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Viral Genetic Determinants of T-Cell Killing and Immunodeficiency Disease Induction by the Feline Leukemia Virus FeLV-FAIDS

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Within the fatal immunodeficiency disease-inducing strain of feline leukemia virus, FeLV-FAIDS, are viruses which range in pathogenicity from minimally (clone 61E is the prototype) to acutely pathogenic, most of the latter of which are also replication defective (clone 61C is the prototype). Mixtures of 61E and 61C virus and chimeras generated between them, but not 61E alone, killed feline T cells. T-cell killing depended on changes within a 7-amino-acid region near the C terminus of the gp70 *env* gene or was achieved independently by changes within a 109-amino-acid region encompassing the N terminus of gp70. The carboxy-terminal change was also sufficient for induction of fatal immunodeficiency disease in cats. Other changes within the 61C gp70 gene enhanced T-cell killing, as did changes in the long terminal repeat, the latter of which also enhanced virus replication. T-cell killing correlated with high levels of intracellular unintegrated and proviral DNA, all of which were blocked by treatment of infected cells with sera from 61C-immune cats or with a neutralizing monoclonal antibody. These findings indicate that T-cell killing is a consequence of superinfection and that the mutations in *env* critical to pathogenicity of the immunosuppressive variant result in a failure to establish superinfection interference in infected cells.

Domestic cats persistently infected with feline leukemia virus (FeLV) have a high incidence of both cytopathic-cytosuppressive and cytoproliferative diseases (reviewed in reference 13). Immunodeficiency syndrome, recognized as a major consequence of FeLV infection for 20 years, provides a model to explore mechanisms of immunosuppressive (and cytopathic) diseases caused by retroviruses (1, 31; reviewed in reference 22). Retroviruses from all three subfamilies have been shown to induce cytopathic diseases or cytopathic changes in cells: (i) oncoviruses, including FeLV, certain avian leukosis viruses, avian reticuloendotheliosis virus, and mink cell focus-forming murine virus; (ii) lentiviruses, including human immunodeficiency viruses (HIV), feline immunodeficiency virus, simian immunodeficiency viruses, and visna virus; and (iii) spumaviruses, including human and feline foamy viruses (11, 18, 19, 30, 46). Only the spumaviruses as yet have no known disease association. The mechanisms by which these viruses kill cells are not understood, although the viral envelope (*env*) gene product has been implicated in some cases (reviewed in reference 47). One event mediated by the *env*-encoded surface glycoprotein is superinfection interference (2, 6, 47, 48); should this process be delayed or disrupted in retrovirus-infected cells, massive reinfection and cell killing may ensue. This hypothesis, originally put forth by Temin and colleagues (17, 49) in studies of avian reticuloendotheliosis and leukosis viruses, is supported by the observed accumulation of unintegrated viral DNA (UVD) coincident with cytopathogenicity in vitro and in vivo induced by avian viruses as well as by visna virus and HIV type 1 (HIV-1) (12, 29, 36–38, 50) and pathogenicity in vivo induced by the FeLV strain FeLV-FAIDS (21, 24).

Fatal immunodeficiency disease, characterized by profound lymphoid depletion, wasting syndrome, and opportunistic infections, occurs in nearly 100% of viremic cats inoculated with the FeLV-FAIDS strain (15). Persistent infection in these animals is associated with the appearance of a common-form viral genome in bone marrow, so named because it persists throughout the life of the animal. Disease is prefigured in these animals by early deficits in colony-forming T cells, depletion of circulating T cells, impairment of antibody response, and the appearance of variant viral genomes. Variant genomes are recognizable as distinct from the common-form virus by a *Kpn*I restriction site polymorphism in the *env* gene and are found initially in bone marrow and blood cells (15, 25, 27, 35).

A molecularly cloned representative of the common-form genome (called F6A or 61E) was found to be replication competent and induced viremia but not immunodeficiency disease in inoculated cats (5, 25). In contrast, each of 10 variant-form virus clones was found to be replication defective in feline fibroblast and T-cell lines (25, 25a). When four of these variant clones were rescued through complementation in *trans* by 61E, fatal immunodeficiency disease developed (23, 25, 25a). Pathogenicity in vivo could be attributed to changes in the 3′ portion of the variant viral genome, since a chimera containing the 5′ 5.8 kb from 61E and the 3′ 2.6 kb from the prototype variant clone 61C (chimera EECC) was replication competent and induced acute-onset fatal immunodeficiency syndrome (25).

In this report, we identify the mutational changes responsible for the pathogenic phenotype of 61C, identify killing of a feline T-cell line as an in vitro correlate of disease induction, and delimit aspects of the mechanism of T-cell killing.

