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Antigenic Analysis of Equine Infectious Anemia Virus (EIAV) Variants by Using Monoclonal Antibodies: Epitopes of Glycoprotein gp90 of EIAV Stimulate Neutralizing Antibodies†

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Monoclonal antibodies produced against the prototype cell-adapted Wyoming strain of equine infectious anemia virus (EIAV), a lentivirus, were studied for reactivity with the homologous prototype and 16 heterologous isolates. Eighteen hybridomas producing monoclonal antibodies (MAbs) were isolated. Western blot (immunoblot) analyses indicated that 10 were specific for the major envelope glycoprotein (gp90) and 8 for the transmembrane glycoprotein (gp45). Four MAbs specific to epitopes of gp90 neutralized prototype EIAV infectivity. These neutralizing MAbs apparently reacted with variable regions of the envelope gp90, as evidenced by their unique reactivity with the panel of isolates, suggesting recognition of at least three different neutralization epitopes. The conformation of these epitopes appears to be continuous, as they resisted treatment with sodium dodecyl sulfate and reducing reagents. Monoclonal antibodies that reacted with conserved epitopes on gp90 or gp45 failed to neutralize EIAV. Our data also demonstrated that there was a large spectrum of possible EIAV serotypes and confirmed that antigenic variation occurs with high frequency in EIAV. Moreover, the data showed that variation is a rapid and random process, as no pattern of variant evolution was evident by comparison of 13 isolates from parallel infections. These results represent the first production of neutralizing MAbs specific for a lentivirus glycoprotein and document alterations in one or more neutralization epitopes of the major surface glycoprotein among sequential isolates of EIAV recovered during persistent infection.

Equine infectious anemia virus (EIAV), a lentivirus, causes an important disease in horses which is characterized by viral persistence, immunologically mediated lesions, and a variable clinical course (7, 11). Biochemically, the virus contains two major surface glycoproteins (gp90 and gp45) and four major nonglycosylated internal proteins designated p26, p15, p11, and p9 (15, 21). One possible mechanism for viral persistence with EIAV involves the evolution of antigenic variants which allow the virus to temporarily elude established host immune defenses. Antigens displayed on the surface of the virion serve as potential targets for neutralizing antibodies (14, 26). Variants of the lentiviruses EIAV and visna virus contain alterations confined to the envelope glycoproteins (6, 17, 25, 27). In this instance, the emerging mutant viruses have altered envelope glycoproteins, and these alterations are purportedly sufficient to allow the virus to temporarily escape immunological inactivation. Although it would be premature to predict the role of antigenic variation in human immunodeficiency virus (HIV), isolates of this virus from different individuals differ genotypically, and this genomic heterogeneity is greatest in the region of the *env* gene (2, 24, 31). Recently, Hahn et al. (10) suggested that sequential HIV isolates from persistently infected individuals have evolved in parallel from a common progenitor virus. This type of variation may be common to all members of the subfamily *Lentivirinae*. If these genotypic and phenotypic changes are mirrored antigenically, they will undoubtedly pose serious problems for vaccine development.

The discriminatory power of monoclonal antibodies (MAbs) makes them ideal reagents for investigation of antigenic relatedness between viral proteins. Therefore, we produced MAbs to study antigenic variation of EIAV and to define epitope alterations in virion surface glycoproteins. These data provide additional information on alteration of antigenicity which complements the structural and genomic variations previously observed with EIAV variants (17, 22). In addition, MAbs against the EIAV surface glycoproteins have been used to identify antigenic determinants important for neutralization. Highly conserved determinants of EIAV glycoproteins have been identified which may serve as potential targets for the development of a group-specific immunogen.

