Journal of General Virology (1990), **71**, 739–743. *Printed in Great Britain*

Intracellular proteins of feline immunodeficiency virus and their antigenic relationship with equine infectious anaemia virus proteins

Herman F. Egberink,[1] Joke Ederveen,[1] Ronald C. Montelaro,[2] Niels C. Pedersen,[3] Marian C. Horzinek[1] and Marck J. M. Koolen[1]*

[1] *Department of Virology, Institute of Infectious Diseases and Immunology, Veterinary Faculty, State University, Yalelaan 1, De Uithof, 3584 CL Utrecht, The Netherlands,* [2] *Department of Biochemistry, Louisiana State University, Baton Rouge, Louisiana 70803 and* [3] *School of Veterinary Medicine, University of California, Davis, California 95616, U.S.A.*

Feline immunodeficiency virus (FIV) grown in cat lymphocyte and thymocyte cultures was labelled with L-[^{35}S]methionine or [^{3}H]glucosamine and virus-coded proteins were identified using immunoprecipitation. Polypeptides with apparent M_r values of 15K, 24K, 43K, 50K, 120K and 160K were detected. An additional polypeptide of 10K was detected by Western blot analysis. The two highest M_r species sometimes appeared as one band, of which only the 120K polypeptide was glycosylated. In the presence of tunicamycin gp120 was no longer detectable and a non-glycosylated precursor of 75K was found instead. Pulse–chase experiments suggested that the smaller polypeptides p24 and p15 are cleavage products of both p160 and p50. Western blot analysis using a rabbit serum directed against p26 of equine infectious anaemia virus (EIAV) and an anti-EIAV horse serum from a field case of infection revealed a cross-reactivity with p24 of FIV. Cat sera collected late after experimental FIV infection recognized p26 of EIAV, indicating a reciprocal cross-reactivity.

Recently a lentivirus infection was described as the cause of an immunodeficiency-like syndrome in cats (Pedersen *et al.*, 1987). The virus can be isolated in lymphocyte cultures from viraemic cats, where it induces syncytia. It contains a reverse transcriptase (RT) with a divalent magnesium cation preference and is morphologically similar to the human immunodeficiency virus (HIV), the lentivirus that causes AIDS in man. Based on its physical, biochemical and morphological characteristics the cat virus was named feline T-lymphotropic lentivirus and is now generally referred to as feline immunodeficiency virus (FIV), although the disease is frequently quoted as 'feline AIDS'.

Well studied members of the animal lentivirus subfamily are equine infectious anaemia virus (EIAV), visna virus (VV) and caprine arthritis–encephalitis virus (CAEV) and sequence homologies between the *gag* and *pol* genes of these viruses and HIV have been reported (Stephens *et al.*, 1986). In addition, a non-reciprocal antigenic cross-reactivity was observed between the *gag* gene products p24 of HIV and p26 of EIAV. No cross-reactivity was detected between VV or CAEV and EIAV or HIV, respectively (Goudsmit *et al.*, 1986). In a recent study no antigenic relationships between FIV and established lentiviruses HIV-1, HIV-2, simian immunodeficiency virus (SIV), CAEV and VV were detected when antibodies from naturally infected hosts were used (Yamamoto *et al.*, 1988). However, in a later study rabbit sera to CAEV and VV were found to react with the putative core protein of FIV (Olmsted *et al.*, 1989). In the present paper, we report on the identification of the intracellular proteins of FIV grown in lymphocyte cultures and the reciprocal heterologous recognition of the major core proteins between FIV and EIAV.

FIV was propagated in thymocytes or peripheral blood lymphocytes from specific pathogen-free cats; the cells were stimulated with concanavalin A (5 μg/ml) and recombinant human interleukin-2 (100 units/ml), as previously described (Pedersen *et al.*, 1987). Radioactive label was added to thymocyte and lymphocyte cultures at 5 and 8 days, respectively, when RT activity was high (1×10^6 to $1{\cdot}5 \times 10^6$ c.p.m./ml). About 2×10^6 viable cells were pelleted, washed once in phosphate-buffered saline (PBS) and resuspended in 1 ml methionine-deficient minimal essential medium (MEM) supplemented with 2% foetal calf serum. After 1 h, 60 μCi of L-[^{35}S]methionine was added. The cells were incubated overnight (16 h), pelleted and lysed (Rottier *et al.*, 1981).