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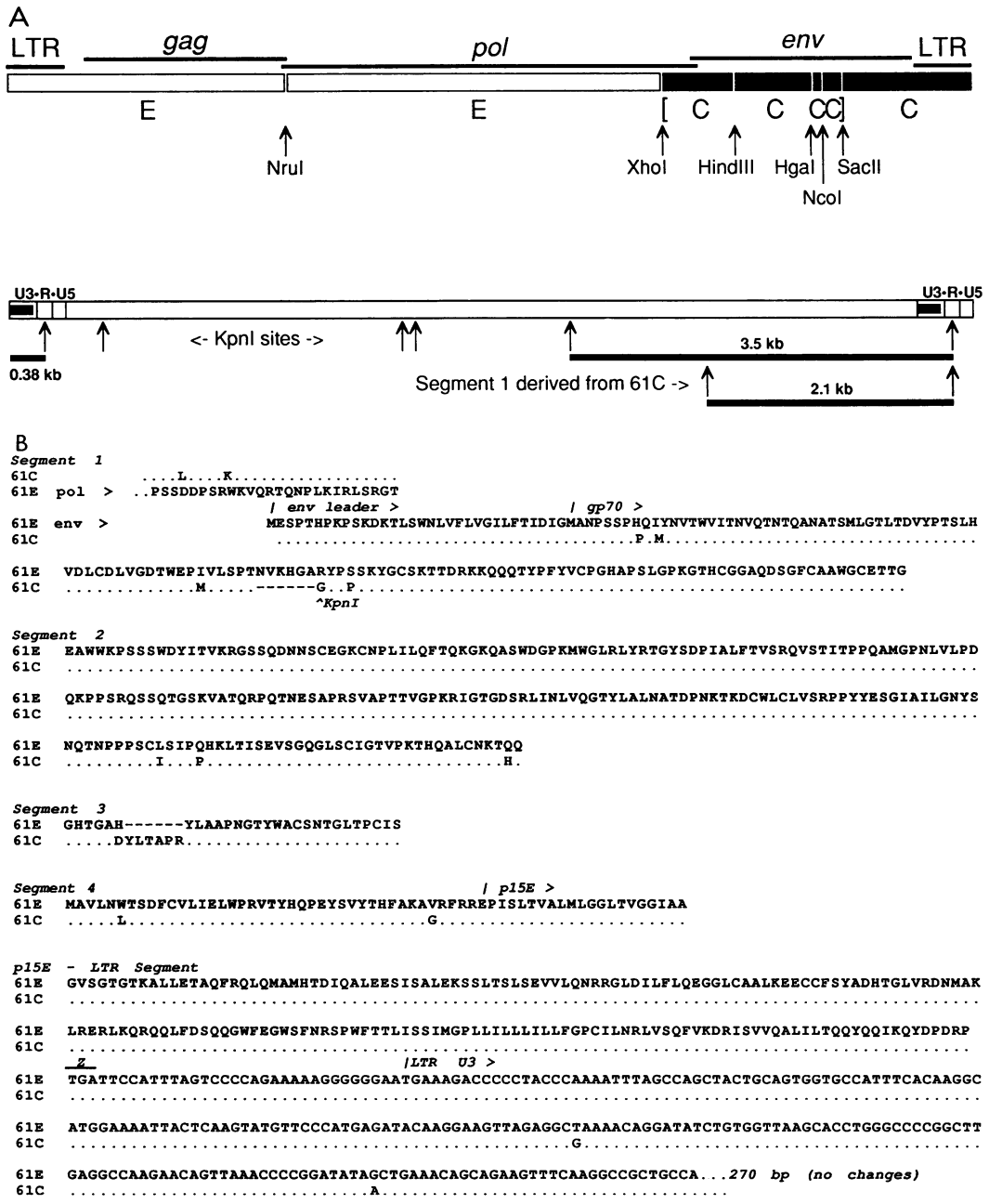


FIG. 1. FeLV gene and restriction map (A) and alignment of 61E and 61C sequences in the 3′ region of the viral genome (B). (A) Schematic diagram of the FeLV gene map and genome is shown along with restriction sites critical to generation of chimeras (upper portion of panel) and *Kpn*I fragments detected following Southern blot analysis (lower portion of panel). The region of hybridization with the exU3 probe, which is specific for the U3-LTR region of exogenous FeLV (24), is shown as a filled-in box. The 3.5- and 2.1-kb bands detected following Southern blot analysis (see Fig. 4) are indicated and correspond to internal virus bands derived from both integrated and unintegrated viral DNA. *Kpn*I fragments derived from the 3′ end of the viral genome are 3.5 kb long when the first sector of a chimera is derived from 61E and 2.1 kb long when the sector is derived from clone 61C. The 0.38-kb band is derived from the portion of the viral genome from a free 5′ end to the *Kpn*I restriction site in the U5 region of the 5′ LTR and therefore indicates the presence of UVD (20, 21). (B) The sequence shown extends from the *Xho*I site (position 5818) through the 3′ end of the proviral LTR (5, 25). Predicted primary amino acid sequences of 61E are shown in single-letter code together with sites of 61C substitutions. Deletions are indicated by dashed lines. Five regions are shown: four spanning the surface protein gp70, and one containing most of p15E and the entire LTR. Region 1 also contains a small amount of overlapping *pol* sequence. The *Kpn*I site polymorphism described above is shown in segment 1. Z corresponds to the stop codon which terminates the transmembrane protein. Beginning at this position, DNA sequence alignments are shown. We previously reported that three nucleotide changes distinguish 61E from 61C in the LTR (25). More recently, we detected an error in the 61C sequence that reduces the number of nucleotide changes to two and have now corrected it in the alignment shown here.