MATERIALS AND METHODS

Virus production, propagation, and purification. All virus isolates used for this study were produced by serial passage of the prototype strain of EIAV in Shetland ponies (19). Virus isolates were designated by the letter P followed by a three-digit number with the first, second, and third digit corresponding to the passage number, pony number, and isolate number, respectively (e.g., P1.1-1). The prototype cell-adapted Wyoming strain of EIAV (13) and isolates P2.1-1, P2.1-6, P3.1-1, P3.1-2, P3.1-3, P3.1-4, P3.2-1, P3.2-2, P3.2-3, P3.2-4, P3.2-5, P3.3-1, P3.3-2, P3.3-3, and P3.3-4 (19; A. Orrego, Ph.D. dissertation, Louisiana State University, Baton Rouge, 1983; Rwambo, unpublished data) were propagated in primary fetal equine kidney (FEK) cells and

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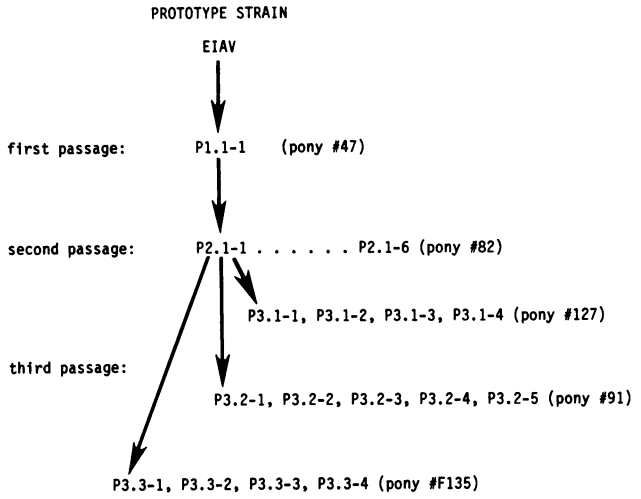

FIG. 1. Inoculation scheme followed for the production of EIAV isolates in experimentally infected Shetland ponies. Each of the three ponies received 1 ml of plasma collected from pony 82 during its first febrile episode. Plasma infectivity was $10^{4.5}$ $TCID_{50}$/0.5 ml.

purified by previously described procedures (15). The virus isolates obtained from plasma collected during the indicated sequential febrile episodes of experimentally infected Shetland ponies were purified by endpoint dilution in FEK cells to ensure isolation of the predominant virus population (17). Isolates P2.1-1 and P2.1-6 were obtained from an experimentally infected pony during the first and sixth febrile episodes (Fig. 1). The remaining 13 isolates were recovered from three Shetland ponies infected with the same virus inoculum (1 ml of plasma collected from pony 82 during its first febrile episode, with plasma infectivity of $10^{4.5}$ 50% tissue culture-infective doses [$TCID_{50}$] per 0.5 ml). These isolates are identified as follows: four consecutive isolates (P3.1-1 through P3.1-4) from pony 127; five consecutive isolates (P3.2-1 through P3.2-5) from pony 91; and four consecutive isolates (P3.3-1 through P3.3-4) from pony F135 (25).

Glycoprotein purification. The glycoproteins of EIAV were purified by affinity chromatography over a column of lentil lectin bound to Sepharose in the presence of detergent as described previously (16, 17).

MAb production. Two-month-old female BALB/c mice were injected subcutaneously and intraperitoneally with either 100 μg of purified EIAV glycoproteins or 200 μg of intact virus or deoxycholate-disrupted virus in Freund complete adjuvant. The mice were injected on days 14 and 28 with the antigen preparations (same concentrations and routes as above) in Freund incomplete adjuvant. The final injection was given on day 88 with antigen in 0.01 M phosphate buffer, either intraperitoneally or intravenously, 4 days before fusion. Three days after the last injection, serum samples from each mouse were taken and assayed for EIAV antibodies in enzyme-linked immunosorbent assay (ELISA), Western blot (immunoblot), and neutralization tests. Mice with high antibody titers were used as a source of spleen cells on day 4 after the last injection.

The procedures for fusion and selection of the hybridoma cells producing antibodies against EIAV glycoproteins were based on those of Galfré and Milstein (9) and Shulman et al. (28). Selected hybridomas producing anti-EIAV antibodies were subcloned at least twice by limiting dilution. Ascitic fluid was obtained from pristane-primed mice inoculated with 1×10^6 to 2×10^6 hybridoma cells into the peritoneal cavity. The isotype of immunoglobulin produced by the hybridomas was determined by immunodiffusion test.

