

Natural Feline Leukemia Virus Variant Escapes Neutralization by a Monoclonal Antibody via an Amino Acid Change Outside the Antibody-Binding Epitope†

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We have molecularly cloned a natural variant of feline leukemia virus subtype B. This isolate is unique in that it is not neutralized by a monoclonal antibody which neutralized all other feline leukemia virus isolates tested, including members of the A, B, and C subtypes. Western immunoblotting indicated that the monoclonal antibody was less able to bind to the gp70 of the resistant isolate (designated λ B1) than to the gp70s of susceptible viruses. Nucleotide sequence analysis of the envelope gene of λ B1 revealed a high degree of homology with the susceptible Snyder-Theilen, Gardner-Arnstein, and Rickard subtype B isolates, including the presence of a 5-amino-acid minimal binding epitope required for binding by the neutralizing monoclonal antibody. The only change within the vicinity of this epitope was in a single nucleotide, and this difference changed a proline residue to leucine three amino acids from the N terminus of the binding epitope. Competitive binding studies with synthetic peptides indicated that substitution of leucine for proline resulted in a 10-fold decrease in the ability of the peptide to compete for antibody binding to native antigen. The results are consistent with the interpretation that this amino acid change lowers the affinity of antibody binding, resulting in failure of the antibody to neutralize the variant virus.

We recently reported the identification of several neutralizing sites on the envelope gene of Gardner-Arnstein feline leukemia virus (FeLV), defined by antibodies to synthetic peptides (4). Fortuitously, one of the peptides containing a neutralizing site was recognized by a neutralizing monoclonal antibody, C11D8 (8), prepared against intact virions. By preparing a nested set of peptides around this region, we were able to define a contiguous 5-amino-acid epitope required for binding by C11D8 (4). As expected, this 5-amino-acid epitope was conserved in viruses neutralized by C11D8, including members of FeLV subtypes A, B, and C. However, a natural variant of FeLV subtype B was resistant to neutralization by C11D8. Herein, we report the nucleotide sequence of the envelope gene of the resistant variant and the unexpected observation that the C11D8-binding epitope is conserved in this isolate. The results indicate that a single amino acid change outside the minimal antibody-binding epitope causes a reduction in antibody affinity and failure of the antibody to neutralize the virus.

MATERIALS AND METHODS

Virus strains and cell lines. Viruses were propagated on the dog thymus-derived Cf2Th cell line (ATCC CRL 1430). Viruses used include a molecularly cloned transfected isolate of Gardner-Arnstein FeLV subtype B (5), a molecularly cloned transfected type B isolate from cells infected with Rickard virus (6), and three prototype isolates representing subgroups A, B, and C which were obtained from Oswald Jarrett and subsequently molecularly cloned in our laboratory. The last viruses were transfected into Cf2Th cells, and the resultant virus progeny was used for subsequent studies.

The cloned subtype B virus, designated λ B1, was unique among the viruses we tested in its resistance to neutralization by C11D8 monoclonal antibody and is the primary subject of this report.

Peptide synthesis and nucleotide sequence analysis. Peptides derived from the sequence of Gardner-Arnstein envelope gene (5) and λ B1 were prepared as described elsewhere (11, 16). Nucleotide sequence analysis of the envelope gene of λ B1 was performed as described elsewhere (15).

Viral infectivity and neutralization. In vitro virus infectivity and titers were determined by a modified immunoblotting procedure as previously described (7, 12), except that virus-infected cells were detected with rabbit anti-p27 antiserum and peroxidase-conjugated goat anti-rabbit immunoglobulin instead of anti-gp70 antibody and ¹²⁵I-labeled *Staphylococcus aureus* protein A (Sigma Chemical Co., St. Louis, Mo.). Peroxidase conjugate was detected with 4-chloro-1-naphthol dye (9). Prepared antisera were tested with the corresponding prebleed sera for ability to neutralize FeLV in vitro by preincubating virus supernatants for 30 min at 37°C prior to dilution and addition to cell monolayers.