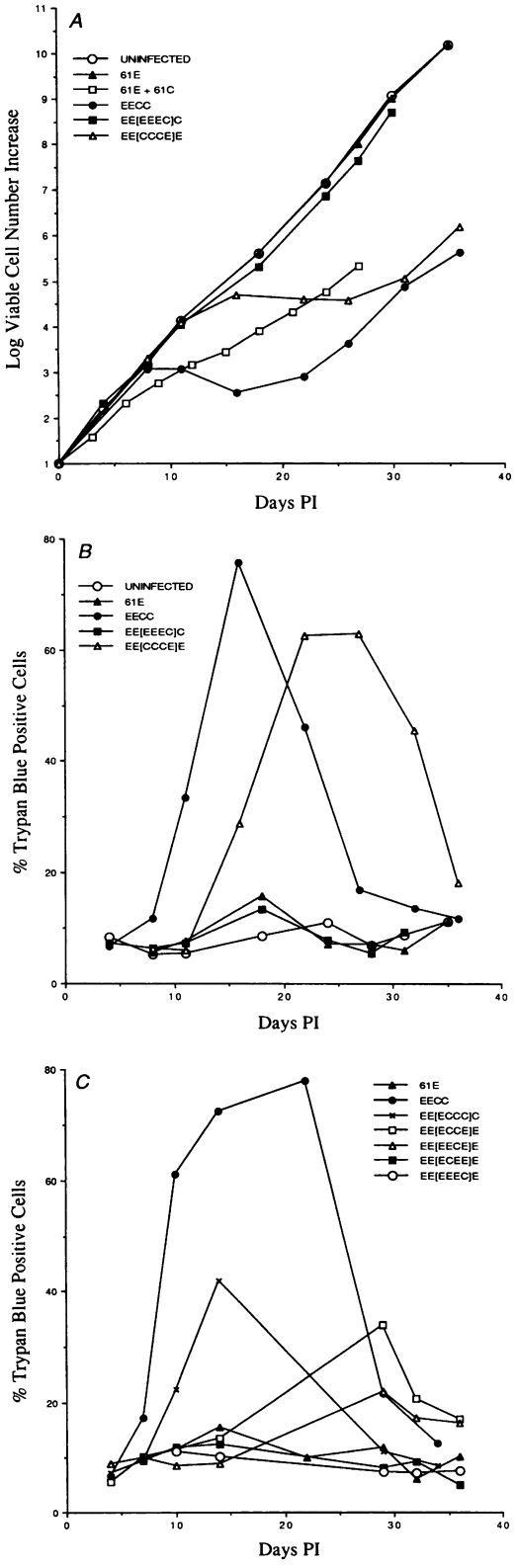


FIG. 2. Cytopathic effects induced by molecularly cloned FeLV-FAIDS viruses in feline 3201 T cells. Total numbers of viable cells (A) or percent trypan blue-positive (nonviable) cells (B and C) are given at the indicated days postinfection (PI). Each curve represents the average of duplicate infections of 2×10^6 cells with 10^5 infectious units of virus, thus, a multiplicity of infection of 0.05. Virus chimeras containing the 61C LTR were detected by 7 days postinfection; those containing the 61E LTR were first positive by ELISA at 14 days postinfection.

MATERIALS AND METHODS

Nucleic acid manipulations. Conserved restriction sites between clones 61E and 61C were used to generate fragments and to exchange 61C sequence segments into the complete 61E genome backbone. Segment 1 was bordered by an *Xho*I site in *pol* and a *Hin*dIII site in the gp70 gene, segment 2 by *Hin*dIII and *Hga*I sites in the gp70 gene, segment 3 by *Hga*I and *Nco*I sites in the gp70 gene, and segment 4 by *Nco*I and *Sac*II sites in the p15E gene (Fig. 1A). The structure of each chimera was confirmed by restriction site polymorphisms for segments 1 and 3, by the length of flanking DNA for the p15E-U3 segment, and by DNA sequencing for segments 2 and 4. Standard procedures were used for the generation and recovery of plasmid DNAs and for double-stranded DNA sequencing (40). Southern transfer and hybridization analysis was conducted as described previously (20, 24). Autoradiograms were quantitated by densitometry, using as a standard DNA from a single-cell clone-derived cell line of FeLV-infected human RD cells (20). The most intense band in these cells corresponds to 22 viral DNA copies per cell, and the weaker bands are single-copy, host-virus junction bands (20) (see Fig. 4).

3201 T-cell killing assays. Transfection was conducted by electroporation (40), and virus was detected by a capsid protein (p27) enzyme-linked immunosorbent assay (ELISA) (Synbiotics). 3201 T cells were a gift from W. D. Hardy, Jr., and E. E. Zuckerman, Memorial Sloan-Kettering Institute, New York, N.Y. (38, 43), and were grown at a density of 0.2 $\times 10^6$ to 4 $\times 10^6$ cells per ml in 1:1 L15-RPMI medium (GIBCO)–20% fetal calf serum supplemented with penicillin, streptomycin, and L-glutamine. Vi-rus ti ters from infected 3201 cells were determined in triplicate by end-point dilution and ELISA on 3201 cells.

For analysis of live and dead cells, 3201 cells were separated with a Ficoll-sodium diatrizoate mixture (LSM; Organon Teknica) (3) by layering cultured cells over an equal volume of LSM. Banded live cells and pelleted dead cells were recovered and analyzed separately by Southern transfer and hybridization.