0000-9136 © 1990 SGM

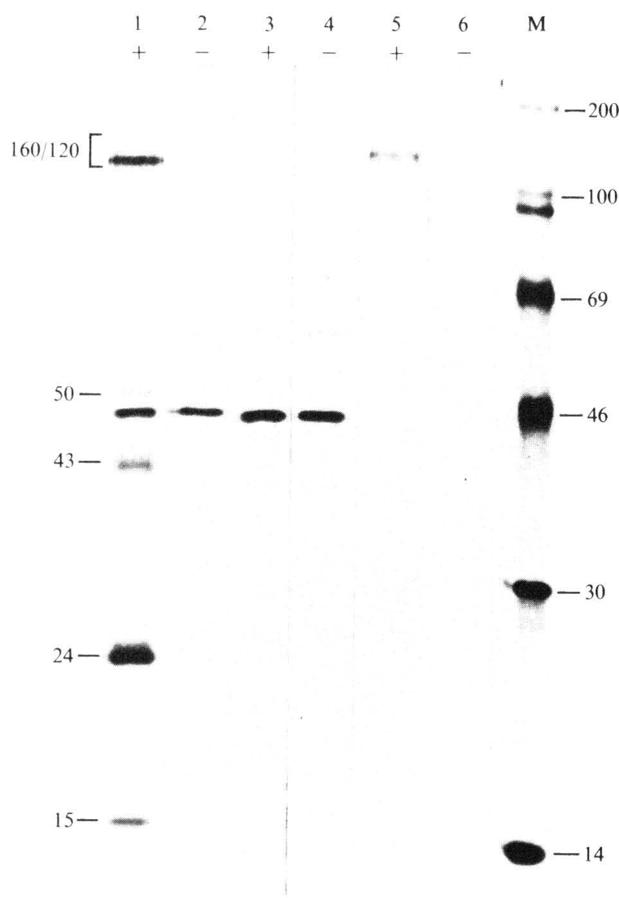

Fig. 1. Electrophoretic analysis of proteins in FIV-infected (lanes 1, 2, 5 and 6) and mock-infected (lanes 3 and 4) lymphocytes labelled with [^{35}S]methionine (lanes 1 to 4) and [^{3}H]glucosamine (lanes 5 and 6) after immunoprecipitation using pre- (−) and post-infection (+) sera. $M_r \times 10^{-3}$ of the virus-specific proteins (on the left) and the ^{14}C-labelled marker proteins (lane M) are shown. Analysis was done in a 15% SDS–polyacrylamide gel.

For glycoprotein labelling, cells were incubated in MEM containing 1/10 the standard amount of glucose, 2% foetal calf serum and [^{3}H]glucosamine hydrochloride (34·6 Ci/mmol).

Lysates of labelled infected cells were incubated with sera from cats before and after experimental infection with FIV. Preparations containing about 200000 c.p.m. L-[^{35}S]methionine (10 µl) and 300000 c.p.m. [^{3}H]glucosamine were diluted with 300 µl lysis buffer and mixed with 5 µl undiluted serum. Immune complexes were allowed to form overnight at 4 °C and subsequently precipitated with formaldehyde-fixed *Staphylococcus aureus* cells (Pansorbin, Calbiochem) for 45 min at 4 °C. The precipitates were washed three times and finally resuspended in Laemmli sample buffer as previously described (Rottier *et al.*, 1981). The proteins were analysed by SDS–PAGE and visualized by fluorography (Laskey & Mills, 1975).

As shown in Fig. 1, the serum from an FIV-infected cat recognized proteins with apparent M_r values of 15K, 24K, 43K, 50K, 120K, and 160K in lymphocyte lysates (lane 1), and these are therefore considered as virus-specific. In contrast to p120 the 160K polypeptide was not always detectable. The 120K protein can be labelled with [^{3}H]glucosamine (lane 5). When FIV-infected lymphocytes were grown in the presence of tunicamycin, an inhibitor of *N*-linked glycosylation, gp120 was absent and a new polypeptide of about 75K appeared in the gel (Fig. 2); these data suggest that p75 is the unglycosylated precursor of gp120.