ELISA. A solid-phase assay for hybridoma screening was modified from that described by Voller et al. (30) and O'Sullivan et al. (20). Immulon-1 96-well plates (Dynatech Laboratory) were coated with 1 μg of protein per well in TEN coating buffer (Tris [0.05 M], EDTA [0.001 M], NaCl [0.15 M]). The antigen was fixed with paraformaldehyde (4% [wt/vol], pH 7.2) for 5 min, and nonabsorbed antigen was washed away with TEN buffer. Plates were blocked with 5% bovine serum albumin in phosphate-buffered saline. Hybridoma supernatant or ascitic fluid was detected by peroxidase-labeled goat anti-mouse immunoglobulins (Cappel Laboratories, Cochranville, Pa.). Plates were developed with the substrate 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid), diammonium salt (Sigma). The reaction was continued for 10 to 20 min, and the optical density at 490 nm was recorded with a spectrophotometer (Dynatech).

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 10% slab gels by the Laemmli procedure (12). Procedures used for sample preparation and SDS-PAGE in continuous sodium phosphate buffer have been described previously (15).

Western blot assay. After separation by SDS-PAGE, proteins were electrophoretically transferred to nitrocellulose membranes at 0.25 A for approximately 18 h with a transfer buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3 (4, 29). The nitrocellulose membrane was then saturated with 3% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) in 10 mM Tris hydrochloride (pH 7.4) with 150 mM sodium chloride for 30 min at room temperature with continuous agitation. The membrane was incubated for 90 min with gentle shaking at room temperature in a 1:100 dilution of ascitic fluid or serum from immunized mice. The nitrocellulose membranes were washed in at least three changes of Tris-saline containing 0.05% Nonidet P-40 (Particle Data Laboratories, Elmhurst, Ill.). The nitrocellulose membrane was then incubated for 1 h in Tris-saline containing a 1:1,000 dilution of peroxidase-conjugated goat anti-mouse immunoglobulins. After incubation with the second antibody, the membrane was washed as above and incubated with the color development solution. This solution was prepared by dissolving 60 mg of 4-chloro-1-naphthol (Sigma) in 20 ml of ice-cold methanol and then mixing it with 100 ml of Tris-saline plus 60 μl of 30% (wt/vol) hydrogen peroxide. The immunoreactive proteins stained purple with this reagent.

Neutralization assay. MAbs and immune mouse sera were evaluated for their ability to neutralize the cytopathic activity of prototype EIAV in a microtiter assay. Samples to be used in neutralization assays were filtered (0.45 μm) and heated at 56°C for 30 min before mixing with the virus. Serial twofold dilutions of ascitic fluids were mixed with an equal volume (50 μl) of virus suspension (100 $TCID_{50}$) in minimal essential medium and incubated for 1 h at 37°C. Cells derived from fetal donkey dermal cultures (10,000/well) were added in 100-μl volume to each well as indicators for EIAV infectivity. Incubation was carried out for 7 to 12 days, and cells were observed for cytopathic effect (CPE). The neutralization titer was expressed as the reciprocal of the highest dilution which inhibited the cytopathic effect of 100 $TCID_{50}$ of the prototype EIAV in fetal donkey dermal cell cultures. Neutralization tests were also carried out by the virus dilution-constant ascitic fluid method with FEK cells

TABLE 1. Characterization of anti-EIAV MAbs .

MAb	Isotype	Immunizing antigen	Viral protein immunoblotted	ELISA titer (log_{10})	Neutralization (titer)[a]
86-1E3	IgG1	Disrupted virus	gp90	3.9	−
95-1G8	IgG1	Disrupted virus	gp90	4.2	−
114-3A7	IgG1	Disrupted virus	gp90	3.9	−
85-1E11	IgG1	Disrupted virus	gp90	3.6	−
71-1A9	IgG1	Glycoproteins	gp90	3.3	−
87-1E7	IgG1	Disrupted virus	gp90	3.3	−
82-1C2	IgG1	Disrupted virus	gp90	3.6	+ (128)
98-1D1	IgG1	Disrupted virus	gp90	3.3	+ (64)
115-3D7	IgG1	Disrupted virus	gp90	3.6	+ (128)
128-2B9	IgA	Whole virus	gp90	3.6	+ (128)
75-1F2	IgG1	Glycoproteins	gp45	3.6	−
92-1E6	IgG1	Disrupted virus	gp45	4.5	−
101-2F10	IgG1	Disrupted virus	gp45	4.5	−
109-1A6	IgG1	Disrupted virus	gp45	4.8	−
117-1C5	IgG1	Whole virus	gp45	3.9	−
120-1H9	IgG1	Whole virus	gp45	4.8	−
105-3C8	IgG2b	Disrupted virus	gp45	4.5	−
90-1C1	IgG1	Disrupted virus	gp45	4.5	−