Immunoblotting procedures. Peptides were bound to nitrocellulose in the presence of carrier bovine serum albumin (0.1%) by fixation in 0.8% glutaraldehyde in phosphate-buffered saline. The blots were washed extensively with deionized water and then blocked and reacted with antibody essentially as described for Western blotting with nonfat dry milk (BLOTTO; 12) as diluent. Immune reactivity was detected by using either peroxidase-conjugated anti-immunoglobulin or ¹²⁵I-labeled *S. aureus* protein A.

Peptide competition assays. To assess the degree of epitope "mimicry" in synthetic peptides relative to the epitope in intact virus gp70, competition experiments were performed. Virus was affixed to 96-well tissue culture plates and then reacted with either untreated C11D8 monoclonal antibody or

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C11D8 which had been treated with various concentrations of synthetic peptides containing the epitope bound by C11D8 (4). The amount of antibody bound to the virus-coated plates was then assessed by enzyme-linked immunosorbent assay (3) with goat anti-mouse antibody conjugated to peroxidase. Inhibition was then assessed and quantitated as the peptide concentration which gave 50% inhibition of maximal antibody binding to virus.

RESULTS

The nucleotide sequence and predicted amino acid sequence of the envelope gene of a natural variant of FeLV subtype B is shown in Fig. 1. This isolate, designated λ B1, is unique among the isolates we have examined in its resistance to neutralization by the C11D8 monoclonal antibody described above. The structure of the envelope gene of λ B1 is typical of that of other FeLVs examined (5, 6, 14, 19, 22) and comprises two envelope proteins, gp70 and p15E. The predicted amino acid sequence indicates an envelope polyprotein of 662 amino acids composed of a 32-amino-acid leader sequence followed by a gp70 of 432 amino acids and a p15E molecule of 197 amino acids. The sequence contains no deletions or insertions relative to previously reported subtype B envelope genes (5, 6, 19) and most closely resembles the Snyder-Theilen isolate (19). Comparison of the nucleotide sequences of the envelope genes of λ B1 and Snyder-Theilen isolate (Fig. 1) indicates that there are 35 nucleotide changes between these two isolates, resulting in an overall homology of 98.4%. However, this comparison also indicates that λ B1 is unique and does not arise via a subtle point mutation of the Snyder-Theilen virus. The distribution of nucleotide changes is particularly intriguing because only 5 changes occur in gp70, while the remaining 27 changes occur in p15E. It is also of interest that of the five changes in gp70, four result in amino acid changes (Fig. 1). Of the 27 changes in p15E, only 2 result in amino acid changes (Fig. 1). Barring the existence of a higher mutation rate within the sequences encoding p15E relative to gp70 (a conclusion not evident from the published sequences), the results imply that λ B1 arose via recombination between the Snyder-Theilen isolate and an as yet unreported FeLV isolate. Others (22) have proposed that the subtype B viruses arise via recombination of subtype A viruses and viral elements endogenous to the cat. Possibly, the p15E sequences of λ B1 were contributed by these endogenous elements, but final interpretations must await publication of the endogenous virus sequences.

Of particular interest to this report is the sequence in proximity to the amino acid-binding epitope of the C11D8 antibody (Fig. 1). Only one nucleotide change occurs near this region (nucleotide 786, Fig. 1) in the neutralization-resistant λ B1 gp70. Comparisons of the predicted amino acid sequences of the gp70s of λ B1 and three other FeLV subtype B isolates are shown in Fig. 2. The Snyder-Theilen (19), Gardner-Arnstein (5), and Rickard (6) isolates are all susceptible to neutralization by the C11D8 monoclonal antibody (4; data not shown). Only two amino acids were totally unique to λ B1 relative to the other three subtype B gp70s (Fig. 2). One of these unique amino acids resulted from the single nucleotide change noted above. Thus, λ B1 contains leucine instead of proline at position 242, three amino acids from the N terminus of the binding epitope (Fig. 2). Since this was the only change near the antibody-binding epitope, we have concentrated specifically on defining the influence of this change on binding by C11D8.

