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Posttranslational Modifications Distinguish the Envelope Glycoprotein of the Immunodeficiency Disease-Inducing Feline Leukemia Virus Retrovirus

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The envelope glycoprotein (gp70) of a molecularly cloned, replication-defective feline leukemia virus (FeLV-FAIDS clone 61C) carries determinants for induction of fatal immunodeficiency disease, whereas the gp70 of its companion replication-competent, probably parent virus (clone 61E) does not. Immunoprecipitation analysis of the extracellular glycoproteins of 61E and EECC, a replication-competent viral construct composed of the 61C *env* and 3′ long terminal repeat fused to the 61E *gag-pol* genes, demonstrated that the gp70 of EECC could be distinguished from that of 61E by both feline immune serum and a murine monoclonal antibody. Molecular weights of both the envelope precursor polyprotein (gp80) and the mature extracellular glycoprotein (gp70) of 61E were smaller than the corresponding proteins from the pathogenic EECC. Both the molecular weight disparity and monoclonal antibody discrimination of the two gp80s were abolished by inhibition of envelope protein glycosylation with tunicamycin, whereas the apparent gp70 size differences were resolved by enzymatic removal of N-linked oligosaccharides. Pulse-chase studies in EECC-infected cells demonstrated that processing of gp80 to gp70 was delayed and that this retardation of envelope glycoprotein processing could be simulated in 61E-infected cells by treatment with the glucosidase inhibitor *N*-methyldeoxynojirimycin, a compound that causes retention of oligosaccharides in the high-mannose form. The resultant 61E gp70 then could be recognized by sera from EECC-immunized cats. The presence of a higher content of sialic acid on the apathogenic 61E gp70 indicated that oligosaccharides of 61E and EECC gp70 were processed differently. These data suggested that the unique biochemical properties which distinguish the envelope glycoproteins of the FeLV-FAIDS variant from its companion apathogenic parent virus were responsible for T-cell cytopathicity and induction of immunodeficiency disease. Further biochemical characterization of these glycoproteins should be useful in understanding the pathogenic mechanisms of immunodeficiency disease induced by retroviruses.

Feline leukemia viruses (FeLV) induce a myriad of proliferative and degenerative diseases in naturally and experimentally infected cats. Perhaps the most significant pathogenic property of FeLVs, however, is induction of severe and progressive immunodeficiency. A recent FeLV isolate (FeLV-FAIDS), consistently induces fatal immunodeficiency disease characterized by severe lymphoid depletion, wasting syndrome, enteropathy, and opportunistic infections in infected animals (16, 29). Southern blot analysis of DNA from bone marrow of infected cats indicates that FeLV-FAIDS is composed of at least two major viral genomes: (i) a common form of the FeLV genome (61E), characterized by a 3.45-kilobase-pair *Kpn*I 3′ internal virus band, that appears in bone marrow shortly after infection and persists throughout the disease course; and (ii) a variant virus genome (61C), characterized by acquisition of a *Kpn*I site which confers a signature 2.1-kilobase-pair internal virus restriction fragment, that appears in the marrow and other tissues primarily as unintegrated viral DNA just prior to the onset of clinical immunodeficiency disease (29). Both this new restriction site and an additional variant-specific *Sac*II site are located in the *env* gene of the variant virus.

Prototypes of the common form and variant FeLV-FAIDS genomes have been molecularly cloned and their biological behavior studied in vivo and in vitro. The common form of the virus (61E) is capable of inducing viremia in cats but has not resulted in immunodeficiency disease during an 800+-day observation period. This virus can also infect (but is noncytopathic) feline T lymphocytes (E. A. Hoover et al., unpublished data). In contrast, the variant clone (61C), when rescued with 61E or employed as a construct (EECC) prepared by joining the 5′ long terminal repeat (LTR) and the *gag* and *pol* regions of 61E with the *env* and the 3′ LTR of 61C (30) reproduces the severe immunodeficiency syndrome seen with the original uncloned FeLV-FAIDS inoculum and is T-cell cytopathic in vitro.