[a] Reciprocal of highest dilution of ascitic fluid that inhibited the CPE of 100 $TCID_{50}$ of EIAV in fetal donkey dermal cell cultures. −, <1:4 dilutions of ascitic fluid.

and virus production as an indication of infection as described previously (1) for those isolates which did not produce reliable CPE in fetal donkey dermal cell cultures.

RESULTS

Activity of sera from immunized mice. Mice were inoculated with purified glycoprotein, intact virus, or detergent-disrupted virus to ascertain the optimum antigen presentation for inducing neutralizing antibody. Sera obtained from immunized mice were tested in ELISA and Western blot assays against EIAV proteins and in neutralization tests against prototype EIAV. Sera obtained from mice immunized with lectin-purified glycoprotein preparations reacted predominantly with gp90 and gp45. Sera from mice immunized with dissociated virus plus adjuvant were primarily reactive with gp90, p26, and p15; they reacted only slightly with gp45. Sera from mice inoculated with whole virus plus adjuvant reacted mostly with gp90, gp45, p26, and p15. Recipients of both native EIAV and dissociated virus solubilized with deoxycholate developed high antibody titers by the neutralization assay (neutralization titer ranged from 80 to 320). Spleens of these mice were used as the source of B lymphocytes for fusion with SP2/O myeloma cells.

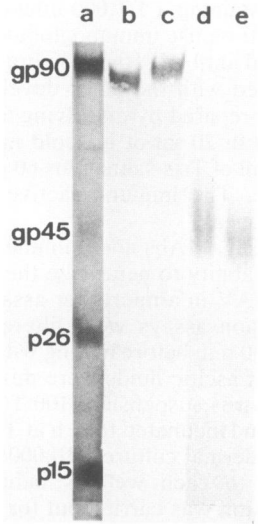

FIG. 2. Imunoblot analysis of EIAV: polypeptides of prototype (lanes a, b, and d) and isolate P2.1-1 (lanes c and e) viruses were separated by SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with serum from mice immunized with disrupted virus preparations (a), neutralizing monoclonal antibody 82-1C2 (b and c), or monoclonal antibody 105-3C8 (d and e).

Reactivity of MAbs with the homologous virus strain. Of the 12 hybridizations performed, 150 hybridomas producing MAbs to EIAV were detected by ELISA screening. From these a total of 18 stable independently cloned hybrid cell lines producing antibody against EIAV glycoproteins were evaluated in Western blot analysis (Table 1). Ten of these MAbs were specific for gp90 and eight for gp45 of EIAV. The most frequent isotype was IgG1, followed by IgG2b and IgA. Indirect ELISA titers of ascitic fluids ranged from $10^{3.3}$ to $10^{4.8}$ (Table 1).

Since the glycoproteins (gp90 and gp45) are located in the envelope of EIAV and represent the two main surface proteins, it was of interest to examine the MAbs for neutralizing activity. Although all 18 of the MAbs bound to either gp90 or gp45 in immunoblots, only 4 of these neutralized the infectivity of the virus to an appreciable titer (Table 1). Each neutralizing MAb was directed against an epitope of gp90. The neutralizing ability of ascitic fluids and mouse sera was also evaluated in the presence of a complement source (pooled guinea pig serum; Miles Laboratories, Inc.) to see whether complement enhanced the neutralization of sensitized virus. The addition of complement had no effect on the neutralization induced by these samples or reference EIAV-positive horse serum (data not shown).