We performed Western blot analyses under denaturing conditions to determine whether C11D8 could bind to λ B1

gp70 (Fig. 3). Binding to gp70 of λ B1 was, in fact, detectable (Fig. 3A, lane 1). However, binding of C11D8 to gp70 of λ B1 was less (approximately 100-fold lower) than to gp70 of Gardner-Arnstein FeLV (Fig. 3A, lane 2) and was negligible under stringent conditions (Fig. 3B, lane 1). Similar results were obtained with C11D8 versus Gardner-Arnstein FeLV and λ B1 in enzyme-linked immunosorbent plate assay under nondenaturing conditions (data not shown). The results suggested that C11D8 binds to λ B1 with lower affinity than it does to the susceptible Gardner-Arnstein isolate. Apparently, the proline-to-leucine amino acid change outside the binding epitope induced a conformational change, resulting in a reduction of antibody affinity. To test this hypothesis, we developed a competition assay whereby synthetic peptides were used to compete for antibody binding to intact virus affixed to tissue culture plates (Fig. 4). This assay measured the degree to which a given peptide mimics the presentation of the binding epitope in the native antigen. Moreover, the assay determined the consequences of amino acid changes on the ability of the peptides to compete for antibody binding to native antigen. Peptide I-26 (4) and another peptide (I-26L), equivalent to the I-26 peptide but containing the proline-to-leucine amino acid change unique to the λ B1 isolate, were synthesized and tested. The best results were obtained with the I-26 peptide, which caused 50% inhibition of antibody binding to virus at a peptide concentration of 1.0 nM. In contrast, the I-26L peptide produced 50% inhibition at 10 nM, a 10-fold decrease relative to that of the I-26 peptide (Fig. 4). This decrease is not as great as the decrease observed in binding of C11D8 to the native molecule of the resistant λ B1 isolate. However, we do not consider this surprising because short peptides can assume multiple conformations in solution (13). It would be expected that the epitope would be considerably more restricted in the native molecule. Taken together, these results indicate that, although absolute binding by the C11D8 antibody requires only the 5-amino-acid binding epitope, the degree of binding is strongly influenced by amino acid sequences outside the binding epitope.

At present, no data are available regarding the three-dimensional structure of the gp70 molecule, so one cannot accurately predict the structure of this epitope in the native molecule. However, analysis of this region based on the predictions of Chou and Fasman (2) (Fig. 5) suggests that changing proline to leucine may have a profound consequence on the beta-turn potential around the epitope. The MGPNL epitope (Fig. 5) has a high propensity for reverse-turn formation, as does the region three amino acids to the left in the susceptible Gardner-Arnstein virus gp70 (Fig. 5A). However, the latter region in the λ B1 gp70, in which proline has been substituted by leucine, has a low propensity for reverse-turn formation (Fig. 5B). Such one-dimensional analysis cannot take into account influences of one region of the molecule on another and may or may not reflect the actual configuration of the epitope within native gp70. However, the data are consistent with the hypothesis that a conformational change occurs within this region of resistant λ B1 gp70, resulting in decreased antibody affinity and failure of the antibody to neutralize the virus.

DISCUSSION

The envelope genes of FeLV are highly polymorphic and contain both constant and variable regions (5, 6, 14, 19, 22). Nucleic acid sequence analyses have revealed that, in particular, the gp70s of these viruses contain hypervariable