Sequence analysis of the entire genome of the FeLV-FAIDS common form virus (4) and the *env* gene of the variant virus (30) has predicted that divergence at the nucleotide level of the 61C *env* should lead to a 6-amino-acid deletion near the amino terminal portion, a 6-amino-acid insertion near the carboxy terminus, and 11 scattered single-amino-acid changes in the extracellular glycoprotein of the pathogenic 61C variant compared with 61E. Sequence divergence of the 61E and 61C envelope genes and the association of pathogenicity with the 61C *env*-3′ LTR region indicate that the envelope protein, gp70, is a major effector of immunodeficiency and lymphocytopathicity.

Envelope glycoproteins are responsible for a variety of phenomena characteristic of retrovirus infection, including cell fusion (5, 24, 27, 31, 39), both efficacious and detrimental induction of the host immune response (1, 2, 6, 15, 19, 20, 28), and cellular transformation (Friend murine leukemia spleen focus-forming virus [23, 25, 26] and subgroup F avian leukosis virus [38]). Many of these properties correlate with alterations in posttranslational modification of the proteins,

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including the rate of precursor processing (14, 40, 42) or the type of carbohydrate chains attached to the protein backbone (7, 12, 13, 27, 32, 36). These posttranslational modifications are a function of both the protein primary structure and the repertoire of host cell processing enzymes (17, 18, 35), either or both of which may influence the biological behavior of the viral envelope proteins.

In this report, we compare the envelope glycoproteins from the molecularly cloned, infectious but nonpathogenic FeLV-FAIDS common form virus (61E) with the pathogenic virus chimera (EECC), which contains the *env* gene and 3′ LTR of variant 61C. We demonstrate that the genomic changes documented in the *env* gene result in antigenic, molecular weight, and processing differences in the pathogenic variant envelope glycoprotein compared with that of its apathogenic putative parent genome.

MATERIALS AND METHODS

Cells and viruses. Feline embryo fibroblasts (AH927), infected with cloned viruses or uninfected, were maintained at 37°C in 5% CO_2 in Dulbecco minimum essential medium supplemented with 1% glutamine–1% penicillin-streptomycin–10% fetal calf serum.

Production of molecularly cloned parent virus, 61E, and construct EECC which contains the envelope and 3′ LTR portion of the replication-defective, pathogenic 61C has been described previously (4, 30). These viruses were originally transfected into AH927 cells, and supernatants from these cells were used to infect low-passage AH927 cells.

Radioimmunoprecipitation assay. Cells were labeled for time periods specified in the text with 100 μCi of [^{35}S] methionine plus cysteine (ICN Pharmaceuticals, Inc., Irvine, Calif.) per ml after a 30-min incubation in methionine-cysteine-deficient RPMI 1640. In metabolic inhibition experiments, the preincubation period also included the inhibitor, 10 μg of tunicamycin (Sigma Chemical Co., St. Louis, Mo.) per ml or 1 mM *N*-methyldeoxynojirimycin (M-DNJ) (Genzyme Corp., Boston, Mass.) in the labeling medium. Cells were then washed three times in ice-cold phosphate-buffered saline and lysed with lysing buffer (0.05 M Tris [pH 7.2], 0.15 M NaCl, 0.1% sodium dodecyl sulfate (SDS), 0.1% Triton X-100, 1% sodium deoxycholate, and 1 mM phenylmethylsulfonyl fluoride) for 20 min on a rotary shaker. Lysates were centrifuged at 100,000 × *g* for 1 h and then were precleared overnight with 0.2 ml of protein A Sepharose beads per ml of lysate. Beads coupled with 15 μl of the appropriate antiserum or monoclonal antibody were incubated for 4 h with 100 to 200 μl of precleared lysate. The beads were then washed five times with lysing buffer (without sodium deoxycholate). The sample was suspended in sample buffer (0.08 M Tris [pH 6.8] 2% SDS, 0.1 M dithiothreitol, 10% glycerol), boiled for 3 min, and resolved by SDS-polyacrylamide gel electrophoresis (PAGE) (21). Gels were treated with En^{3}Hance (New England Nuclear Corp., Boston, Mass.) and were analyzed by fluorography.