Reactions of MAbs with a panel of EIAV variants. MAbs reacting to EIAV gp90 or gp45 were tested in Western blot assays against a panel of 16 EIAV variants (Fig. 1) to identify the pattern of glycoprotein antigenic variation. Variation in electrophoretic mobility of both gp90 and gp45 from several isolates was evident when immunoblotted with a given MAb (Fig. 2), as reported previously (17, 25). A serological comparison of the viral glycoproteins of all 16 EIAV isolates is summarized in Fig. 3. Six patterns of immunoreactivity

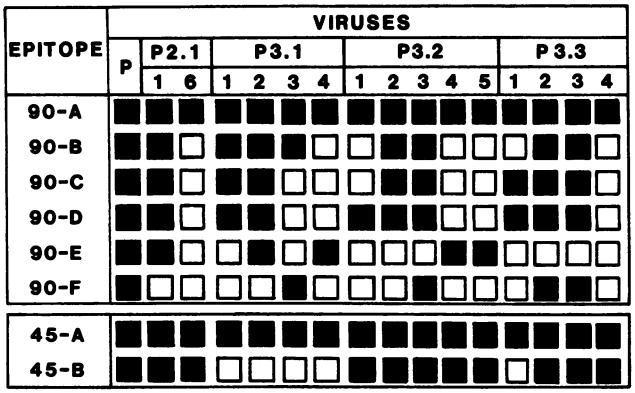

FIG. 3. Immunoreactivity pattern of gp90- and gp45-specific MAbs with polypeptides of 16 EIAV isolates. Polypeptides of the prototype (P), P2.1-1, P2.1-6, P3.1-1, P3.1-2, P3.1-3, P3.1-4, P3.2-1, P3.2-2, P3.2-3, P3.2-4, P3.2-5, P3.3-1, P3.3-2, P3.3-3, and P3.3-4 viruses were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with each of the following MAbs: 86-1E3, 95-1G8, and 114-3A7 (90-A); 87-1E7 (90-B); 82-1C2 and 98-1D1 (90-C); 115-3D7 (90-D); 128-2B9 (90-E); 85-1E11 and 71-1A9 (90-F); 75-1F2, 92-1E6, 101-2F10, 109-1A6, 117-1C5, 120-1H9, and 105-3C8 (45-A); and 90-1C1 (45-B).

were observed for gp90 and two for gp45 when all 18 MAbs were tested in Western blot assays. Each of these immunoreactivity patterns could be assigned to a potential epitope (epitopes 90-A, 90-B, 90-C, 90-D, 90-E, and 90-F for gp90-specific MAbs). Similarly, epitopes 45-A and 45-B were assigned for gp45-specific MAbs.

Epitope 90-A (recognized by MAbs 86-1E3, 95-1G8, and 114-3A7) was a highly conserved epitope of gp90 identified on all 16 EIAV isolates. Epitopes 90-B (recognized by MAb 87-1E7), 90-C (recognized by neutralizing MAbs 82-1C2 and 98-1D1), 90-D (recognized by neutralizing MAb 115-3D7), 90-E (recognized by neutralizing MAb 128-2B9), and 90-F (recognized by MAbs 71-1A9 and 85-1E11) were present only in certain isolates. The four MAbs that had neutralizing activity (82-1C2, 98-1D1, 115-3D7, and 128-2B9) reacted with gp90 from 16 EIAV isolates in three different patterns (Fig. 3). Epitope 45-A (recognized by MAbs 75-1F2, 92-1E6, 101-2F10, 109-1A6, 117-1C5, 120-1H9, and 105-3C8) was a highly conserved epitope of EIAV gp45, whereas epitope 45-B (recognized by MAb 90-1C1) was unique for some of the isolates.

None of the MAbs that reacted with all EIAV isolates neutralized the infectivity of the prototype strain, and none of the isolates reacted with all the MAbs tested. Most isolates could be distinguished antigenically on the basis of their differential reactivity with the panel of glycoprotein-specific MAbs. Nine different groups of isolates were noted on the basis of reaction with gp90-specific MAbs. However, some of the isolates which had identical immunoreactivity with gp90-specific MAbs differed in their gp45 reactivity. Only two immunoreactivity patterns were seen for the gp45-specific MAbs. The first group contained the majority of isolates (11 of 16). Seven of eight gp45-specific MAbs recognized a highly conserved epitope on all 16 EIAV isolates (epitope 45-A). A second epitope on gp45 (epitope 45-B) appeared to be unique to the prototype and some other variants, e.g., four sequential isolates from pony 127 (P3.1-1, P3.1-2, P3.1-3, and P3.1-4). Although the gp45 components of these isolates had common changes, they could all be differentiated on the basis of their gp90 reactivity. Twelve different antigenic variants were therefore identified among 16 virus strains with respect to both glycoproteins (gp90 and gp45).