CTGCAGGGCCAACCAACATCAGGACCCCTCAGAAGGCCCGAGCTCGGACGATCCATCGAGATGGAAGGTCCAACGCACCCAAAACCCCTCTAAGATAAG 100
Leader
M E G P T H P K P S K D K
 T F S W D L M I L Y G V L L R L D V G M ^{GP70} A N P S P H Q I Y N V T W 200
 ACTTCTCGTGGGACCTAATGATTTTGGTGGGGTCTTATTAAGACTAGACGTGGGAATGGCCAAACCTAGTCCGCACCAGATATAATGTAACCTGGA
 T I T N L V T G T K A N A T S M L G T L T D A F P T M Y F D L C D I 300
 CAATAACCAACCTTGTAACTGGAACAAAGGCTAATGCCACCTCCATGTTGGAAACCCGTGACAGACGCTTCCCTACCATGTATTTGACTTATGTGATAT
 I G N T W N P S D Q E P F P G Y G C D Q P M R R W Q Q R N T P F Y 400
 AATAGGAAATACATGGAACCCCTCAGATCAGGAACCATCCAGGGTATGATGTGATCAGCCTATGAGGAGGTGGCAACAGAGAAACACACCCCTTTTAT
C
 V C P G H A N R K Q C G G P Q D G F C A V W G C E T T G E T Y W R 500
 GTCTGTCCAGGACATGCAACCGGAAGCAATGTGGGGGCCACAGATGGGTTCTGCGCTGTATGGGGTTCGAGACCACCGGGAAACCTATTGGAGAC
 P T S S W D Y I T V K K G V T Q G I Y Q C S G G G W C G P C Y D K A 600
 CCACCTCCTCATGGGACTACATCACAGTAAAAAAGGGTTACTCAGGGAATATATCAATGTAGTGGAGGTGGTTGGTGGGCCCTGTTACGATAAAGC
 V H S S I T G A S E G G R C N P L I L Q F T Q K G R Q T S W D G P 700
 TGTTCACTCCTCGATAACGGGAGCTAGTGAAGGGGGCCGGTGCAACCCCTTGATCTTGCAATTTACCCAAAAGGGAAGCAAAACATCTTGGGATGGACCT
 K S W G L R L Y R S G Y D P I A L F S V S R Q V M T I T L P Q A ^M 800
 AAGTCATGGGGCTACGACTATACCGTTACGATATGACCCATAGCCCTGTTCTCGGTATCCCGCAAGTAAAGACCATTACGCTGCCACGGCAATGG
C
G P N L V L P D Q K P P S R Q S Q I E S R V T P H H S Q G N G G T P 900
GACCAAACTAGTCTCGCTGATCAAAAACCCCATCCAGGCAATCTCAATAGAGTCCGAGTAACACCTCACCATTCCCAAGGCAACGGAGGCCCC
 G I T L V N A S I A P L S T P V T P A S P K R I G T G N R L I N L 1000
 AAGTATAACTCTGTTAATGCTCCATTGCCCTCTAAGTACCCCTGTCAACCCCGCAAGTCCCAACGTATAGGGACAGGAAATAGGTTAATAAATTTA
 V Q G T Y L A L N V T N P N K T K D C W L C L V S R P P Y Y E G I 1100
 GTGCAGGGACATATAGCTTTAAATGTCATAACCCCAACAAAACCTAAAGACTGTGGCTCTGTCTGGTCTCCGACCGCCCTATTATGAAGGAATTG
A
 A V L G N Y S N Q T N P P S C L S D P Q H K L T I S E V S G Q G S 1200
 CGGTATTGGGAATACAGCAACCAACCAACCCCCCTCCTGCCTATCCGACCCACAACATAAACTGACTATATCAGAAGTGTCCGGCAAGGTT
T
 C I G T V P K T H Q A L C K K T Q K G H K G T H Y L A A P S G T Y 1300
 TGTCATGGGACTGTTCTAAGACCCACCAAGCTTTGTGCAAAAAGACAAAAAGGACATAAAGGGACTCACTATCTAGCAGCCCTAGCGGTACCTAC
 W A C N T G L T P C I S M A V L N W T S D F C V L I E L W P R V T 1400
 TGGCATGCAACACCGGGCTAACCCATGCATTCCATGGCAGTGCTCAATGGACCTCTGATTTTGTGCTAATCGAATTGTGGCCAGAGTGACCT
T
 Y H Q P E Y V Y T H F D K T V R L R R ^{P15E} E P I S L T V A L M L G G L T 1500
 ACCATCAACCGAATATGTTTACACACATTTGACAAAACCTGTCAGGCTCCGAGAGAACCAATATCGCTAACCGTTGCCCTTATGTTAGGAGGACTCAC
A
 V G G I A A G V G T G T K A L L E T A Q F G Q L Q M A M H T D I Q 1600
 TGTAGGGGCATAGCCGAGGGTGGAAACGGGACTAAAGCCCTCCTCGAAAAGCCAGTTCGGACAACTACAAATGGCCATGCACACAGATATCCAG
A C
 A L E E S I S A L E K S L T S L S E V V L Q N R R G L D I L F L Q 1700
 GCCCTGGAAGAGTCAATTAGTGCCTTAGAAAAATCCCTGACCTCCCTCTGAGGTAGTCTTACAAAACAGGCGAGGCTGGATATTCTGTCTTACAGG
A G A I A
 E G G L C A A L K E E C C F Y A D H T G L V R D N M A K L R E R L K 1800
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C C G C T C A C
 Q R Q Q L F D S Q Q G W F E G W F N K S P W F T T L I S S I M G P 1900
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A
 L L I L L L I L L F G P C I L N R L V Q F V K D R I S V V Q A L I 2000
 TTACTTATCCTGCTCCTAATTCCTCTTCCGCCATGCATCCTTAACCGATTGGTGCAATTCGTAAGACAGAATATCTGTGGTACAAGCCTTAATTT
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 L T Q Q Y Q Q I K Q Y D P D Q P 2047
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A G

