Characterization and Significance of Delayed Processing of the Feline Leukemia Virus FeLV-FAIDS Envelope Glycoprotein

MARY L. POSS,¹ SANDRA L. QUACKENBUSH,¹ JAMES I. MULLINS,² AND EDWARD A. HOOVER^{1*}

Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523,¹ and Department of Microbiology and Immunology, Stanford University Medical School, Stanford, California 94305-5402²

Received 29 January 1990/Accepted 5 June 1990

FeLV-FAIDS, an immunodeficiency-inducing isolate of feline leukemia virus, is composed of a pathogenic but replication-defective genome (molecular clone 61C) and a replication-competent but non-immunodeficiency-inducing variant genome (molecular clone 61E). The chimeric virus EECC, composed of the 5' *gag-pol* of 61E fused to the *env*-3' LTR of 61C, also induces immunodeficiency. The 61C (or EECC) gp80 can be distinguished from that of 61E on the basis of antigenic recognition, size, and rate of posttranslational processing. We found that the nascent precursor polypeptides of the two viruses were the same size; however, the 61E gp80 rapidly shifted to a smaller size and was subsequently cleaved to gp70, whereas EECC gp80 maintained its nascent size and was cleaved to gp70 only after a prolonged time. Endo- β -*N*-acetyl glucosaminidase H and *N*-glycanase digestions of newly formed glycoproteins resulted in a similar banding pattern for both viruses, indicating that both contained the same number of oligosaccharide side chains and that all of these were high mannose sugars. The metabolic inhibitors of glycosylation, castanospermine or *N*-methyldeoxynojirimycin, prevented both the rapid trimming of 61E gp80 and its cleavage to gp70. Treatment with mannosidase inhibitors, however, did not affect 61E gp80 processing or size, suggesting that retention of glucose residues on EECC was responsible for these distinguishing properties of the glycoprotein. The pathological consequence of aberrant viral glycoprotein processing was evaluated in feline 3201 T lymphocytes, which are infectable by both 61E and EECC but are killed only by EECC. As in fibroblasts, the EECC glycoprotein produced in lymphocytes was larger, antigenically distinct, and processed more slowly than was the glycoprotein of 61E. Castanospermine treatment of 61E-infected 3201 T cells, however, not only abrogated the antigenic differences between the 61E and EECC glycoproteins but also resulted in a cytopathic effect. Our results suggest that (i) intracellular accumulation of EECC envelope glycoprotein may occur consequent to retention of glucose residues on carbohydrate side chains and (ii) a strong correlation exists between delayed glycoprotein processing and cytopathicity in FeLV-FAIDS-infected T lymphocytes.

The FeLV-FAIDS isolate of feline leukemia virus (FeLV) consistently induces fatal immunodeficiency disease in inoculated cats (11, 20, 21). Several full-length viral genomes have been cloned directly from tissue DNA of FeLV-FAIDS-infected animals (26). A replication-competent clone, 61E, while highly infectious *in vivo*, does not induce immunodeficiency disease, whereas a replication-defective clone, 61C, derived from the same tissue sample, induces a fatal immunodeficiency syndrome when coinoculated with 61E (21, 26). A virus chimera generated between these two viral genomes containing the 61E 5' long terminal repeat (LTR)-*gag-pol* region and the 61C *env*-3' LTR produces a replication-competent, immunodeficiency disease-inducing virus, EECC (26). *In vivo* and *in vitro* assays employing EECC and a series of viral chimeras containing more limited segments of 61C *env* demonstrate that critical pathogenic determinants of FeLV-FAIDS reside in the envelope glycoprotein (26).

Whereas the transmembrane protein p15E is 100% homologous between the two viruses, the 61C external glycoprotein, gp70, contains a predicted 6-amino-acid deletion and a 6-amino-acid insertion near the amino and carboxy termini, respectively, and 11 single amino acid changes relative to the 61E gp70 (26). These changes render the envelope glycoproteins distinct from one another on the basis of size, antigenic

recognition, rate of processing, and type of carbohydrate constituents (28). Although there are no differences in potential *N*-linked glycosylation sites between the two viral glycoproteins, all of the distinguishing characteristics can be attributed to differences in posttranslational processing of the glycoprotein precursor (28).

Variation in glycoprotein processing is implicated in the pathogenic mechanism of several viruses. Altered infectivity and virulence of several mutant viruses when compared with those of the wild-type strains have been attributed to delayed precursor processing or glycosylation differences of the envelope glycoproteins (17, 19, 20, 34, 35, 42, 43). Interference with glycosylation of human immunodeficiency virus gp120 alters syncytium formation and production of infectious virions (2, 10, 41).

The array of carbohydrates on a given glycoprotein can change during cell development or transformation (3, 22, 24, 29) and may differ for the same protein from different types of cells (27). This implies that oligosaccharides can be important functional determinants of a protein and has led to a resurgence of interest in glycosylation pathways and carbohydrate structure. Preassembled oligosaccharides in the form glucose₃, mannose₉, *N*-acetylglucosamine₂, are added to available asparagine (*N*-linked) residues found in the signal sequence asparagine-X-threonine (serine), where X can be any amino acid except proline (12). Each of the three glucose residues and at least one of the mannose residues are cleaved prior to exiting the rough endoplasmic reticulum

* Corresponding author.