The 15K and 24K proteins probably represent core proteins with their 50K precursor (*gag* gene products) and evidence for this assumption came from pulse–chase experiments. Cells were pulse-labelled with L-[^{35}S]methionine for 30 min, washed with PBS, incubated further at 37 °C in RPMI medium containing 10% foetal calf serum and twice the amount of methionine, and chased for various periods of time. During a 0·5 to 4 h chase period the intensity of the 50K band decreased, whereas the intensity of both the 24K and 15K bands increased (Fig. 3). In HIV a highly glycosylated precursor protein (gp160) is processed to yield an outer membrane protein of 120K and a transmembrane protein of 41K (Veronese *et al.*, 1985). In the pulse–chase experiment the 160K band was only faintly visible during the first 30 min of the chase period and specific processing products were not detected. We therefore assume that the p160 polypeptide is a translational readthrough product of the *gag–pol* gene (Jacks *et al.*, 1988). Preliminary results obtained with monoclonal antibodies directed against the 50K and 24K proteins indicated that the p160 polypeptide coprecipitates in radioimmunoprecipitation assays. The decrease in intensity of gp120 during the chase may be due to release of the protein from the cells.

The 43K species is considered to be the transmembrane protein (*env* gene product). In contrast to the gp41 protein of HIV, the 43K protein of FIV in lymphocytes could not be labelled with [^{3}H]glucosamine. Recently, Talbott *et al.* (1989) characterized the molecular structure of FIV. Three open reading frames, corresponding to the *gag*, *pol* and *env* gene coding frames, are evident. The M_r of the FIV-specific proteins described here are in agreement with the predicted polypeptides encoded by the open reading frames.

Our search for an antigenic relationship between the FIV-specific proteins and those of established lentiviruses focused on EIAV, which had not been included in an earlier comparative study (Yamamoto *et al.*, 1988). When using a rabbit immune serum directed against the p26 of EIAV (Hussain *et al.*, 1988) a weak reaction was noticed in immunofluorescence assays on FIV-infected Crandell feline kidney cells. Therefore, Western blot