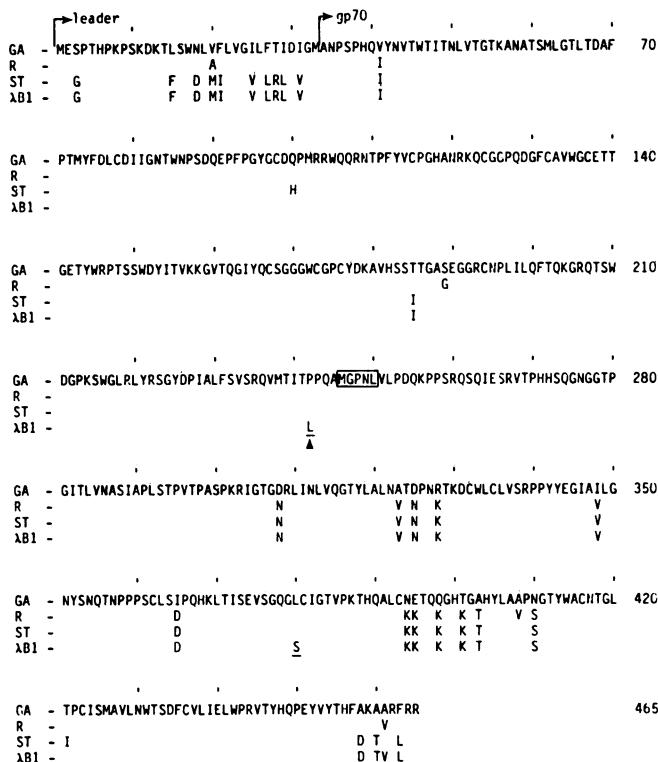


FIG. 2. Comparison of the amino acid sequences of λ B1 gp70 and gp70s of three other subtype B FeLVs. GA, Gardner-Arnstein (5); R, Rickard subtype B (6); and ST, Snyder-Theilen (19). Gardner-Arnstein gp70 is shown in its entirety; only changes are shown for other isolates. Boxed amino acids constitute the 5-amino-acid binding epitope for monoclonal antibody C11D8 (8), which neutralizes Gardner-Arnstein, Rickard subtype B, and Snyder-Theilen isolates but not λ B1. Underlined amino acids are changes unique to λ B1. Arrow denotes change in λ B1 near the antibody-binding epitope.