(RER) (14, 40). Further mannose cleavage and addition of other sugars can occur in the Golgi apparatus (14, 40). Metabolic inhibitors of each of these cleavage steps have been identified and used to investigate the functional alterations conferred on proteins carrying the untrimmed carbohydrate chains. These modified cellular proteins may be functionally unaltered, but most display aberrant intracellular transport or function (1, 5, 25, 30, 36, 38).

The virus chimera EECC infects and kills T-lymphocyte cell line 3201, whereas 61E infects but does not kill these cells. Killing of 3201 cells thus provides a bioassay correlated with disease-inducing capacity (P. R. Donahue, S. L. Quackenbush, C. M. G. deNoronha, M. V. Gallo, J. Overbaugh, E. A. Hoover, and J. I. Mullins, submitted for publication) and an *in vitro* system to study the underlying mechanism of T-cell killing by this virus. Infection of 3201 cells with EECC leads to death of most cells in the culture. A few cells survive the acute cytopathic infection, however, and repopulate the culture as persistently infected cells. Virus derived from these recovered cultures is infectious and capable of instituting another round of cytopathicity if inoculated onto naive cultures.

In this report, we extend our characterization of FeLV-FAIDS 61E and EECC envelope glycoproteins to determine the features of the EECC envelope precursor gp80 that lead to its delayed processing. We find that retention of glucose residues may lead to the differences in size and the antigenic and processing properties that distinguish EECC and 61E glycoproteins. Furthermore, the apathogenic infection of 61E in 3201 cells can be rendered cytopathic by preventing glucose trimming on the 61E glycoprotein, suggesting that this event may be in part responsible for the T-cell cytopathic properties of FeLV-FAIDS.

MATERIALS AND METHODS

Cells and viruses. Production of molecularly cloned 61E and construct EECC has been described previously (26). Viruses were grown in AH927 feline embryo fibroblasts that were maintained in Dulbecco minimum essential medium supplemented with 1% glutamine, 1% penicillin-streptomycin, and 10% fetal calf serum. Supernatants from these infected cells were used to infect 3201 cells maintained in a 50:50 mixture of L-15 and RPMI 1640 (GIBCO) containing 20% fetal calf serum, 1% glutamine, and 1% penicillin-streptomycin.

Pulse-chase radioimmunoprecipitation assay. Cells seeded into a 6-well plate were preincubated for a total of 4 h with the indicated metabolic inhibitor at the following concentrations: castanospermine (CSTP), 200 μ g per ml; *N*-methyldeoxynojirimycin (M-DNJ), 1 mM; 1-deoxymannojirimycin, 5 mM; and swainsonine (SWSN), 5 μ g per ml. All inhibitors were purchased from Genzyme Corp., Boston, Mass. For the final hour of preincubation, cells were placed in cysteine-methionine-deficient medium containing the inhibitor. Cells were labeled for 15 min with 100 μ Ci of [35 S]cysteine (Amersham Corp., Arlington Heights, Ill.)–[35 S]methionine (ICN Pharmaceuticals, Inc., Irvine, Calif.) (50:50) per ml of cysteine-methionine medium washed three times with ice-cold phosphate-buffered saline, and incubated for 1 or 2 h in medium containing a fivefold excess of cysteine and methionine. Radioimmunoprecipitation (RIPA) procedures were performed as previously described (28). Apparent molecular sizes were calculated from a standard curve of relative mobility (M_r) versus $\log_{10}$ molecular size. Conditions for pulse-chase studies in 3201 cells were as described for AH927 (28). To evaluate antibody recognition

of viral proteins, infected 3201 cells were labeled for 6 h following a 1-h preincubation period in cysteine-methionine medium. Lysates were prepared as previously described (28), except for the addition of 1 mg of DNase to the lysis buffer.

Glycosidase digestions. Endo- β -*N*-acetylglucosaminidase H (Endo H) digestions were performed on immunoprecipitated glycoproteins either attached to protein A-Sepharose beads (Sigma Chemical Co., St. Louis, Mo.) or dissociated from the beads by boiling. Both methods of glycoprotein preparation gave similar results. Following washing procedures specified for RIPA (28), beads were washed twice in 0.05 M phosphate (pH 5.7)–0.25% sodium dodecyl sulfate (SDS) and resuspended in 0.05 ml of this buffer. For partial digests, beads were incubated with 0.04 U of Endo H (Genzyme) per ml for 2 h at 37°C. Complete digestion of glycoproteins was accomplished by incubating samples for 12 h with additional portions of 0.04 U of Endo H per ml added at 5 and 10 h. *N*-glycanase (Genzyme) reaction mixtures contained 0.1 M phosphate (pH 8.0)–0.75% Triton X-100–0.1% SDS and 0.5 to 1.0 U of *N*-glycanase, and the reactions were conducted at 37°C for 12 h. Digests were suspended in 0.08 M Tris (pH 6.8)–2% SDS–0.1 M dithiothreitol–10% glycerol and resolved on 7.5% SDS-polyacrylamide gels.