Purification of gp70. Medium from ^{35}S-labeled, infected cells was collected and clarified at 10,000 × *g* for 15 min, and the virus was pelleted at 100,000 × *g* for 1.5 h. Virus pellets were suspended in 0.025 M Tris (pH 7.8)–0.1 M NaCl–0.1% Triton X-100; the pellets were then vortexed and applied to an affinity column prepared with CNBr-activated Sepharose and monoclonal antibody C11D8 that recognizes a linear epitope in a conserved region of FeLV envelope proteins (11). The column was extensively washed in this buffer and eluted with 4 M guanidine HCl; the recovered eluate was desalted immediately on a G-25 column.

Soluble gp70 was obtained from virus-free medium by NH_4SO_4 fractionation (35 to 70%); it was desalted on a G-25 column and eluted from a Sephacryl S-200 column (2.6 by 70 cm) in 0.02 M Tris (pH 7.4)–0.25 M NaCl. Fractions containing gp70 were concentrated and applied to an affinity column as described above.

Enzyme digests. The reaction mixture for digestion with *N*-glycanase (Genzyme) contained 0.2% SDS, 0.2 M phosphate (pH 8.6), 1.25% Nonidet P-40, 10 U of *N*-glycanase per ml, and 10 to 20 μl of gp70. gp70 was digested with 1 U of neuraminidase (Genzyme) per ml in 0.02 M phosphate (pH 6.0) with 0.001 M calcium acetate for 1 h.

RESULTS

Antigenic analysis of envelope glycoproteins from 61E and EECC. Antigenic differences between envelope glycoproteins of 61E and the pathogenic viral construct EECC were

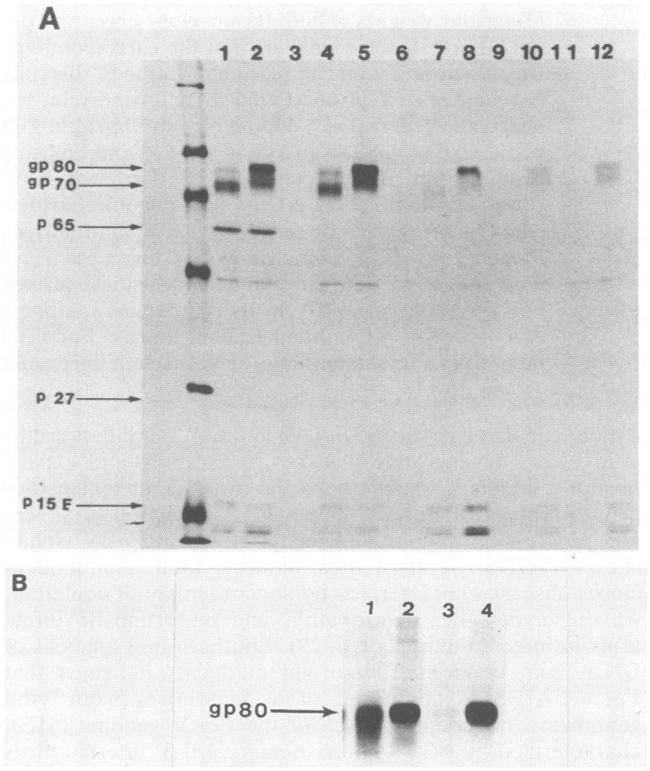

FIG. 1. Radioimmunoprecipitation of lysates of 61E- or EECC-infected or uninfected cells. (A) Cells were incubated for 7 h with 100 μCi of [^{35}S]methionine plus cysteine per ml and lysed as described in Materials and Methods. Lysates were precipitated overnight and analyzed on a 10% gel. Lanes 1, 4, 7, 9, and 11 represent lysates from 61E-infected cells; lanes 2, 5, 8, 10, and 12 were from EECC-infected cells; and lanes 3 and 6 were from uninfected cells. Antibodies used to precipitate viral proteins were goat polyclonal anti-FeLV (lanes 1 to 3), monoclonal antibody C11D8 (lanes 4 to 6), monoclonal antibody C5E5 (lanes 7 and 8), and antisera from cats 1755 (lanes 9 and 10) and 1768 (lanes 11 and 12) that were vaccinated with cell-associated, replication-defective 61C. Molecular size markers are, from top to bottom, 200, 97.4, 68, 43, 29, and 18.4 kilodaltons. (B) To accentuate the label in 61E gp80, cells were labeled for 1 h and lysed as described in the text. Lanes 1 (61E) and 2 (EECC) were precipitated with polyclonal anti-gp70 antibody, and lanes 3 (61E) and 4 (EECC) were precipitated with monoclonal antibody C5E5.