Serum protection experiments were conducted by incubating cells in a variety of dilutions of immune serum from a cat immunized with 61C virus-transfected cells (25) or of the virus-neutralizing C11D8 monoclonal antibody (a gift from C. K. Grant) (10), and viral DNA was analyzed by Southern transfer and hybridization. The fetal calf serum used in these experiments was heat inactivated.

In vivo studies. Cultured virus supernatant (1 ml) was inoculated intraperitoneally, usually 10^4 to 10^6 tissue culture infectious units, as described previously (15). The clinical course of experimental feline immunodeficiency syndrome observed here and previously (14, 15, 25, 34), as well as that of the naturally occurring disease in cats, is similar to the clinical course of human AIDS and is characterized by

persistent infection, intractable diarrhea, progressive weight loss and emaciation, severe lymphoid depletion, and opportunistic infections.

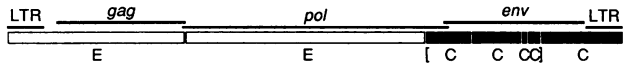
RESULTS

Generation of FeLV-FAIDS chimeras. To localize deductively the viral determinant(s) and to explore the underlying mechanisms of immunodeficiency disease induction by FeLV-FAIDS in cats, we initially generated a series of virus chimeras. A chimera we previously described (25), EECC, is composed of segments contributed by the minimally pathogenic clone 61E and the pathogenic clone 61C (Fig. 1A). The first two letters, EE, describe the origin of the *gag* and *pol* regions, respectively, which in this and all subsequent chimeras is derived from clone 61E. The 61C-derived components of EECC encompass the sequences extending from the extreme 3′ end of the polymerase (*pol*) gene through the entire *env* gene, including sequences encoding the surface glycoprotein (gp70) and transmembrane (p15E) proteins, and the 3′ long terminal repeat (LTR) (from which the U3 region of each LTR in daughter viruses would derive following reverse transcription). The chimeras generated in this study subdivide the latter portion of the genome to localize the regions within the EECC chimera contributed by 61C which are essential to its pathogenic phenotype. The third letter in each chimera designation corresponds to the regions occupied by the gp70 gene and is described by a single letter when derived wholly from one parent sequence and by four letters within brackets when subdivided into segments derived from both parent sequences; e.g., using the latter convention, clone EECC would be designated EE[CCCC]C. The differences between 61E and 61C within the first gp70 segment include two amino acid substitutions near the extreme 3′ end of the integrase-coding sequence of *pol* and five amino acid substitutions and a six-amino-acid deletion within the 5′ end of the 61C gp70 gene (Fig. 1B). The remaining three segments of the 61C gp70 contain the following differences: segment 2, three amino acid substitutions; segment 3, one amino acid substitution and a six-amino-acid insertion; and segment 4, two amino acid substitutions. The final letter of each chimera designation reflects the origin of the p15E-LTR segment, in which 61C differs from 61E only by two nucleotide substitutions in the U3 region in the LTR. The structures of all chimeric viral genome-containing plasmids were verified by restriction mapping and, when required, DNA sequencing, and the plasmids were then used for transfection of feline 3201 T cells. Infectious, virus-containing supernatants were harvested 2 to 4 weeks following transfection, and equivalent infectious titers were used in subsequent assays.

T-cell killing by FeLV-FAIDS chimeras. To identify an in vitro correlate of immunodeficiency disease induction, we assessed the affects of 61E plus 61C virus mixtures, chimera EECC, and 61E alone on growth and viability of the FeLV-negative, $CD4^+/CD8^+$ (15a) feline T-cell lymphoma line 3201 (38, 43). Transfection or infection of 3201 cells with either 61E plus 61C or EECC but not 61E alone resulted in a plateau or decrease in cell number (Fig. 2A) and death of a large percentage of the cell population, as measured by uptake of the vital dye trypan blue (Fig. 2B).

Cytopathic effects, defined by a decrease in viable number and/or an increase in trypan blue-stained (i.e., dead) cell numbers, were observed in 3201 cells infected with the EE [CCCE]E chimera but not in cells infected with the EE[EE EC]C chimera, indicating that changes within the 3′ end of *pol* and/or gp70 of 61C were essential for T-cell killing, whereas changes within the LTR and extreme C terminus of gp70 were not sufficient (Fig. 2A and B). All subsequent chimeras containing the third segment of gp70 derived from 61C were cytopathic, including EE[EECE]E, which differed from 61E by only a single amino acid change (His to Asp) and an immediately adjacent six-amino-acid (18-bp) insertion, corresponding to an imperfect direct repeat (Fig. 2C and 3). The efficiency of T-cell killing by EE[EECE]E was relatively low, indicating that surrounding sequences in gp70 and/or the LTR enhance the efficiency of cell killing.