T-cell cytopathicity assay. A feline T-cell lymphoma line, 3201 (a gift from W. D. Hardy, Jr. [37]), was inoculated with 1 ml of tissue culture supernatant from virus-infected AH927 cells. Cells were incubated with virus for 1 h and washed, and 2×10^6 cells were suspended in fresh medium in 25-cm² flasks. After 3 days at 37°C in 5% CO₂, live cells were enumerated by trypan blue staining. A total of 2×10^6 cells were reseeded, as described above. This procedure was repeated for 60 days, and data were presented as cumulative total live cell numbers.

Evaluation of CTSP-treated 3201 cell growth kinetics. Persistently infected or uninfected 3201 cells were seeded at 5×10^6 cells per flask (25 cm²) with or without the daily addition of 100 μ g of CSTP per ml. Prior to treatment, the percentage of virus-infected cells was determined by surface labeling with a fluorescein isothiocyanate (FITC)-conjugated FeLV gp70 specific monoclonal antibody C11D8 (9). Briefly, an aliquot of 3201 cells was washed once in phosphate-buffered saline containing 5% fetal calf serum and 0.2% sodium azide and incubated with a 1:20 dilution of 0.286 mg of C11D8-FITC per ml for 20 min. Cells were washed three times, resuspended in phosphate-buffered saline, and analyzed on an EPICS Profile II flow cytometer (Coulter Electronics, Inc., Hialeah, Fla.). The efficacy of CSTP treatment was monitored by similar labeling with a 1:25 dilution of 5 mg of concanavalin A-FITC (Sigma) per ml. Treatment was considered effective if a twofold increase in mean channel intensity was present in CSTP-treated cells relative to that in the respective untreated cells. The total number of live cells was determined by manual counting of two replicate samples in a hemacytometer, and the number of viable cells in CSTP versus the corresponding non-CSTP treatment group (percent control) was determined. Results presented represent the median value of percent control for four experiments. Data for each virus-infected or uninfected culture and its corresponding CSTP-treated culture were statistically evaluated by chi-square analysis.

RESULTS

Analysis of early glycoprotein processing events in 61E- and EECC-infected cells. We have previously shown that molec-

FIG. 3. Processing of 61E and EECC envelope glycoproteins in AH927 cells treated with M-DNJ. Cells were preincubated for 4 h with 1 mM M-DNJ, and lysates were prepared as described in Materials and Methods. Endo H digests (lanes +) were conducted for 2 h with 40 mU of Endo H. Uninfected cells (lanes A) and 61E (lanes E)- and EECC (lanes C)-infected cell lysates are as indicated.

charide chains as did the glycoprotein produced in uninhibited infected cells. The ladder of bands resulting from partial digestion indicated at least eight Endo H-susceptible bands in both 61E and EECC gp80, but these bands were better defined and more widely spaced than those of partially digested native gp80 (data not shown). Thus, the increased size of the glycoprotein precursor appears to be due to retained sugar moieties and not to additional oligosaccharide chains.

Results of kinetic experiments conducted in the presence of 5 μ g of SWSN, an inhibitor of Golgi apparatus mannosidase II (6) are shown (Fig. 4). The behavior of viral glycoproteins produced in the presence of this inhibitor was essentially the same as those produced in uninhibited cells. Both 61E and EECC gp80 had similar mobilities following pulse-labeling, but 61E gp80 resolved into a 79- to 81-kDa band by 1 h. Processing of gp80 to gp70 in 61E-infected lysates was also apparent at this time point. Complete Endo H digests yielded major 59- and minor 63-kDa bands at both the pulse and 1-h chase for 61E gp80 and a single 59-kDa

FIG. 4. Processing of 61E (lanes E) and EECC (lanes C) envelope glycoproteins in AH927 cells treated with SWSN. Cells were preincubated for 4 h with 5 μ g of SWSN per ml, and lysates were prepared as described in Materials and Methods. Endo H digests (lanes +) were done for 12 h at 37°C, with the addition of 40 mU of Endo H per ml at 5 and 10 h. Deglycosylated proteins produced in tunicamycin-treated cells (lanes TM) are also shown.

FIG. 5. Antigenic recognition of envelope glycoproteins from 61E and EECC produced in a T-lymphoblastoid cell line. 3201 cells were labeled for 6 h with 100 μ Ci of [35 S]methionine plus [35]cysteine per ml in the presence or absence of 100 μ g of CSTP per ml, lysed, and precipitated with monoclonal antibody C11D8 or C5E5. Lysates from CSTP-treated cells (lanes +), uninfected cells (lanes A), and 61E (lanes E)- and EECC (lanes C)-infected cells are as indicated.

band for Endo H-treated EECC gp80 at all time points. Treatment of cells with 1-deoxymannojirimycin, a mannosidase I inhibitor (18), produced essentially the same results, except glycoproteins produced in cells treated with this inhibitor were slightly larger than were those produced in SWSN-treated cells (data not shown).