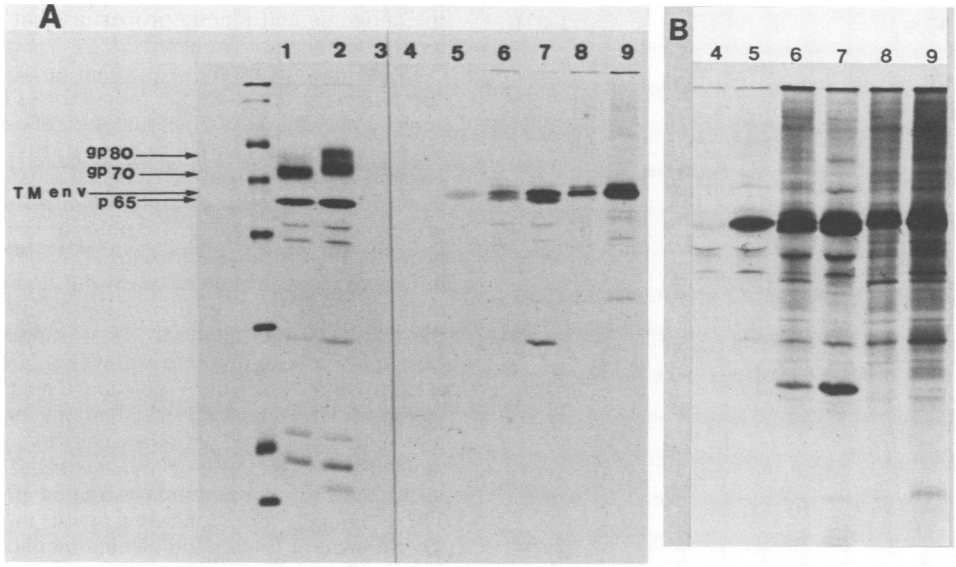

FIG. 2. (A) Radioimmunoprecipitation of tunicamycin-treated cells infected with 61E or EECC. Cells were preincubated 1 h in 10 μg of tunicamycin per ml in methionine- and cysteine-free medium. This medium was supplemented with 100 μCi of [^{35}S]methionine + cysteine per ml, and the cells were maintained for 7 h. Lysates were precipitated overnight, and samples were analyzed on a 12.5% gel. Lanes 1 to 3 represent untreated cell lysates infected with 61E (lane 1) or EECC (lane 2) or uninfected (lane 3) and precipitated with polyclonal anti-FeLV antibody. Tunicamycin-treated cell lysates of 61E are represented in lanes 4, 6, and 8; and lysates of EECC-infected cells are shown in lanes 5, 7, and 9. Lanes 4 and 5 were precipitated with antiserum from cat 1755, lanes 6 and 7 were precipitated with the polycloned goat anti-FeLV, and lanes 8 and 9 were precipitated with monoclonal antibody C5E5. (B) Lanes 4 to 9 are an overexposure of the corresponding lanes in panel A, to emphasize the difference in recognition of cat 1755 antiserum (lanes 4 and 5) for the unglycosylated envelope proteins of 61E (lane 4) and EECC (lane 5). At this exposure, the p65 *gag* precursor band blends with that of the unglycosylated envelope polyprotein in lanes 6 and 7 which was precipitated with polyclonal anti-FeLV antibody.

