. V. Adams. 1982. Virulence and *in vitro* growth of a cell-adapted strain of equine infectious anemia virus after serial passage in ponies. *Am. J. Vet. Res.* **43**:1556-1560.
 36. Pauza, C. D., J. Galindo, and D. D. Richman. 1988. Human immunodeficiency virus infection of monoblastoid cells: cellular differentiation determines the pattern of virus replication. *J. Virol.* **62**:3558-3564.
 37. Payne, S. L., O. Salinovich, S. M. Nauman, C. J. Issel, and R. C. Montelaro. 1987. Course and extent of variation of equine infectious anemia virus during parallel persistent infections. *J. Virol.* **61**:1266-1270.
 38. Plata, H., F. Garcia-Pons, A. Ryter, F. Lebarry, M. M. Goode, M. H. Q. Dat, B. Autran, and C. Mayaud. 1990. HIV-1 infection of lung alveolar fibroblasts and macrophages in humans. *AIDS Res. Hum. Retroviruses* **8**:979-986.
 39. Rice, N. R., A. S. Lequarre, J. W. Casey, S. Lahn, R. M. Stephens, and J. Edwards. 1989. Viral DNA in horses infected with equine infectious anemia virus. *J. Virol.* **63**:5194-5200.
 40. Salinovich, O., S. L. Payne, R. C. Montelaro, K. A. Hussain, C. J. Issel, and K. L. Schnorr. 1986. Rapid emergence of novel antigenic and genetic variants of equine infectious anemia virus during persistent infection. *J. Virol.* **57**:71-80.
 41. Schmitt, M. P., J. L. Gendral, C. Schweitzer, A. M. Steffan, C. Beyer, C. Royer, D. Jaeck, J. L. Pasquali, A. Kirn, and A. M. Aubertin. 1990. Permissivity of primary cultures of human Kupffer cells for HIV-1. *AIDS Res. Hum. Retroviruses* **6**:987-991.

42. **Shott, S.** 1990. Statistics for health professionals. The W. B. Saunders Co., Philadelphia.
43. **Stephens, R. M., J. W. Casey, and N. R. Rice.** 1986. Equine infectious anemia virus gag and pol genes: relatedness to visna and AIDS virus. *Science* **231**:589-594.
44. **Stoler, M. H., T. A. Eskin, S. Benn, R. C. Angerer, and L. M. Angerer.** 1986. Human T-cell lymphotropic virus type III infection of the central nervous system. A preliminary *in situ* analysis. *J. Am. Med. Assoc.* **256**:2360-2364.
45. **Vallee, H., and H. Carre.** 1904. Sur la nature infectieuse de l'anémie du cheval. *C.R. Acad. Sci.* **139**:331-333.
46. **Vazeux, R., N. Brousse, A. Jarry, D. Henin, C. Marche, C. Vedrenne, J. Mikol, M. Wolff, C. Michon, W. Rozenbaum, J. F. Bureau, L. Montagnier, and M. Brahic.** 1987. AIDS subacute encephalitis. Identification of HIV-infected cells. *Am. J. Pathol.* **126**:403-410.
47. **Von Laer, D., F. T. Hufert, T. E. Fenner, S. Schwander, M. Dietrich, H. Schmitz, and P. Kern.** 1990. CD34+ hematopoietic progenitor cells are not a major reservoir of the human immunodeficiency virus. *Blood* **76**:1281.
48. **Whetter, L., D. Archambault, S. Perry, A. Gazit, L. Coggins, A. Yaniv, D. Clabough, J. Dahlberg, F. Fuller, and S. Tronick.** 1990. Equine infectious anemia virus derived from a molecular clone persistently infects horses. *J. Virol.* **64**:5750-5756.