To summarize, both the properties of delayed processing and larger size that characterize the EECC envelope precursor could be conferred on the 61E gp80 if the protein were produced in the presence of glucosidase but not mannosidase inhibitors. Thus, retention of glucose, but not mannose, residues on some oligosaccharide chains of the maturing EECC glycoprotein contributed to its distinctive biochemical features.

Processing and antigenic recognition of 61E and EECC envelope proteins in a cytopathically infected target cell. All of the experiments described above assessed the viral proteins produced in persistently infected fibroblast cells. In order to determine the influence of cell type on posttranslational modifications of viral envelope proteins and also to investigate the biological relevance of the distinct properties ascribed to the EECC glycoprotein, antigenic recognition and processing of viral glycoproteins produced in a cytopathically infected T-lymphocytoid cell line (3201 cells; a gift from W. D. Hardy, Jr.) (37) were examined. The results (Fig. 5) were comparable to those observed for viral envelope proteins produced in fibroblasts. The monoclonal antibody C11D8 (a gift from C. K. Grant) (9) that recognizes a conserved epitope in the gp70 of most FeLVs precipitated the envelope precursor from both viruses. Monoclonal antibody C5E5 (also from C. K. Grant) (9) that recognizes an epitope near the amino-terminal portion of p15E (C. K. Grant, personal communication) precipitated only the gp80 from EECC-infected 3201 cells. When the infected cells were treated with CSTP, however, gp80 from both viruses was recognized by this antibody. Processing of gp80 to gp70 was also examined in the 3201 cells in a pulse-chase experiment (Fig. 6). Both the shift to a faster-migrating form of 61E gp80 and delayed processing of EECC gp80 occurred in infected 3201 cells. gp70 is not apparent during the chase, because it is inefficiently precipitated from 3201 cell lysates by the polyclonal anti-FeLV serum used in this experiment. Thus, the antigenic distinction, size difference, and processing delay that characterized the EECC envelope glycopro-

FIG. 6. Kinetics of glycoprotein processing in 61E- and EECC-infected 3201 cells. Cells were pulsed with 100 μ Ci of [35 S]methionine plus [35 S]cysteine per ml for 15 min, washed, and suspended in medium containing a fivefold excess of cysteine and methionine for 1 and 3 h. Lysates were prepared as described in Materials and Methods, precipitated with polyclonal anti-FcLV serum, and analyzed on a 7.5% polyacrylamide gel by SDS-polyacrylamide gel electrophoresis. Uninfected cells (lanes A) and 61E (lanes E)- and EECC (lanes C)-infected cells are shown.

tein produced in fibroblasts were also properties of this protein in a T-lymphocyte cell line.

Effect of CSTP on cell killing in a virus-infected and uninfected T-lymphocyte cell line. The above data indicated that the distinguishing biochemical properties of the EECC envelope protein could be conferred to the envelope protein of the avirulent 61E by producing the protein in cells treated with glucosidase inhibitors. To determine whether these envelope properties also correlate with cytopathicity, the effect of CSTP on the growth of infected 3201 cells was evaluated. EECC efficiently infects and kills 3201 T cells,

FIG. 7. Acute cytopathic effect of chimera EECC versus 61E in feline 3201 T cells and development of a persistently infected survivor EECC cell population. Cells were incubated with tissue culture supernatant from infected AH927 cells for 3 h and washed, and 2×10^6 cells were suspended in fresh medium. Every 3 days, cells were enumerated and 2×10^6 cells were seeded in fresh medium. Results are expressed as the cumulative cell number for each virus-infected and uninfected group. Symbols: $\square$, control (uninfected); $\blacklozenge$, 61E infected; $\blacksquare$, EECC infected.

FIG. 8. Effect of CSTP on growth kinetics of persistently EECC- or EECC-infected and uninfected 3201 cells. Virus-infected or uninfected cells were seeded at 5×10^5 cells per flask with or without 100 μ g of CSTP per ml. Live cell numbers were determined daily for 4 days by counting with a hemacytometer. Medium containing fresh CSTP was added daily. Data represent the median value for four experiments of percent control (number of CSTP-treated cells per number of untreated cells) for virus-infected and uninfected cells. Symbols: $\square$, EECC infected; $\blacklozenge$, EECC infected; $\blacksquare$, control (uninfected). CSTP treatment of EECC cells significantly affected cell growth, as determined by chi-square analysis at $P < 0.005$ and $P < 0.05$ for uninfected cells. There was no significant effect on EECC-infected cells.

whereas 61E can infect but does not kill these cells. Following an acute cytopathic event initiated 7 to 14 days postinfection with EECC, a population of persistently infected cells developed (Fig. 7). Despite the continued presence of EECC replication in the persistently infected T cells, no further overt cytopathicity was observed, although the culture growth rate was slower than that of either 61E-infected or control cells (Fig. 7).