Three chimeras containing the first segment of gp70 from 61C (EE[CEEE]E, EE[CEEE]C, and EE[CCEE]C) failed to



Virus	T-cell Killing in vitro	Number of cats Inoculated	Viremic	FAIDS	Mean Survival (days)	Viremic cats euthanized without disease (days)
EECC	++++	12	11	11	27	
EECE	+++	0				
EE[CCCE]E	+++	8	3	3	36	
EE[ECCC]C	++	4	4	4	53	
EE[ECCC]E	+	0				
EE[ECCE]C	++	0				
EE[ECCE]E	+	4	2	1	225	1 - 231
EE[EECC]C	++	8	6	6	152	
EE[EECC]E	+	0				
EE[EECE]C	+	6	6	6	263	
EE[EECE]E	+	8	8	5	230	3 - 203
EE[CECE]C	++++	8	0			
EE[CCEC]C	++	0				
EE[CEEC]C	++	4	0			
EE[CEEE]C	defective					
EE[CCEE]C	defective					
EE[CEEE]E	defective					
EE[ECEC]E	-	0				
EE[ECEC]C	-	0				
EE[ECEE]C	-	0				
EE[ECEE]E	-	4	4	0		4 - 210
EE[EEEC]C	-	0				
EE[EEEC]E	-	4	3	1	62	2 - 203
EEEC	-	0				

FIG. 3. In vitro and in vivo analyses of chimeras. Each chimera's designation provides the position of 61C sequences within the *env* and LTR sequences. For simplicity, some chimeras are referred to with a four-letter designation, i.e., EECC is the same as EE[CC CC]C, EECE is EE[CCCC]E, and EEEC is EE[EEEE]C. The composition of virus constructs was confirmed by restriction site polymorphisms within some segments, by DNA sequencing, or by differences in the length of 3′-flanking DNA. The in vitro T-cell cytopathicity of each chimera relative to EECC is indicated by one to four pluses or a minus (noncytopathic): ++++, a peak of >70% cell killing; +++, 50 to 70% cell killing; ++, 30 to 50% cell killing; +, 20 to 30% cell killing. The background level of dead cells in uninfected or 61E-infected cells was usually 5 to 15%. Induction of fatal immunodeficiency disease in vivo by the chimeras tested is also shown, including the number of cats inoculated, the number that became viremic, and the number that developed fatal immunodeficiency syndrome. Average survival time of cats with disease is indicated, as is the observation period for cats that remained healthy throughout the study. All chimeras were tested in vitro by infection with equivalent infectious units, except for EE[ECEC]C, which was tested following transfection only.

generate infectious virus. The inclusion of segment 3 or 4 from 61C (i.e., EE[CEEC]C, EE[CECE]C, and EE[CCEC]C), but not segment 2 (i.e., EE[CCEE]E), restored replication competence. Thus, changes near the C-terminal portion of the 61C gp70 complement changes near the N terminus to permit the production of infectious virus. Each of the last infectious constructs also was cytopathic, indicating that a second determinant for T-cell killing resides within the first segment of 61C (Fig. 3).

A chimera which contained both segments 1 and 3 from 61C had significantly augmented cytopathicity relative to the chimeras containing only segment 3. Segments 2 and 4 did not encode cytopathic determinants; however, segment 4 augmented the cytopathicity determined by segment 3 and also complemented segment 1 in the formation of infectious cytopathic virus. The two nucleotide changes within the U3 region of the 61C LTR enhanced cytopathicity in some chimeras and accelerated the time course for detection of virus produced by all chimeras (Fig. 2).

Genetic determinants of immunodeficiency disease induction. To identify the viral genetic requirements for fatal immunodeficiency disease induction in vivo, we inoculated weanling-age specific-pathogen-free cats with many of the chimeras discussed above (Fig. 3). Some cats included in this study were previously described (34). Nearly all cytopathic chimeras tested also induced immunodeficiency disease, and in general, the efficiency and latency of disease induction varied directly with the efficiency of T-cell killing in vitro. For example, chimeras EECC, EE[CCCE]E, and EE[ECCC]C were highly cytopathic and induced short-latency disease in all inoculated viremic animals. The EE[ECCE]E, EE[EECC]C, EE[EECE]C, and EE[EECE]E chimeras induced immunodeficiency disease at a lower frequency and with prolonged latency, consistent with their attenuated cytopathicity in vitro. Some cytopathic chimeras containing the first *env* gene section of 61C (EE[CECE]C and EE[CEEC]C), while replication competent in vitro, failed to induce viremia in cats (Fig. 3). The reason for this is unclear but likely involves the well-established age- and macrophage-related restriction of growth of some strains of FeLV (16). This restriction can be bypassed in some cases by intra-bone marrow inoculation (7), but this was not attempted during these studies.

The noncytopathic chimeras tested, with one exception, failed to induce disease in any of eight inoculated viremic cats. One cat inoculated and viremic with EE[EEEC]E died from the typical form of immunodeficiency disease after 62 days. However, in contrast to each of the 36 other cats that developed immunodeficiency disease during this study, only low levels of viral DNA were detected by Southern blot analysis in this animal (data not shown). Thus, we are unable to assess the involvement of FeLV-FAIDS in the development of disease in this animal.

T-cell killing and unintegrated viral DNA. We have previously described an association between immunodeficiency disease induction and the detection of high levels of viral DNA, largely as UVD, in bone marrow and other cells (21, 24, 25, 35). To assess the possible link between accumulation of high levels of viral DNA and cell killing in vitro, we examined viral sequences present in 3201 cells following infection with cytopathic and noncytopathic chimeras. A large increase in viral DNA copy number, much of it as UVD, was invariably detected coincident with the onset of cell killing (Fig. 4A; see also Fig. 1A for a description of probes and fragments detected). A total of 250 to 400 copies of viral DNA per cell were measured just before and during acute cell killing induced by EECC and EE[ECCC]C, whereas 30 to 100 copies per cell were measured during cell killing induced by the more attenuated chimeras. When dead and live cells were fractionated on Ficoll gradients, dead cells contained seven to nine times more UVD and up to 700 copies of viral DNA per cell (Fig. 4B). Following the acute phase, an oligoclonally infected culture of surviving cells was established that contained up to about 20 copies of viral DNA and very little UVD (Fig. 4B). In contrast, cells infected with noncytopathic viruses accumulated UVD only transiently during initial stages of infection and developed an average of one to four integrated proviruses per cell (EE[EEC]C in Fig. 4A). The decrease in viral DNA copy number in noncytopathic infections may reflect a low level of cell killing or the extrachromosomal degradation of unintegrated DNA.