evaluated by radioimmunoprecipitation assay, using antisera from virus-infected (61C) cats, goat anti-FeLV and anti-gp70 sera, and monoclonal antibodies produced against FeLV gp70 (11). Most infected cats which succumb to immunodeficiency disease generate a poor anti-FeLV antibody response. However, cats 1755 and 1768 that were vaccinated intramuscularly with the cell-associated pathogenic defective variant (61C) produced antibodies that recognize only the EECC gp70 envelope glycoprotein (Fig. 1A, lanes 9 to 12). Differential recognition of these two viral gp70s by the polyclonal (lanes 1 to 3) or monoclonal antibody (C11D8) (lanes 4 to 6) was not observed. A monoclonal antibody (C5E5) (11) to the transmembrane protein p15E, which is 100% homologous between the two viruses, precipitated the gp80 precursor protein of EECC but not of 61E (lanes 7 to 8), indicating that the target epitope was buried in the envelope polyprotein of the apathogenic virus. Because the amount of gp80 in 61E-infected cells metabolically labeled for prolonged periods is low, precipitation with C5E5 was repeated on lysates from cells labeled for 1 h. Results of this experiment, shown in Fig. 1B, recapitulated the antigenic distinctness of these viral polyproteins. Thus, the envelope protein from EECC carries unique epitopes compared with the 61E gp70, indicative of either divergence of primary structure, differences in posttranslational modifications, or both.

Analysis of envelope glycoproteins of 61E and EECC produced in tunicamycin-treated cells. The results in Fig. 1 also demonstrate that both gp80 and the mature glycoprotein gp70 from EECC run slightly slower on SDS-PAGE than do the corresponding proteins from 61E. Since the predicted overall length of the proteins is the same and gels were run under reducing conditions, the larger size of EECC glycoproteins could be due to differences in their carbohydrate contents. To evaluate this possibility, the glycoproteins were harvested from virus-infected cells grown in the presence of tunicamycin, a metabolic inhibitor that blocks the addition of N-linked sugars by preventing formation of an obligatory lipid-carbohydrate intermediate (22). Immunoprecipitation of these proteins with polyclonal anti-FeLV antibody demonstrated that the unglycosylated precursor proteins from the two viruses had similar molecular weights as determined by SDS-PAGE (Fig. 2A, lanes 6 and 7). The p15E epitope on the envelope polyprotein of 61E, previously inaccessible to monoclonal antibody C5E5, was exposed in the unglycosylated protein (see lanes 8 and 9). Serum from cat 1755, however, was unable to recognize the modified envelope protein from 61E (Fig. 2A and B, lanes 4 and 5). Thus, the oligosaccharide components appear to impose the molecular weight differences observed between the glycoproteins of EECC and 61E and to be responsible, in the case of C5E5 epitope recognition, for imparting a unique structure to the gp80 precursor glycoprotein.

Kinetics of envelope polyprotein processing in 61E- and EECC-infected cells. Lysates of EECC-infected cells labeled for 6 h or less contained a higher gp80 to gp70 ratio than did lysates of 61E-infected cells (see Fig. 1A, lanes 1 and 2), suggesting that processing of the precursor envelope protein to gp70 and p15E might be slower in EECC than in 61E-infected cells. Kinetics of gp80 processing in infected AH927 cells, as determined by pulse-chase experiment, are shown in Fig. 3. The 30-min pulse labeled the precursor viral proteins gp80 and p65. After a chase of 3 h, over 50% of the label was present in the 61E gp70, whereas the majority of label persisted in gp80 of EECC. There was little change in the amount of labeled gp70 of EECC at 6 h compared with the amount at 3 h, whereas in 61E, gp70 contained the majority of label at 6 h. Shorter pulse and chase times also demonstrated that gp80 of 61E undergoes a shift to a