The biological significance of altered processing of EECC gp80 was examined by comparing the growth kinetics of 3201 cells persistently infected with EECC (a viral chimera that contains the entire 61E genome except for the addition of the 61C LTR) or EECC and uninfected 3201 cells in the absence or presence of CSTP (Fig. 8). To eliminate differences in protein expression that occur under the influence of the more active 61C LTR (Donahue et al., submitted; M. L. Poss, unpublished data), EECC replaced 61E in this experiment. Persistently infected cells were used in order to discriminate between virus- and drug-induced cytopathicity. Data presented in Fig. 8 represent four separate experiments in which either virus-infected or uninfected 3201 cells were maintained for 4 days in medium containing 100 μ g of CSTP per ml. Cell cultures were greater than 97% infected, as assessed by flow cytometric analysis of cell surfaces labeled with C11D8-FITC (data not shown). CSTP-treated cells infected with EECC grew as well as or better than (difference was not statistically significant) untreated, EECC-infected cells over the 4-day observation period. In contrast, CSTP treatment of EECC-infected cells caused a significant ($P < 0.005$) decrease in the number of treated cells compared with untreated EECC-infected cells. This effect was apparent within 24 h of initiating treatment. CSTP slowed the growth of

uninfected 3201 cells (significant at $P < 0.05$), however the decrease in cell numbers in treated cultures was not evident before day three of treatment.

Thus, CSTP treatment of EECC-infected 3201 cells induced a rapid and significant decrease in culture growth. While CSTP treatment did eventually cause a significant decrease in growth of uninfected 3201 cells, this effect required more prolonged treatment. These data inferred, therefore, that altered glycoprotein processing due to constraints either imposed by protein structure (as was the case with EECC infection of 3201) or induced by inhibition of processing enzymes (as demonstrated above for EECC-infected 3201 cells) was cytopathic to a T-lymphocyte cell line.

DISCUSSION

Virus-induced cytopathology can result from the interaction of a virus and a cell at any stage of the virus replication cycle. In some cases, viral pathogenic determinants have been localized to a structural protein (4, 17, 26, 31, 39). To understand the mechanism by which a viral protein can alter cell metabolism, the structure, intracellular interactions, and transport of the protein must be known. FeLV-FAIDS provides an excellent model system to investigate lymphocyte killing resulting from interaction of the viral envelope with T cells, because the pathogenic determinants of the virus EECC are carried within the extracellular envelope glycoprotein that is otherwise over 98% homologous to the envelope glycoprotein of an infectious but apathogenic parent virus, 61E (26; Donahue et al., submitted). Thus, comparison of biochemical characteristics of the two viral glycoproteins may identify properties unique to EECC that also correlate with cell killing. Furthermore, the significance of any biochemical divergence of EECC glycoproteins from those of 61E can be tested in vitro by conferring the distinguishing property to the 61E glycoprotein and determining if this results in cytopathicity.

The most distinctive biochemical differences between the glycoproteins of 61E and EECC are in size and rate of processing. Immediately posttranslation, however, envelope precursors of 61E and EECC were the same size, which for glycoproteins of the same predicted length, indicated that they also contained the same number of carbohydrate chains. Removal of high mannose sugars by Endo H digestion of these nascent envelope precursors resulted in similar partial (data not shown) and complete digestion profiles for both viral proteins, providing further evidence that the total number of carbohydrate chains initially added to gp80 was the same. All of these sugars were high mannose, as evidenced by similar digestion profiles resulting from *N*-glycanase and Endo H treatment. These experiments do not indicate whether the glycosylation sites used are the same on both proteins.

61E gp80 was rapidly processed to a smaller molecular mass form, with subsequent cleavage to p15E and gp70, whereas EECC gp80 retained its posttranslational size and proteolytic cleavage to gp70 and p15E was delayed. Treating virus-infected cells with either of the glucosidase inhibitors, CSTP or M-DNJ, resulted in production of 61E gp80 that failed to undergo both the molecular mass shift and cleavage to gp70 and p15E. We have previously shown that polyclonal sera and a monoclonal antibody unable to recognize native 61E gp80 can precipitate gp80 produced in cells treated with M-DNJ (28). Kinetics and processing of viral glycoproteins in cells treated with SWSN did not differ from those ob-

served in uninhibited cells. Thus, retention of glucose residues may lead to delayed transport to the Golgi apparatus, where proteolytic cleavage of gp80 to p15E and gp70 is thought to occur.

Both viral gp80s were represented as slower-migrating and more heterogeneous glycoproteins when produced under the influence of either of the glucosidase inhibitors, although these effects were less pronounced for EECC than for 61E gp80. This could reflect the utilization of an additional glycosylation site that was exposed because of a more open conformation of the untrimmed proteins. Alternatively, either incomplete inhibition of glucosidases or activation of minor glycosylation pathways present in the cell may result in differential trimming of residues or addition of an incomplete oligosaccharide precursor that circumvents the glucosidase steps (8, 15). These alternatives could not be resolved from our data, although it seems unlikely that alternative pathways would be up regulated during the 4-h exposure to the inhibitor.