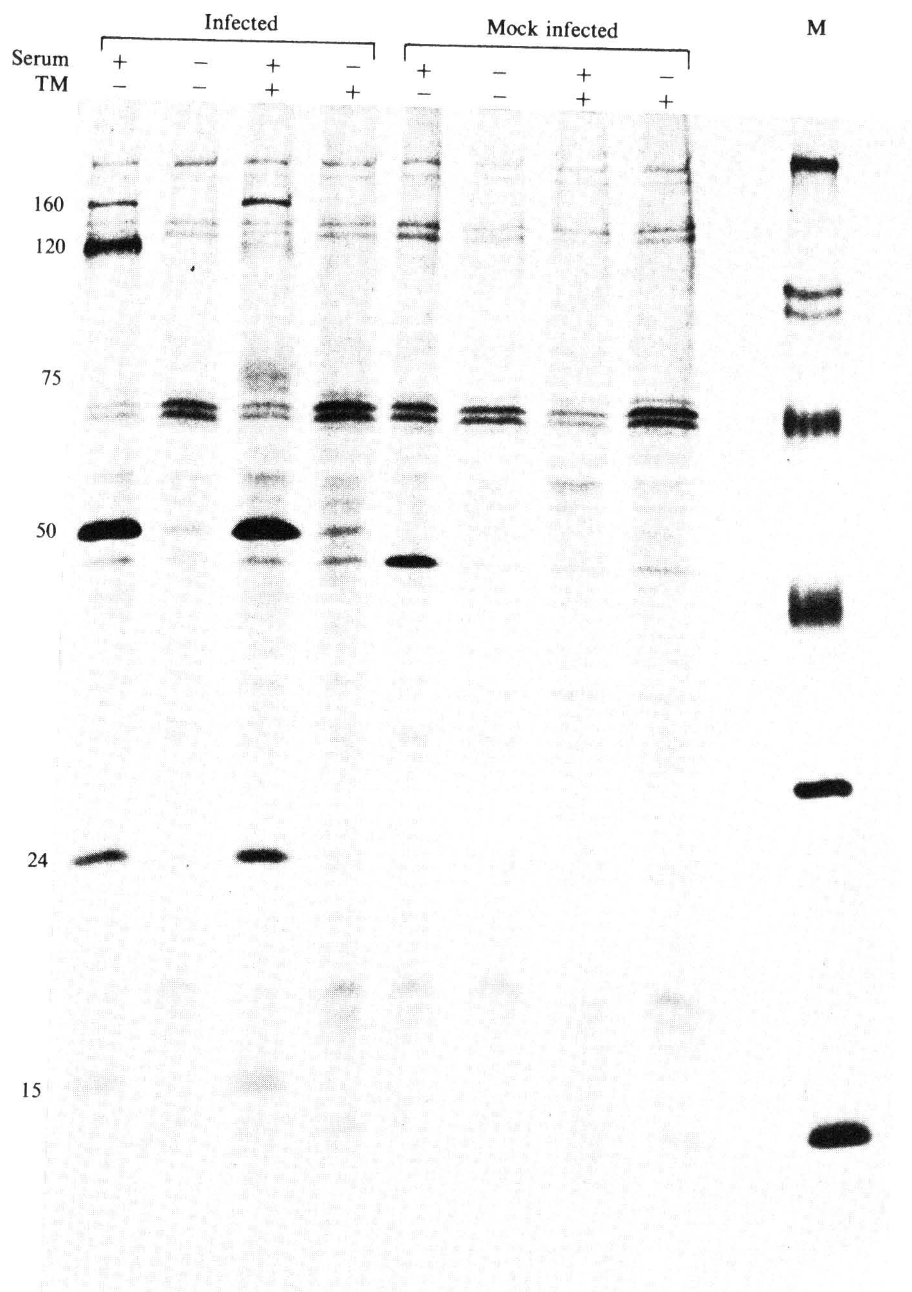

Fig. 2. Effect of tunicamycin on virus-specific intracellular glycoproteins. FIV-infected and mock-infected lymphocytes, 5 days post-infection, were labelled with [^{35}S]methionine and [^{35}S]cysteine for 3 h in the absence (−) or in the presence (+) of tunicamycin (TM) (20 μg/ml). The intracellular viral proteins were precipitated using FIV-positive and -negative cat sera and analysed in a 15% SDS–polyacrylamide gel. $M_r \times 10^{-3}$ of the virus-specific proteins are shown (left). Lane M contains ^{14}C-labelled marker proteins.

ELISAs were performed to examine the reaction of homologous and heterologous sera with the proteins of gradient-purified EIAV and FIV. Electrophoresis and transfer of the proteins to nitrocellulose filters was performed as previously described (Zhou *et al.*, 1988). The nitrocellulose filters were blocked with PBS containing 0·5% pig skin gelatin and 0·1% Triton X-100 (blot buffer), The antisera and the horseradish peroxidase-conjugated anti-IgG were diluted in blot buffer and incubations were performed for 1 h. The protein bands were visualized using 4-chloro-1-naphthol as a chromogen. As shown in Fig. 4, the anti-FIV cat serum recognized the 24K and 15K proteins and an additional minor protein of about 10K. The latter protein, presumably another cleavage product of the *gag* gene product precursor was not detectable in L-[^{35}S]methionine-