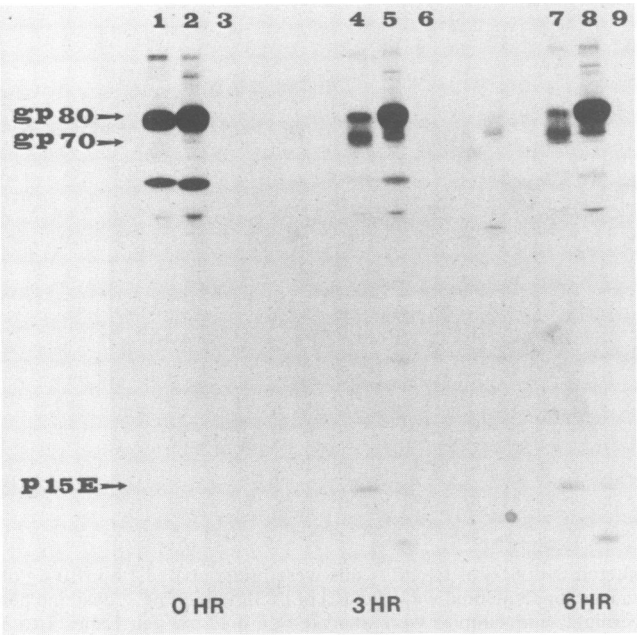

FIG. 3. Kinetic analysis of envelope protein processing in 61E- or EECC-infected AH927 cells. Cells were pulse-labeled for 30 min with 100 μCi of [^{35}S]methionine plus cysteine per ml. Lysates were prepared at the indicated times and were precipitated with polyclonal anti-FeLV antibody. Lanes 1, 4, and 7 are 61E-infected cell lysates; lanes 2, 5, and 8 represent EECC-infected cell lysates; and lanes 3, 6, and 9 are uninfected cell lysates.

faster-migrating form at the earliest examined chase time of 30 min (data not shown). These results indicated that there is a delay in processing the EECC envelope precursor which might be due to failure of gp80 to undergo proper folding or glycosylation at an early stage of posttranslational processing.

Effect of M-DNJ on antigenic recognition and kinetics of processing of the envelope proteins from 61E and EECC. One of the early events in protein glycosylation is trimming of glucose and mannose residues from high-mannose oligosaccharides, a necessary step for further processing to complex carbohydrate chains (9, 41). M-DNJ is a specific inhibitor of the glucosidases responsible for the first steps of this alteration (34). If the apparent defect in processing of EECC gp80 results from omission of these cleavage steps, then it can be theorized that metabolic inhibition of this step in 61E-infected cells might impart some of the antigenic and kinetic characteristics of the EECC gp80 to the envelope precursor of 61E. To test this hypothesis, a pulse-chase experiment was conducted on virus-infected cells in the presence of 1 mM M-DNJ. Figure 4 shows that the gp80s of 61E and EECC labeled during the 25-min pulse had molecular sizes of 94 and 96 kilodaltons, respectively. There were equivalent amounts of labeled gp80, which represent the majority of the label remaining in both 61E- and EECC-infected cell lysates after 2.5 h. Untreated, infected cell lysates were included at 2.5 h for comparison. Lanes 7 to 10 show lysates of a 6-h label of infected cells precipitated with anti-FeLV or serum from cat 1755. Retention of label in the 61E precursor glycoprotein was still evidence at this time. Furthermore, serum from cat 1755 precipitated gp80 from both viruses. These results indicate that defective modification of the precursor oligosaccharide side chains of EECC may confer the antigenic and kinetic properties that distinguish it from its nonpathogenic parent, 61E.

Glycosidase digestion of purified gp70 of 61E and EECC. Alterations in the envelope precursor of 61E and the pathogenic construct EECC should be reflected in the mature envelope protein from these two viruses. To investigate this possibility, gp70 from 61E and EECC was purified and digested with specific glycosidase enzymes. Because EECC produces an abundance of non-virion-associated gp70, a property of several retroviruses (3, 33, 37), this soluble form of the envelope protein also was purified and compared with the virion-associated gp70 of 61E and EECC. Figure 5 shows the results of this analysis. As was predicted by the tunicamycin studies, complete removal of N-linked oligosaccharides by digestion with *N*-glycanase negated the differences in molecular weight observed in the native proteins. Removal of sialic acid residues, however, accentuated this molecular weight difference, causing 61E gp70 to migrate faster than the neuraminidase-treated protein from EECC. These findings are consistent with the observation that interference in processing of high-mannose to complex carbohydrates in the EECC envelope precursor protein results in biochemical distinction of the mature envelope glycoprotein, gp70.