T-cell killing is blocked by immune sera. Internally synthesized envelope glycoproteins are thought to mediate superinfection interference by rendering their cognate receptor unavailable for reattachment and/or entry of extracellular virus (reviewed in reference 48). Accumulation of high levels of proviral and unintegrated viral DNA might occur as a result of a failure or delay in the establishment of superinfection interference, that is, a failure to limit subsequent rounds of reinfection (reviewed in reference 47). To test this possibility, 3201 cells were infected with the cytopathic chimera EECC or the noncytopathic chimera EEEC at relatively high multiplicities (approximately 1 infectious unit per cell) and then grown in the presence or absence of cat immune sera in an effort to block reinfection of cells (Fig. 5). Indirect immunofluorescence assays with a gp70-specific monoclonal antibody indicated that 60 to 80% of the cells in each culture were infected by 2 days postinfection (data not shown). EECC-infected cells not maintained in immune serum underwent cytopathic depletion beginning at day 4 postinfection. In contrast, all EEEC-infected cells and EECC-infected cells incubated in 61C-immunized cat serum displayed normal growth kinetics over a 2-week interval. EECC-infected cells protected by immune serum accumulated only about 0.75 copy per cell at day 4 and 2 copies per cell by day 10 postinfection (data not shown). When immune serum was removed at day 7, cell killing commenced (Fig. 5). A coincident increase of viral DNA to >100 copies per cell was observed (data not shown). Cell killing could be blocked for at least 1 month by continually replenishing the culture with 61C-neutralizing cat serum or with the FeLV-neutralizing monoclonal antibody C11D8 (10) but not with nonimmune cat serum (data not shown).

DISCUSSION

A functional map of the critical 3′ end of the EECC genome is shown in Fig. 6. These findings emphasize that wide ranges of retroviral pathogenic potential exist in viruses with subtle differences in sequence and that hidden within a small degree of genetic variation there may be changes responsible for the conversion of a minimally pathogenic virus to a virulent pathogen. In this case, we found that critical changes in the extracellular envelope glycoprotein led to a low level of T-cell killing and immunodeficiency disease-inducing capacity, but as found in the animal (each virus had been directly cloned from tissue DNA [25]), some of the viruses that acquired this change (e.g., 61C) had also acquired additional changes that enhanced their growth kinetics and T-cell-killing capacity. In addition to enhanced T-cell killing, the 61C (and EECC) virus owes some of its