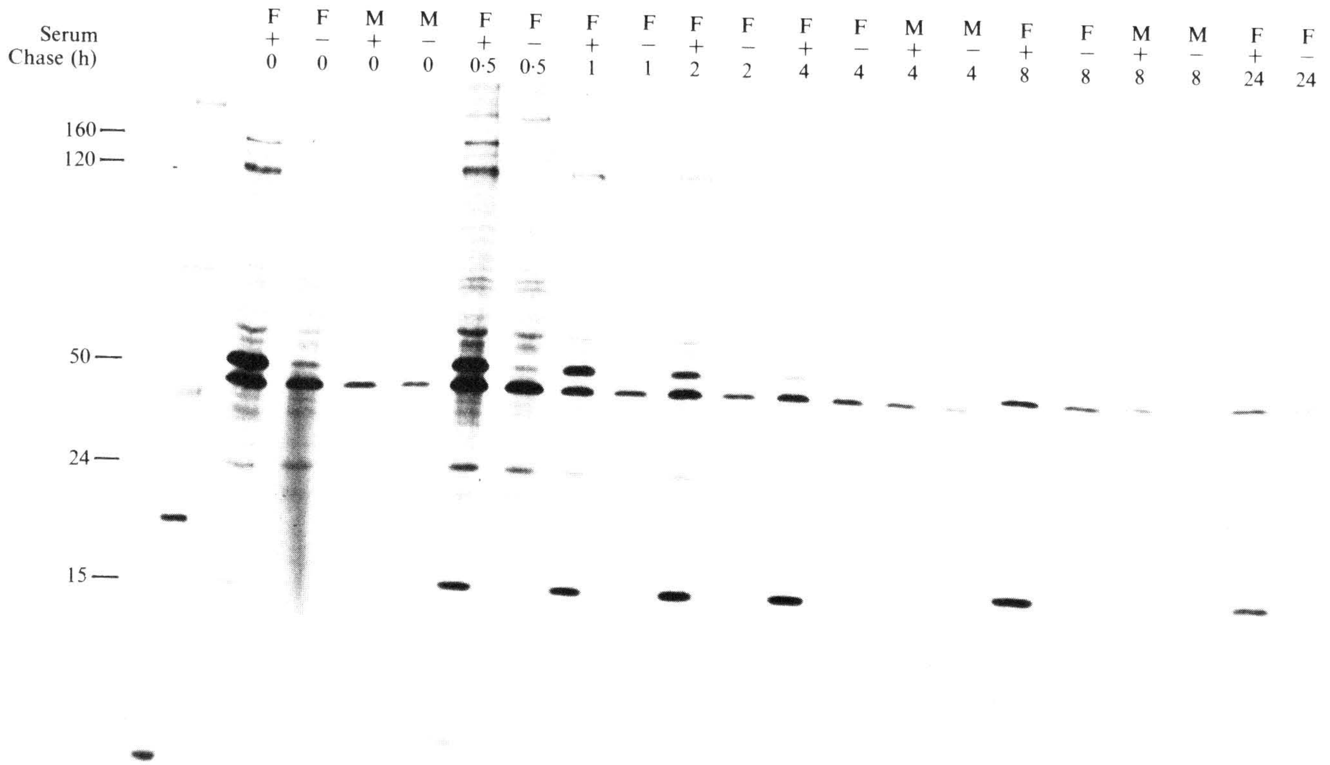

Fig. 3. Pulse–chase labelling of intracellular FIV-specific proteins. FIV-infected (F) and mock-infected (M) cat thymocytes were pulse-labelled with [^{35}S]methionine for 30 min and chased for various times. The cell lysates were immunoprecipitated with anti-FIV-positive (+) and -negative (−) cat sera. The samples were analysed in a 15% polyacrylamide gel.

labelled cell lysates of FIV-infected lymphocytes (Fig. 1). The high M_r proteins were not visualized, probably due to loss of the knobs from the virion membrane during purification and their poor transfer during blotting (Saah *et al.*, 1987). The EIAV-specific proteins were recognized by homologous horse serum. Both the rabbit anti-p26 EIAV and the cat anti-FIV serum recognized the p26 of EIAV. Also, the rabbit and horse anti-EIAV sera recognized the p24 of FIV, which indicates a two-way cross-reactivity. When sera from serial bleedings of cats experimentally infected with FIV were used in immunoblotting it appeared that recognition of p26 of EIAV occurred only after 14 weeks p.i., whereas the homologous reaction was visible from 6 weeks p.i. onward. These observations suggest that the epitopes conserved between FIV and EIAV are not immunodominant and prolonged exposure to the immune system is required for antibodies to develop. The rabbit serum recognized some additional proteins in both the FIV- and EIAV-containing lanes, the specificity of which is presently under study.

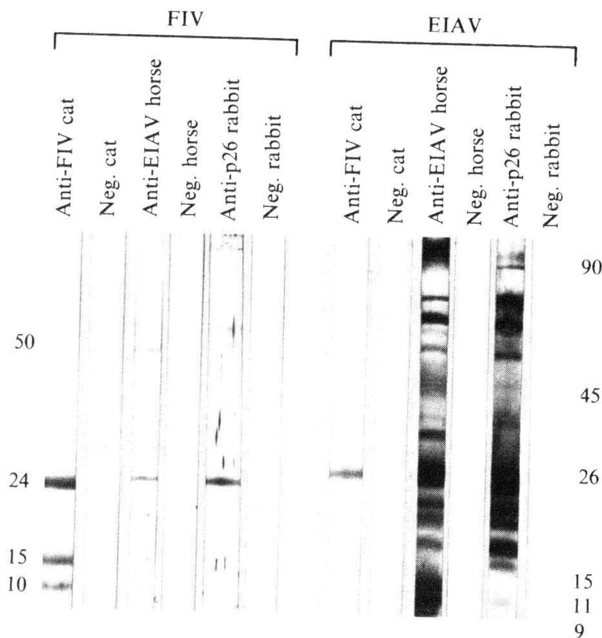

Fig. 4. Antigenic relationship of purified FIV and EIAV virus-specific proteins, as determined by Western blot analysis. Strips were incubated with pre- (−) and post-infection (+) serum of an FIV-infected cat, a horse anti-EIAV and a normal horse serum, a polyclonal rabbit serum directed against p26 of EIAV and a negative rabbit serum. The $M_r \times 10^{-3}$ of FIV-specific proteins are indicated on the left side and of EIAV on the right side.