Glycosylation patterns on viral glycoproteins are specified both by the infected cell and the primary structure of the protein (7, 23, 33). Accumulation of viral precursor proteins is associated with cytopathology in a number of different virus systems (16, 39). FeLV-FAIDS cytopathically infects a T-lymphocyte cell line (3201) in vitro and results in lymphocyte depletion in vivo. Infection of 3201 cells by EECC was also characterized by accumulation of a slower-migrating gp80 with delayed production of gp70. As is the case in 61E-infected AH927 cells, gp80 from CSTP-treated, 61E-infected 3201 cells could be recognized by C5E5. These results demonstrated that, with the cell lines examined, the inability of a cell to efficiently process the EECC envelope glycoprotein was predominantly a function of the protein and not the type of cell infected. On the basis of the similarity in results obtained with antigenic recognition of C5E5 for 61E gp80 from CSTP-treated AH927 and 3201 cells, we infer that the processing delay in 3201 cells may also involve retention of glucose residues on nascent oligosaccharides.

Two important questions are raised by these data. (i) What is the relationship between delayed glycoprotein processing and cytopathology? (ii) Why are some cells in which this phenomenon occurs killed while others are not? Several mechanisms to explain the effect of viral protein accumulation on cell viability can be evoked. Obstruction or alteration of cellular glycoprotein processing may occur due to excess viral protein accumulation in the RER. Alternatively, an altered conformation or the presence of a unique carbohydrate epitope resulting from retention in the RER could confer a distinct structure on the extracellular viral glycoprotein that enhances binding or interferes with a vital cell receptor. Altering the carbohydrates on glycoproteins does not always abrogate function (30, 36), and thus some cells may be more susceptible to virus infection simply because they require a receptor or factor whose function is carbohydrate dependent.

The impact of intracellular viral glycoprotein accumulation on 3201 T cells was determined by following the growth kinetics of 3201 cells (maintained in 100 μ g of CSTP per ml) that were uninfected or persistently infected with virus. A significant decrease in cell number resulted from CSTP treatment of EECC-infected cultures, whereas EECC-infected cultures were unaffected by CSTP treatment. This supported the theory that protein accumulation in cells acutely infected with EECC was related to cytopathicity but did not refute the thesis that the extracellular glycoprotein

gp70 might be the major effector of cell dysfunction. The data did suggest, however, that if an altered gp70 effected cytopathicity, a carbohydrate or carbohydrate-induced epitope was the critical pathogenic determinant, since the primary structure of the EECC glycoprotein is unchanged in this experiment.

It was also interesting that persistently infected cells, recovered from acute cytopathic infection with EECC, were resistant to the effects of CSTP. Cells normally produce a minor population of partial oligosaccharides, and when selected for CSTP resistance they can produce truncated oligosaccharides that do not contain terminal glucose residues or they can activate alternative trimming pathways that eliminate the need for glucosidase I (8, 15). It should be noted, however, that gp80 still accumulates in persistently EECC-infected cells, although this may be quantitatively less than what occurs in acutely infected cells (M. L. Poss, unpublished data). Comparison of oligosaccharides on both viral and cellular glycoproteins from cells acutely and persistently infected with EECC would help to determine the impact of intracellular accumulation of viral glycoprotein on T-cell function.

Our data are consistent with the hypothesis that accumulation of envelope precursor glycoprotein from the lymphocytotoxic FeLV-FAIDS virus chimera EECC occurs consequent to retention of glucose residues on nascent oligosaccharide side chains. These additional residues account for the larger size of the glycoprotein from EECC in comparison with the glycoprotein of its apathogenic parent, 61E. Accumulation of viral glycoprotein in the RER or Golgi apparatus may mediate lymphocytotoxicity, since growth of EECC-infected 3201 cells is adversely affected if proper processing of the envelope glycoprotein of EECC is prevented by the use of metabolic inhibitors. It is important to point out, however, that processing of gp80 to gp70 does occur and may result in a mature envelope glycoprotein or a degradation product of this glycoprotein that could be the effector of cytopathicity. Thus, while detailed understanding of the biochemical mechanism of lymphocytotoxicity induced by FeLV-FAIDS awaits further investigation of other phases of virus-cell interaction, the data presented here demonstrate a direct link between posttranslational modification of the viral envelope protein and pathogenicity. These observations also suggest that careful consideration be given to the therapeutic use of glycosylation inhibitors in human immunodeficiency virus infections because of both the potential cellular resistance to these agents and the possible exacerbation of opportunistic virus infections by such treatment.

ACKNOWLEDGMENTS

We thank Julie Overbaugh, who cloned the original FeLV-FAIDS 61E/61C; Peter Donahue, who prepared constructs EECC and EECC; Christopher K. Grant, who provided monoclonal antibodies C11D8 and C5E5; and Mowafak D. Salmon, who contributed his expertise in statistical analysis.

This work was supported by Public Health Service grants RO1-CA-43216 and RO1-AI-25273. M. L. Poss is the recipient of a National Research Service award.