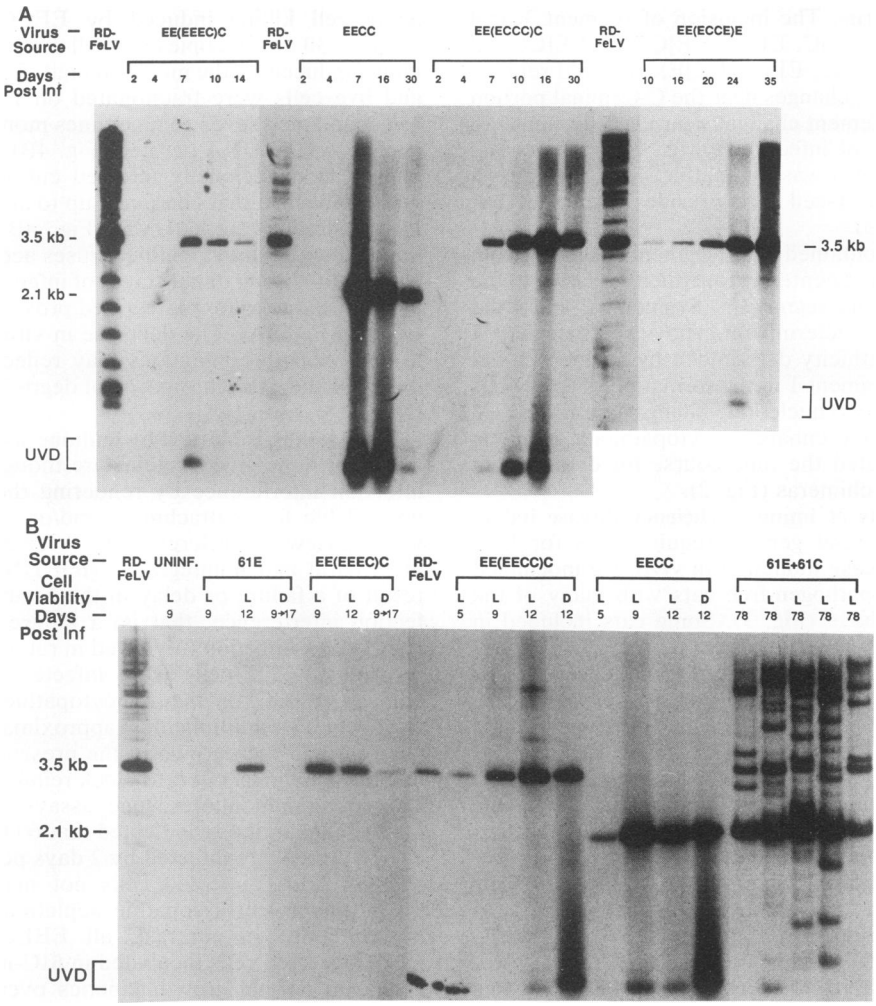


FIG. 4. Viral DNA analysis in 3201 T cells following infection with FeLV-FAIDS chimeras. (A) DNA was extracted from cell samples at the designated days postinfection, and 10 μg was digested with *Kpn*I, electrophoresed, blotted, and probed with the exU3 probe (24). The left lane of each blot provides a copy number standard and is derived from a single-cell clone of the RD-FeLV cell line (20). In these cells, the most intense band corresponds to 22 copies per cell and the weaker bands are single-copy, host-virus junction bands (20). Region 1 has a polymorphism at a *Kpn*I restriction site (Fig. 1A) giving rise to internal hybridizing bands of either 3.5 kb (if derived from 61E) or 2.1 kb (if derived from 61C). Transient accumulation of UVD from the noncytopathic EE[EEEC]C chimera occurred at day 7 postinfection but was undetectable at later times. Two UVD bands are noted in some cells, most likely indicating the presence of circular (upper band) as well as linear viral DNA genomes. Densitometric analysis of bands and comparison with RD-FeLV indicated a steady-state proviral copy number of about two to five copies per cell for EE[EEEC]C, which was typical of all noncytopathic chimeras. The cytopathic constructs EECC and EE[ECCC]C accumulated virus loads of 300 to 500 copies per cell 7 to 16 days after infection. Cytopathicity and UVD accumulation were delayed in EE[ECCE]E-infected cells, consistent with slower virus growth kinetics resulting from the 61E-derived LTR. Elevated levels of UVD were detected for all pathogenic chimeras. (B) Dead cells were purified by Ficoll gradient fractionation; >90% of cells in the "dead" fraction (lanes D) stained with trypan blue, while <1% of the cells in the "live" fraction (lanes L) stained with trypan blue. Dead cells from cultures infected with cytopathic chimeras (e.g., EE[EECC]C and EECC) were enriched for viral DNA and especially UVD. Smears of viral DNA in lanes with dead-cell DNA represent partial degradation of genomic DNA. By 78 days postinfection, oligoclonal T-cell lines had grown out from separate cultures infected with cytopathic viruses (here a 61E-61C mixture; no RD-FeLV copy number standard is shown for this panel); virus produced by these cells are fully cytopathic on naive 3201 cells. The amounts of viral DNA recovered from cultures undergoing cell killing varied, and variable amounts were loaded on these gels. The amounts of cellular DNA loaded were taken into account when determining viral DNA copy number. In two cases (61E and EE[EEEC]C), DNA was pooled from dead cells at days 9 through 17 (9→17).

acutely pathogenic behavior in vivo to an apparent intestinal tropism. The immediate cause of death in these animals is intractable diarrhea, progressive weight loss, and emaciation associated with massive cytopathic virus replication in intestinal tissue (16, 23). Similarly, the most acutely pathogenic simian immunodeficiency virus strain described to date, SIVsmmPBj14, has been shown to lead to death due to diarrhea and severe weight loss superimposed on marked lymphopenia (9). This strain also displays an intestinal tropism determined by subtle changes in the viral genome (4). The intestine-specifying mutations, however, have yet to be determined in either system.

That immune serum prevents the accumulation of high levels of viral DNA and the death of already infected cells strongly suggests that continual reinfection is a critical component of the mechanism of T-cell killing by FeLV-

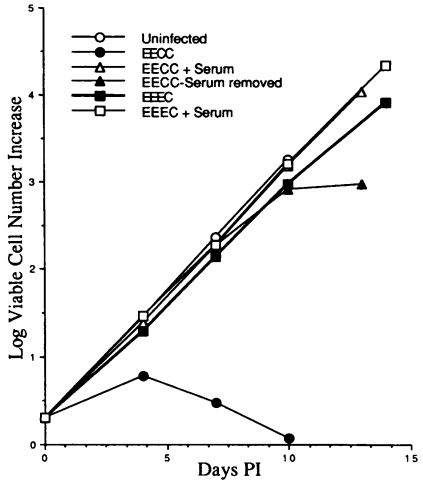


FIG. 5. Protection of virus-infected T cells by 61C-immune serum. Cell growth is represented by a plot of the logarithm of the cumulative viable cell number increase following infection. Each plot represents an average of two infections. For each experiment, 2×10^5 cells were infected at a multiplicity of infection of approximately 1 for 12 h at 37°C. Cells were then pelleted and resuspended in medium with (+ serum) or without (− serum) 61C-immune cat serum (used at a 1:15 dilution). Cells infected with the noncytopathic chimera EEEC and incubated with or without 61C-immune cat serum grew logarithmically (as did uninfected cells); thus, cat serum did not affect cell growth. EECC-infected cells were rapidly killed when not incubated in cat immune serum but remained viable and maintained approximately normal growth kinetics for 2 weeks when incubated in cat immune serum. EECC-infected cells removed from cat serum at day 7 immediately underwent cell killing.