regions. Since gp70 is the major target for neutralizing antibodies, it has been assumed that these hypervariable regions provide the immunological variability which facilitates escape via resistant variants. Certainly, the major A, B, and C subtypes of FeLV can be delineated by amino acid sequences unique to each subtype (22). Since the subtypes can be defined via differential neutralization (20), certain neutralizing responses must be associated with these regions. However, the C11D8 monoclonal antibody used in these studies reacts at an epitope common to all three subtypes and neutralizes across subtype boundaries. Thus, this region provides a good target for the production of a broadly reactive anti-synthetic peptide vaccine. The analysis of the λ B1 isolate described above, however, indicates that single amino acid changes which can facilitate escape can occur, even at relatively conserved sites. The results show that changes outside the antibody-binding site can alter the

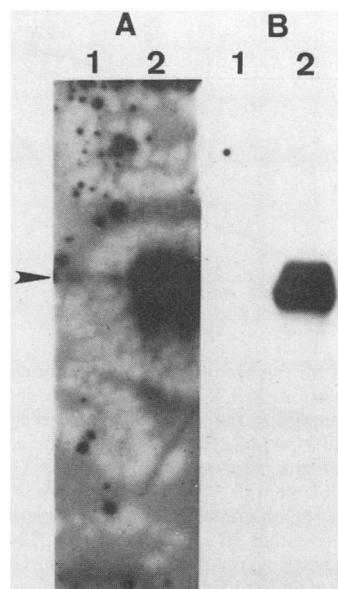


FIG. 3. Western blot of monoclonal antibody C11D8 versus λ B1 and Gardner-Arnstein gp70s under low- and high-stringency conditions. Lanes: 1, λ B1; 2, Gardner-Arnstein isolate. (A) Western blot was performed in the presence of 3% bovine serum albumin in phosphate-buffered saline. Antibody incubations, washing steps, and subsequent reaction with 125 I-labeled *S. aureus* protein A (Sigma) were all carried out in bovine serum albumin in phosphate-buffered saline. (B) Western blot was performed in BLOTTO as previously described (12). After reaction with protein A, the blot was further washed in buffer containing 0.5 M LiCl, 1.0% Nonidet P-40, and Tris hydrochloride (pH 8.5; 21). Arrow denotes detectable binding of C11D8 to λ B1 under low-stringency conditions (panel A, lane 1) that is absent under high-stringency conditions (panel B, lane 1).

structure such that the antibody can no longer neutralize the virus.

The results also show that mere binding to a critical epitope is not sufficient for neutralization, since C11D8 can still recognize and associate with λ B1 gp70 via the 5-amino-acid binding epitope. Apparently, the decrease in alacrity of binding is responsible for failure of the antibody to neutralize λ B1. This assertion is supported by the observation that a peptide containing leucine instead of proline at a position three amino acids from the N terminus of the binding epitope (the difference between susceptible and resistant FeLVs) shows a 10-fold decrease in ability of the peptide to compete for C11D8 binding to native viral antigen.

To fully appreciate the differences between the gp70s of resistant and susceptible viruses, it will be necessary to determine the three-dimensional structures of these molecules. Recent studies of the three-dimensional structure of poliovirus offer interesting parallels to our study. It has been found by comparing the three-dimensional structure of the Mahoney strain of polio (10) to that of the type 3 Sabin strain

FIG. 1. Nucleotide and predicted amino acid sequences of the envelope gene of λ B1 FeLV. The virus was cloned from a partial *Mbol* library in EMBL 3 (stratagene), prepared from DNA of chronically infected Cf2Th cells. An infectious clone was selected and subcloned by utilizing an *Eco*RI site present in the long terminal repeats. A *Pst*I fragment extending from the beginning of *env* (first 6 bases of the sequence shown) to the U3 region of the 3' long terminal repeat was then subcloned and sequenced by chemical sequencing methods (15). The region encoding the 5-amino-acid antibody-binding epitope (MGPNL) of primary interest to this report is boxed. Underlined nucleotides shown below the sequence of λ B1 coincide with nucleotide changes in the Snyder-Theilen envelope gene (19). Asterisks denote nucleotide changes which result in amino acid changes.