LITERATURE CITED

- Atkinson, P., J. Seely, H. Klemcke, and J. Hughes. 1988. Receptor binding and Nb2 cell mitogenic activities of glycosylated vs. unglycosylated porcine prolactin. *Biochem. Biophys. Res. Commun.* **155**:1187-1193.
- Blough, H. A., R. Pauwels, E. De Clercq, J. Cogniaux, S. Sprecher-Goldberger, and L. Thiry. 1986. Glycosylation inhibitors block the expression of LAV/HTLV-III (HIV) glycoproteins. *Biochem. Biophys. Res. Commun.* **141**:33-38.
- Chen, K. Y., and Z. Chang. 1987. A marked increase of fucosylation of glycoproteins in IMR-90 human diploid fibroblasts during senescence in vitro. *Biochem. Biophys. Res. Commun.* **142**:767-774.
- Chung, S., L. Wolff, and S. Ruscetti. 1987. Sequences responsible for altered erythropoietin responsiveness in spleen focus-forming virus strain SFFVp-infected cells are localized to a 678-base-pair region at the 3' end of the envelope gene. *J. Virol.* **61**:1661-1664.
- Duronio, V., S. Jacobs, P. Romero, and A. Herscovics. 1988. Effects of inhibitors of N-linked oligosaccharide processing on the biosynthesis and function of insulin and insulin-like growth factor-I receptors. *J. Biol. Chem.* **263**:5436-5445.
- Elbein, A., T. Szumilo, B. Sanford, K. Sharpless, and C. Adams. 1987. Effect of isomers of swainsonine on glycosidase activity and glycoprotein processing. *Biochimie* **26**:2502-2510.
- Etchison, J., and J. Holland. 1974. Carbohydrate composition of the membrane glycoprotein of vesicular stomatitis virus grown in four mammalian cell lines. *Proc. Natl. Acad. Sci. USA* **71**:4011-4014.
- Foddy, L., and R. Hughes. 1988. Assembly of asparagine-linked oligosaccharides in baby hamster kidney cells treated with castanospermine, an inhibitor of processing glucosidases. *Eur. J. Biochem.* **175**:291-299.
- Grant, C., B. Ernisse, O. Jarrett, and F. Jones. 1983. Feline leukemia virus envelope gp70 of subgroups B and C defined by monoclonal antibodies with cytotoxic and neutralizing function. *J. Immunol.* **131**:3042-3048.
- Gruters, R. A., J. J. Neefjes, M. Tersmette, R. E. Y. de Goede, A. Tulp, H. G. Huisman, F. Miedema, and H. L. Ploegh. 1987. Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. *Nature (London)* **330**:74-77.
- Hoover, E., J. Mullins, S. Quackenbush, and P. Gasper. 1987. Experimental transmission and pathogenesis of immunodeficiency syndrome in cats. *Blood* **70**:1880-1892.
- Hubbard, S. C., and P. Robbins. 1979. Synthesis and processing of protein-linked oligosaccharides in vivo. *J. Biol. Chem.* **254**:4568-4576.
- Kaushal, G., Y. Pan, J. Tropea, M. Mitchell, P. Liu, and A. Elbein. 1988. Selective inhibition of glycoprotein-processing enzymes. *J. Biol. Chem.* **263**:17278-17283.
- Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **54**:631-661.
- Lehrman, M., and Y. Zeng. 1989. Pleiotropic resistance to glycoprotein processing inhibitors in Chinese hamster ovary cells. *J. Biol. Chem.* **264**:1584-1593.
- Machamer, C., and J. Rose. 1988. Vesicular stomatitis virus G proteins with altered glycosylation sites display temperature-sensitive intracellular transport and are subject to aberrant intermolecular disulfide bonding. *J. Biol. Chem.* **263**:5955-5960.
- Machida, C., R. Bestwick, and D. Kabat. 1985. Role of a membrane glycoprotein in Friend virus-induced erythroleukemia: studies of mutant and revertant viruses. *Virology* **144**:158-172.
- McDowell, W., and R. Schwarz. 1988. Dissecting glycoprotein biosynthesis by the use of specific inhibitors. *Biochimie* **70**:1535-1549.
- Montelaro, R., B. Parekij, A. Orrego, and C. Issel. 1984. Antigenic variation during persistent infection by equine infectious anemia virus, a retrovirus. *J. Biol. Chem.* **259**:10539-10544.
- Mullins, J. I., C. S. Chen, and E. A. Hoover. 1986. Disease-specific and tissue-specific production of unintegrated feline leukaemia virus variant DNA in feline AIDS. *Nature (London)* **319**:333-336.
- Mullins, J. I., E. A. Hoover, J. Overbaugh, S. L. Quackenbush, P. R. Donahue, and M. L. Poss. 1989. FeLV-FAIDS-induced immunodeficiency syndrome in cats. *Vet. Immunol. Immunopathol.* **21**:25-37.
- Muramatsu, T. 1988. Alterations of cell-surface carbohydrates during differentiation and development. *Biochimie* **70**:1587-1596.