FAIDS. Parallel results have been reported previously for cell killing associated with avian oncoviruses, HIV-1, and the visna lentivirus (12, 29, 49). In HIV-1, cell surface reactivity of the CD4 receptor molecule is blocked or down-regulated in infected cells, while normal amounts of receptor mRNA are maintained (39, 45). Thus, we may infer from our experiments that changes in conformational aspects of the gp70 envelope glycoprotein critical to its role in superinfection interference are impaired in the T-cell-cytopathic FeLV-FAIDS viruses and that T-cell killing results from failure of gp70 to prevent further attachment or penetration by extracellular virus. We have previously obtained biochemical evidence that the glycoprotein structure of the cytopathic EECC gp70 is significantly altered compared with the gp70 of 61E (32, 33). EECC gp70 has a much slower rate of envelope precursor processing and greater mass that is due to more high-mannose N-linked oligosaccharides, even though both proteins have the same predicted glycosylation sites. Moreover, its association with the p15E transmembrane protein is altered relative to 61E, as indicated by recognition of a p15E epitope normally hidden by association with gp70 (32).

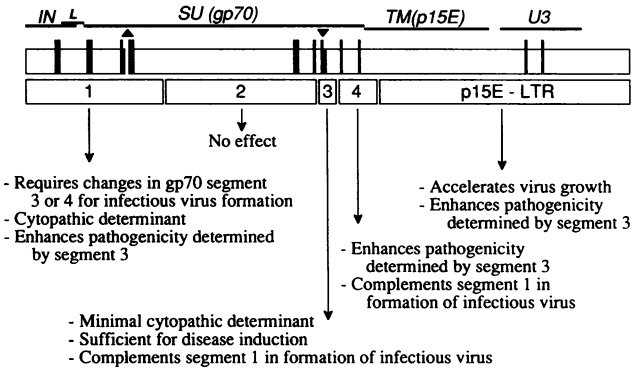


FIG. 6. Functional significance of mutations within the EECC virus chimera. The top of the figure shows the coding and regulatory (U3) sequences in the 61C-derived portion of the EECC chimera. The open bar represents 61E sequence, while vertical lines indicate amino acid substitutions within the predicted 61C *pol* or gp70 protein and nucleotide substitutions in the 61C LTR. Up- and down-pointing triangles indicate the positions of the six-amino-acid insertion and deletion, respectively. The segments numbered 1 through 4 and p15E-LTR correspond to the sequences shown in Fig. 2. The effects of the mutations described in this study are summarized in the lower part of the figure. IN, integrase protein; L, leader peptide; SU, surface protein (gp70); TM, transmembrane protein (p15E).

The biochemical mechanisms by which continual reinfection may lead to cell death remain to be established. However, possibilities include (i) cell membrane disruption by continual virus entry or budding; (ii) insertional mutagenesis of the host genome; (iii) qualitative changes resulting in direct gp70 cytotoxicity; (iv) cytotoxicity resulting from a quantitative increase in viral protein abundance, potentially associated with altered glycosylation patterns; (v) inappropriate cell signaling via continual virus-receptor interaction; and (vi) direct toxicity of viral DNA as UVD. In any of these cases, the cellular mechanism appears to include programmed cell death or apoptosis (24a, 47a), since 3201 cells undergo nuclear condensation and DNA degradation to a nucleosomal pattern during cell killing (5a).

The accumulation of viral DNA and the cytopathic effects of subgroup B, D, and F avian leukosis viruses have been shown to be determined by envelope sequences, and the cytopathic potential of subgroup B avian leukosis virus is dependent on functional interaction with a cell receptor (2, 6, 48). Similarly, we observed low infectivity and weak cytopathicity of EECC in feline fibroblasts, indicating that cell-specific factors partially mediate viral cytopathic potential. Some evidence also links HIV-1 cytopathicity with target cell superinfection, since although *env* protein-induced membrane fusion is an important pathway for cell killing, killing also occurs in the absence of syncytium formation (44, 45). Moreover, elevated levels of UVD have been detected in HIV-infected lymphoid cells in vivo and in vitro (26, 28, 29, 37, 41, 42), at levels comparable to that seen in cytopathic avian oncovirus infections, although not to as high a level as reported here and in visna lentivirus-infected cells (12, 17, 49).

The subtle changes shown here that affect superinfection interference may have a significant impact on the emergence of new virus strains that existed within the initial inoculum, arose de novo, or resulted from reinfection of the host. With HIV, its intermittent detection as UVD in vivo (42) may mark the emergence of new variants and superinfection. A parallel may also be drawn from the analysis of HIV strains isolated during the course of clinical progression to AIDS. Strains obtained during the asymptomatic phase of infection have attenuated growth and syncytium-inducing characteristics in vitro (slow-low viruses), whereas strains isolated later during disease have an accelerated (rapid-high) phenotype (reviewed in reference 8). By analogy, the slow-low viruses have temporal, growth, and cell-killing characteristics in common with the 61E common-form virus, whereas the rapid-high viruses have the characteristics of late appear-

ance, rapid growth, and acute cytopathicity in common with the 61C FeLV-FAIDS pathogen. Whether the slow-low and rapid-high HIV correspond to minimal and acute pathogens in the development of human AIDS remains to be established.

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