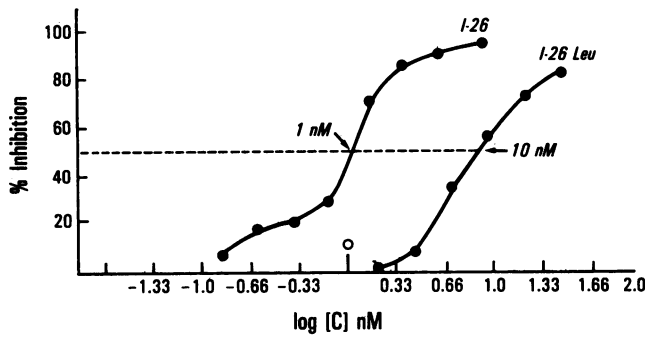


FIG. 4. Curves for the binding of C11D8 monoclonal antibody to intact Gardner-Arnstein FeLV in the presence of competing synthetic peptides containing the antibody-reactive epitope. The I-26 peptide, QVMTITPPQAMGPNLVLP, is predicted from the nucleotide sequence of the neutralization-sensitive Gardner-Arnstein FeLV (5). The I-26L peptide, QVMTITLPQAMGPNLVLP, corresponds to the same region predicted from the nucleotide sequence of the neutralization-resistant λ B1 FeLV (residues 232 to 253 of Fig. 2). Binding measurements were obtained by fixing Gardner-Arnstein FeLV (2 μ g of protein) onto glutaraldehyde-activated 96-well enzyme-linked immunosorbent assay plates in 0.1 M Na_2CO_3 (pH 9.4) for 1 h at 37°C. The nonspecific sites were then blocked (12) overnight at 4°C. Dilutions of each peptide were then added to aliquots (1 μ g) of a 1:700 dilution of C11D8 monoclonal antibody, and the mixtures were then added to the enzyme-linked immunosorbent assay plates and incubated for 2 h at 23°C. After being washed, antibody bound to virus was quantitated by reacting the plates with rabbit anti-mouse antibody followed by alkaline phosphatase-conjugated goat anti-rabbit antibody (Sigma) and monitoring color development with *p*-nitrophenylphosphate as substrate (3). The 50% inhibition values were determined by plotting antibody binding versus log of nanomolar peptide concentration.

(James Hogle, personal communication) that changes involving single proline residues in both VP1 and VP3 result in conformational changes in loop structures which reside immediately C terminal to these residues. We suspect that similar changes are responsible for the observations in the present study of FeLV. As reported recently by Barlow et al. (1), a continuous epitope, such as the binding epitope described in this report, would best be accommodated as a loop structure on the surface of the protein. Alternatively, the proline residue in susceptible strains may be directly involved in secondary binding by the antibody. Such secondary interaction may influence the overall affinity of binding without being required for absolute binding. We are now attempting three-dimensional studies to pursue this issue.

Our present findings are particularly relevant to our previous observations with antibodies directed to synthetic peptides (4). We found that neutralization could be elicited by antibodies to peptides corresponding to several discrete regions of the envelope gene. However, these antibodies exhibited low neutralizing titers despite substantial titers for recognition of native antigen. In particular, antiserum to the I-26 peptide, which contains the binding epitope for C11D8 studied above, neutralized Gardner-Arnstein virus at a titer of 1:60 compared with equivalent neutralization by C11D8 at a titer of 1:600 (4). Competition experiments suggested that the reason for this discrepancy was that the antiserum to the I-26 peptide binds with lower affinity than the C11D8 monoclonal antibody. The results of the present study further indicate that affinity plays a significant role in neutralization. It is of interest that polyclonal anti-peptide antisera neutral-

ized both the Gardner-Arnstein isolate and λ B1 to the same low degree (4), even though λ B1 was totally resistant to the C11D8 monoclonal antibody. This is probably because the panels of antibodies present in the anti-peptide antisera contain components reactive with the epitope in several conformations. The competition experiments reported above indicate that sequences outside of the antibody-binding epitope can influence the degree of antibody reaction, presumably by altering the conformation of the antibody-binding epitope. It is thus feasible to optimize the mimicry of the epitope in the native antigen by changing adjacent sequences or modifying coupling conditions to carrier protein or both. The resultant peptides and conjugates may then be tested in competition assays to determine which configuration best approximates the epitope in the native molecule.