23. Nakamura, K., and R. Compans. 1979. Host cell- and virus strain-dependent differences in oligosaccharides of hemagglutinin glycoproteins of influenza A viruses. *Virology* **95**:8-23.
24. Ogier-Denis, E., P. Codogno, I. Chantret, and G. Trugnan. 1988. The processing of asparagine-linked oligosaccharides in HT-29 cells is a function of their state of enterocytic differentiation. *J. Biol. Chem.* **263**:6031-6037.
25. O'Hara, P., C. Hart, J. Forstrom, and F. Hagan. 1987. Role of carbohydrate in the function of human granulocyte-macrophage colony-stimulating factor. *Biochimie* **26**:4861-4867.
26. Overbaugh, J., P. R. Donahue, S. L. Quackenbush, E. A. Hoover, and J. I. Mullins. 1988. Molecular cloning of a feline leukemia virus that induces fatal immunodeficiency disease in cats. *Science* **239**:906-910.
27. Parehk, R. B., A. Tse, R. Dwek, A. Williams, and T. Rademacher. 1987. Tissue-specific N-glycosylation, site-specific oligosaccharide patterns and lentil lectin recognition of rat Thy-1. *EMBO J.* **6**:1233-1244.
28. Poss, M. L., J. I. Mullins, and E. A. Hoover. 1989. Posttranslational modifications distinguish the envelope glycoprotein of the immunodeficiency disease-inducing feline leukemia virus retrovirus. *J. Virol.* **63**:189-195.
29. Powell, L., E. Bause, G. Legler, R. Molyneux, and G. Hart. 1985. Influence of asparagine-linked oligosaccharides on tumor cell recognition in the mixed lymphocyte reaction. *J. Immunol.* **135**:714-724.
30. Powell, L., K. Smith, and G. Hart. 1987. Site specific glycosylation patterns of H-2K: effects of allelic polymorphism and mitogenic stimulation. *J. Immunol.* **139**:1206-1213.
31. Riedel, N., E. Hoover, R. Dornsife, and J. Mullins. 1988. Pathogenic and host range determinants of the feline aplastic anemia retrovirus. *Proc. Natl. Acad. Sci. USA* **85**:2758-2762.
32. Romero, P., R. Datema, and R. Schwartz. 1983. N-methyl-1-deoxynojirimycin, a novel inhibitor of glycoprotein processing, and its effect on fowl plague virus maturation. *Virology* **130**:238-242.
33. Rosner, M., L. Grinna, and P. Robbins. 1980. Differences in glycosylation patterns of closely related murine leukemia viruses. *Proc. Natl. Acad. Sci. USA* **7**:67-71.
34. Russell, D. L., J. M. Dalrymple, and R. E. Johnston. 1989. Sindbis virus mutations which coordinately affect glycoprotein processing, penetration, and virulence in mice. *J. Virol.* **63**:1619-1629.
35. Salinovich, O., and R. C. Montelaro. 1986. Comparison of glycoproteins by two-dimensional mapping of glycosylated peptides. *Anal. Biochem.* **157**:19-27.
36. Shirosishi, T., G. Evans, E. Appella, and K. Ozato. 1985. In vitro mutagenesis of a mouse MHC class I gene for the examination of structure-function relationships. *J. Immunol.* **134**:623-629.
37. Snyder, H. W., Jr., W. D. Hardy, Jr., E. E. Zuckerman, and E. Fleissner. 1978. Characterization of a tumor-specific antigen on the surface of feline lymphosarcoma cells. *Nature (London)* **275**:656-658.
38. Soderquist, A., and G. Carpenter. 1984. Glycosylation of the epidermal growth factor receptor in A-431 cells. *J. Biol. Chem.* **259**:12586-12594.
39. Szurek, P. F., P. H. Yuen, R. Jerzy, and P. K. Y. Wong. 1988. Identification of point mutations in the envelope gene of Moloney murine leukemia virus TB temperature-sensitive paralytogenic mutant *ts1*: molecular determinants for neurovirulence. *J. Virol.* **62**:357-360.
40. Turco, S., and P. Robbins. 1979. The initial stages of processing of protein-bound oligosaccharides in vitro. *J. Biol. Chem.* **254**:45600-45607.
41. Walker, B. D., M. Kowalski, W. C. Goh, K. Kozarsky, M. Krieger, C. Rosen, L. Rohrschneider, W. A. Haseltine, and J. Sodroski. 1987. Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. *Proc. Natl. Acad. Sci. USA* **84**:8120-8124.
42. Wolff, L., and S. Ruscetti. 1988. The spleen focus-forming virus (SFFV) envelope gene, when introduced into mice in the absence of other SFFV genes, induces acute erythroleukemia. *J. Virol.* **62**:2158-2163.
43. Yuen, P., E. Tzeng, C. Knupp, and P. Wong. 1986. The neurovirulent determinants of *ts1*, a paralytogenic mutant of Moloney murine leukemia virus TB, are localized in at least two functionally distinct regions of the genome. *J. Virol.* **59**:59-65.