DISCUSSION

The pathogenic determinant of the molecularly cloned, immunodeficiency-inducing FeLV-FAIDS virus has been

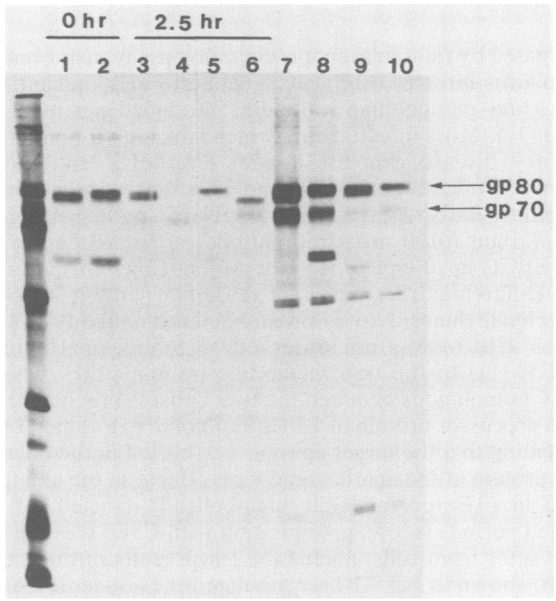

FIG. 4. Analysis of viral glycoproteins produced in infected cells treated with the glucosidase inhibitor M-DNJ. Cells were preincubated in 1 mM M-DNJ for 1 h and then labeled with 100 μCi of [^{35}S]methionine plus cysteine per ml for 30 min (lanes 1, 2, 3, and 5) or 6 h (lanes 7 to 10) in the presence of 1 mM M-DNJ. Lysates were precipitated with polyclonal anti-FeLV antibody (lanes 1 to 8) or with antiserum from cat 1755 (lanes 9 and 10). 61E-infected cell lysates are represented in lanes 1, 3, 4, 7, and 9; EECC-infected cell lysates are in lanes 2, 5, 6, 8, and 10. Lanes 4 and 6 are untreated 61E- and EECC-infected cell lysates, respectively, included for comparison of the extent of processing of these two viruses which occurs in the absence of M-DNJ. Molecular size markers in the far left lane are (top to bottom) 200, 97, 68, 43, 29, 18, and 14 kilodaltons.

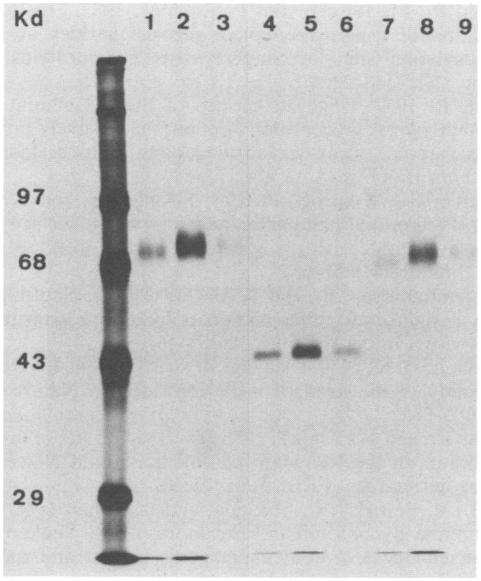

FIG. 5. Enzymatic digestion of purified gp70 from 61E and EECC. gp70 was purified and subjected to digestion with *N*-glycanase (lanes 4 to 6) and neuraminidase (lanes 7 to 9) as described in Materials and Methods. Lanes 1 to 3 represent the undigested proteins. gp70 from 61E is in lanes 1, 4, and 6; virus-associated gp70 from EECC is in lanes 2, 5, and 8; and soluble gp70 from EECC is in lanes 3, 6, and 9.

mapped to the *env* gene or LTR region of the virus (4, 30). Results of our studies indicated that the divergence in the *env* gene nucleotide sequence between 61E and EECC confers distinct structures to the glycoproteins of these viruses.

Distinctiveness of the envelope proteins of the pathogenic and apathogenic viruses was implied by the ability of sera from two cats vaccinated with 61C to recognize the gp70 of EECC but not that of 61E. Folding of the precursor protein gp80 was also different, as suggested by masking of the p15E epitope of 61E gp80 from monoclonal antibody C5E5. Furthermore, the 61E gp80 and gp70 migrated faster on SDS-PAGE than did those from EECC, despite the fact that these proteins are the same length as determined by sequence analysis. Lastly, feline embryo fibroblast cells infected with these molecularly cloned viruses processed the viral envelope proteins at distinctly different rates, indicative of differential accessibility of the cellular glycosylation enzymes to the viral proteins.