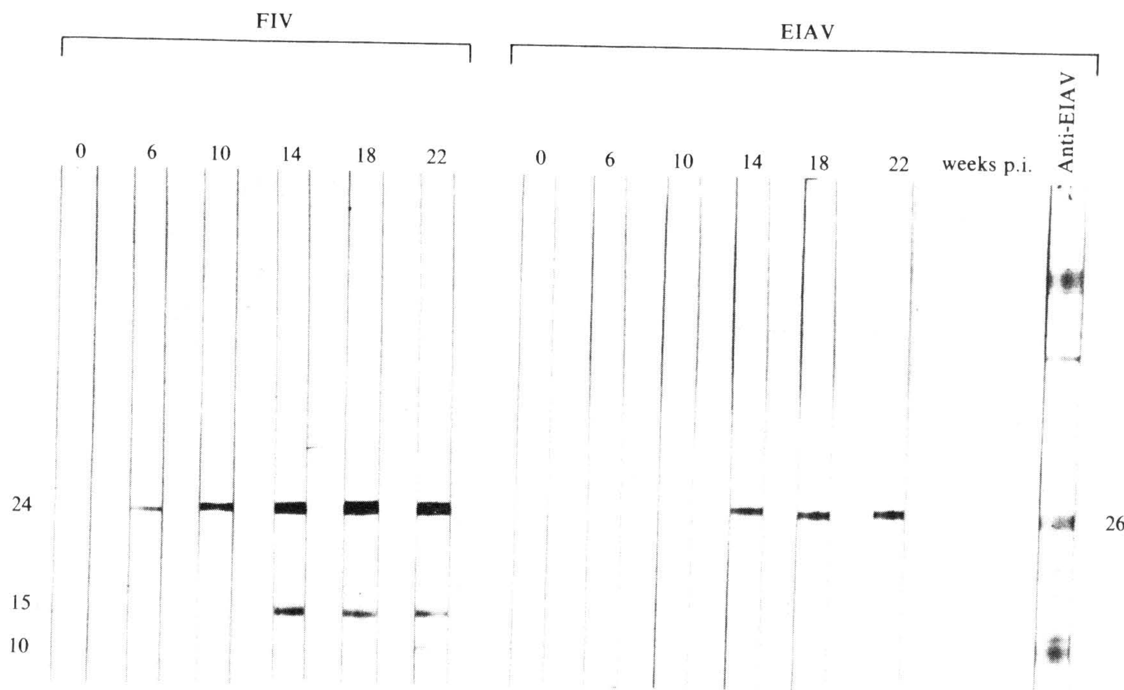

Fig. 5. Western blot analysis of purified FIV and EIAV. Strips were incubated with cat sera collected at different weeks after experimental FIV infection. The reaction of an anti-EIAV horse serum is also shown (lane on the right).

Based upon the serological cross-reactivity at the *gag* gene protein level it was proposed to distinguish two groups within the *Lentivirinae* subfamily, one group including VV, CAEV and pleuropneumonia virus of sheep, and the second group comprising HIV, SIV and EIAV (Goudsmit *et al.*, 1986). Based on our results FIV should be included in the second group. Also, the major core antigen of the bovine immunodeficiency virus (Van der Maaten *et al.*, 1972) is recognized by the rabbit anti-EIAV p26 serum (M. J. M. Koolen, unpublished observations). The FIV–EIAV relationship reported above has been established independently in another laboratory (Steinman *et al.*, 1990).

References

GOUDSMIT, J., HOUWERS, D. J., SMIT, L. & NAUTA, I. M. (1986). LAV/HTLV III *gag* gene product p24 shares antigenic determinants with equine anaemia virus but not with visna virus or caprine arthritis encephalitis virus. *Intervirology* **26**, 169–173.