The λ B1 isolate is a natural variant, i.e., it was not generated via *in vitro* selection in the presence of antibody. Whether immunological selection occurred *in vivo* to pro-

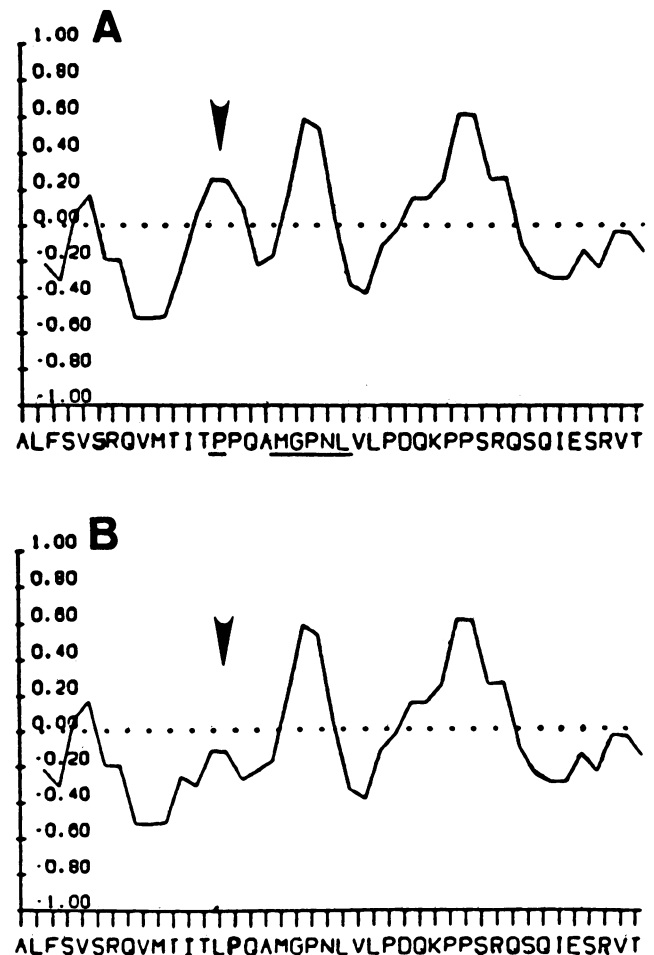


FIG. 5. Analysis of beta-turn potential around the C11D8 antibody-binding epitope in Gardner-Arnstein isolate and λ B1 gp70s. Computer-assisted analyses based on the predictions of Chou and Fasman (2) were done. Sequences shown correspond to amino acid positions 229 to 269 of Fig. 2. (A) Gardner-Arnstein isolate; (B) λ B1. Arrows indicate influence of a single amino acid change from proline (P in Gardner-Arnstein isolate) to leucine (L in λ B1) on the beta-turn potential of this region. The antibody-binding epitope (MGPNL) is underlined.

duce this virus is unknown. The fact that two laboratories have independently prepared neutralizing monoclonal antibodies which react at this epitope (8, 18) implies that it is a major target for a neutralizing immune response. Thus, it is possible that λ B1 was a product of immunological pressure. How much variation can occur in this region without impairing viral function is unknown. However, the high degree of conservation across all the FeLV subtypes around the C11D8 epitope implies a minimal tolerance to change. Thus far only one other isolate contains a change in this region. The feline acquired immunodeficiency syndrome variant (17) contains a single amino acid change which alters the binding epitope from MGPNL to MGPD (J. I. Mullins, personal communication). We have not yet examined this isolate, but we expect that it will not be neutralized or recognized by the C11D8 antibody. The limited variability so far observed in this region suggests that the epitope is still a good target for a synthetic vaccine.

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