Some of these distinguishing properties may result from differences conferred by posttranslational processing of the envelope protein. In support of this tenet were the observations that there were no molecular weight differences detectable by SDS-PAGE between the precursor envelope proteins when N-linked glycosylation was inhibited by tunicamycin treatment of infected cells or when N-linked sugars were removed from purified viral gp70s by *N*-glycanase digestion. Preventing glycosylation of the 61E envelope precursor unmasked the epitope recognized by monoclonal antibody C5E5, so that C5E5 was then able to precipitate the unglycosylated precursor protein from both EECC and 61E. On the other hand, polyclonal antibodies from cats 1755 and 1768 still distinguished the unglycosylated proteins from the two viruses. Perhaps the most convincing evidence was the demonstration that two characteristic properties of the EECC envelope protein, antigenic distinction and delayed processing, could be conferred on the glycoproteins of 61E by inhibition of high-mannose oligosaccharide processing. These data suggested that oligosaccharides introduced via posttranslational changes endowed the viral envelope precursors with distinct structures, although these proteins were apparently distinct in the absence of carbohydrate as well.

Alterations in processing of viral glycoproteins may change the cellular distribution and function of these proteins and, consequently, the pathogenic potential of the virus (12). Intramembranous accumulation of the glycoproteins of *ts*12, a temperature-sensitive mutant of Uukuniemi virus that is incapable of virus particle production, induces the same vacuolization of the Golgi complex that is characteristic of infection with the wild-type virus (10). A defect in gp80 processing and its subsequent intracellular accumulation are associated with hindlimb paralysis in mice infected with a temperature-sensitive (*ts*1) mutant of Moloney murine leukemia virus (40). In other retrovirus systems, defective cleavage due either to amino acid changes at the gp70-p15E cleavage site or to changes in oligosaccharide processing is associated with decreased infectivity and inability to establish interference (8, 32).

Similar to the aforementioned viruses, the FeLV-FAIDS pathogenic variant virus (61C) and its replication-competent viral construct (EECC) differ from the apathogenic parent virus (61E) in the structure, processing, and distribution of the envelope protein gp70. Several mechanisms can be formulated to explain the altered biochemical properties of this glycoprotein and its role in cytopathology. Intracellular accumulation of gp80 in the internal membrane system may cause alteration in processing of a normal cellular glycoprotein essential for cell viability. In this situation, pathogenicity is due more to a mechanical blockage than to a specific effect of the virus. Conversely, delayed processing also leads to structural differences in the mature gp70 which is found either associated with virions or soluble in tissue culture supernatants. The presence of soluble gp70 may be due to specific glycosylation signals which target the protein for secretion, to a more labile interaction of gp70 with the transmembrane protein, or to disorderly assembly of virus particles with subsequent shedding of excess gp70. Although we have not detected differences between the soluble and virion-associated forms of gp70 to date, we cannot yet conclude that they do not exist. Even if the soluble and virion-associated EECC glycoproteins are identical, the relative excess of soluble gp70 or a different conformation conferred as a result of being in solution and unassociated with its transmembrane anchor may endow this protein with different biochemical properties relative to its virion-associated counterpart. It is plausible, therefore, that either virion-associated or soluble gp70 may be the effector of lymphocyte killing induced by this virus, perhaps through competition with an essential growth factor for its receptor or through interference with receptor function.

Nucleotide sequence analyses of FeLV-FAIDS molecular clones 61C and 61E indicate that differences exist in the *env* gene, and viral construct data demonstrate that this gene carries the pathogenic determinants of the virus. Data presented here show that these genomic differences in 61C *env* result in production of an envelope glycoprotein which is distinguishable from the gp70 of the apathogenic probable parent virus 61E by altered kinetics of posttranslational processing, size, carbohydrate constituents, and antigenic recognition. Further biochemical analyses of these proteins

and determination of viral protein-cell interaction should elucidate the mechanism by which this immunodeficiency-inducing retrovirus exerts its effects on target cells.

ACKNOWLEDGMENTS

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