HUSSAIN, K. A., ISSEL, C. J., RWAMBO, P. M., ARNIZAUT, A. B., BALL, J. M., SCHNORR, K. L. & MONTELARO, R. C. (1988). Identification of *gag* precursor of equine infectious anaemia virus with monoclonal antibodies to the major viral core protein, p26. *Journal of General Virology* **69**, 1719–1724.

JACKS, T., POWER, M. D., MASIARZ, F. R., LUCIW, P. A., BARR, P. J. & VARMUS, H. E. (1988). Characterization of ribosomal frameshifting in HIV-1 *gag-pol* expression. *Nature, London* **331**, 280–283.

LASKEY, R. A. & MILLS, A. D. (1975). Quantitative detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. *European Journal of Biochemistry* **56**, 335–341.

OLMSTED, R. A., BARNES, A. K., YAMAMOTO, J. K., HIRSCH, V. M., PURCELL, R. H. & JOHNSON, P. R. (1989). Molecular cloning of feline immunodeficiency virus. *Proceedings of the National Academy of Sciences, U.S.A.* **86**, 2448–2452.

PEDERSEN, N. C., HO, E. W., BROWN, M. L. & YAMAMOTO, J. K. (1987). Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* **235**, 790–793.

ROTTIER, P. J. M., SPAAN, W. J. M., HORZINEK, M. C. & VAN DER ZEIJST, B. A. M. (1981). Translation of three mouse hepatitis virus strain A subgenomic RNA's in *Xenopus laevis* oocytes. *Journal of Virology* **38**, 20–26.

SAAH, A. J., FARZADEGAN, H., FOX, R., NISHANIAN, P., RINALDO, C. R., PHAIR, J. P., FAHEY, J. L., LEE, T.-H., POLK, B. P. AND THE MULTICENTER AIDS COHORT STUDY (1987). Detection of early antibodies in human immunodeficiency virus infection by enzyme-linked immunosorbent assay, Western blot, and radioimmunoprecipitation. *Journal of Clinical Microbiology* **25**, 1605–1610.

STEINMAN, R., DOMBROWSKI, J., O'CONNOR, T., MONTELARO, R. C., TONELLI, Q., LAWRENCE, K., SEYMOUR, C., GOODNESS, J., PEDERSEN, N. & ANDERSEN, P. R. (1990). Biochemical and immunological characterization of the major structural proteins of feline immunodeficiency virus. *Journal of General Virology* **71**, 701–706.

STEPHENS, R. M., CASEY, J. W. & RICE, N. R. (1986). Equine infectious anemia virus *gag* and *pol* genes: relatedness to visna and AIDS virus. *Science* **231**, 589–594.

TALBOTT, R. L., SPARGER, E. E., LOVELACE, K. M., FITCH, W. M., PEDERSEN, N. C., LUCIW, P. A. & ELDER, J. H. (1989). Nucleotide sequence and genomic organization of feline immunodeficiency virus. *Proceedings of the National Academy of Sciences, U.S.A.* **86**, 5743–5747.

VAN DER MAATEN, M. J., BOOTHE, A. D. & SEGER, C. L. (1972). Isolation of a virus from cattle with persistent lymphocytosis. *Journal of the National Cancer Institute* **49**, 1649–1657.

VERONESE, F. D., DEVICO, A. L., COPELAND, T. D., OROSZLAN, S., GALLO, R. C. & SARNGADHARAN, M. G. (1985). Characterization of gp41 as the transmembrane protein coded by the HTLV-III/LAV envelope gene. *Science* **229**, 1402–1405.

YAMAMOTO, J. K., SPARGER, E., HO, E. W., ANDERSEN, P. R., O'CONNOR, T. P., MANDELL, C. P., LOWENSTINE, L., MUNN, R. & PEDERSEN, N. C. (1988). Pathogenesis studies of experimentally induced feline immunodeficiency virus infection. *American Journal of Veterinary Research* **49**, 1246–1258.

ZHOU, Y., EDERVEEN, J., EGBERINK, H. F., PENSAERT, M. & HORZINEK, M. C. (1988). Porcine epidemic diarrhea virus (CV777) and feline infectious peritonitis (FIPV) are antigenically related. *Archives of Virology* **102**, 63–71.

(*Received 21 July 1989; Accepted 17 October 1989*)