Both neutralization and nonneutralization epitopes changed during in vivo passage (epitopes 90-B, 90-C, 90-D, 90-E, 90-F, and 45-B). In addition, some neutralization epitopes conserved their configurational integrity during in vivo passage, e.g., epitope 90-E was only found on the prototype and isolates P2.1-1, P3.1-2, P3.1-4, P3.2-4, and P3.2-5. In a limited study, the four neutralizing MAbs (82-1C2, 98-1D1, 115-3D7, and 128-2B9) were also tested in neutralization assays against EIAV isolates P2.1-1, P2.1-6, P3.1-1, P3.1-2, P3.1-3, and P3.1-4, which displayed different immunoreactivity patterns with the neutralizing MAbs in Western blot analysis (Fig. 3). The virus dilution-constant ascitic fluid method was used since not all the isolates reproducibly produced CPE in cell culture (Issel, unpublished results). The results indicated that MAbs 82-1C2, 98-1D1, 115-3D7, and 128-2B9 had a neutralization index of 2, 2.5, 2.5, and 2, respectively, against the prototype EIAV. However, none of the isolates tested were neutralized by any of the four neutralizing MAbs.

Detailed serological comparisons of the glycoproteins of isolates from ponies with parallel infections (P3.1-1 through P3.1-4, P3.2-1 through P3.2-5, and P3.3-1 through P3.3-4) showed also that they belonged to different groups since we were able to identify 12 different antigenic variants among 16 virus strains. Distinct changes were detected in specific epitopes of EIAV isolates recovered from a disease episode separated by only 2 weeks in a single animal, as shown by the differences in immunoreactivity pattern of isolates P3.2-1 and P3.2-2 or P3.3-1 and P3.3-2.

DISCUSSION

The present investigation enabled us to determine the following factors which may assist in understanding antigenic variation of EIAV: (i) the identification of antigenic sites of the two main surface glycoproteins of EIAV; (ii) the documentation of rapid epitope alterations in the virion surface glycoproteins of EIAV, presumably via genomic point mutations, during persistent infections; (iii) the pattern and extent of serological cross-reactions of EIAV isolates, and an estimation of the minimum number of possible serotypes of EIAV; and (iv) the identification of the determinant(s) involved in stimulating neutralizing MAbs.

We have described the production, characterization, and use of hybridoma MAbs specific for EIAV glycoproteins (gp90 and gp45). Our results demonstrate subtle antigenic differences among EIAV isolates. These were evident by definitive antigenic alterations at one or more epitopes in the surface glycoproteins of EIAV isolates from persistently infected animals. The majority of isolates tested could be distinguished antigenically on the basis of their pattern of reactivity with the panel of MAbs. This suggests a high degree of heterogeneity of these isolates, although highly conserved epitopes (epitopes 90-A and 45-A) were identified. We have previously used oligonucleotide mapping and peptide mapping procedures to compare certain of the isolates used in this study. There were cases when two isolates appeared identical by one method of analysis but differed by others. For example, isolates P3.2-4 and P3.2-5, which appeared to be closely related and could not be distinguished by our battery of MAbs, had totally different oligonucleotide, peptide, and glycopeptide maps (23, 25). In contrast, isolates P3.2-1 and P3.2-4, which could not be distinguished

by oligonucleotide or glycopeptide mapping of gp45, were found to be different by at least one epitope on gp90 (epitope 90-E). Isolates P3.2-2 and P3.2-3, which could not be distinguished by peptide maps, were distinguished by oligonucleotide and glycopeptide maps and by immunoreactivity with our panel of MAbs. These observations document the heterogeneity of all isolates tested and argue against the existence of two identical isolates. All 16 isolates used for this study could be distinguished by the combination of oligonucleotide, peptide, and glycopeptide maps or immunoreactivity pattern with MAbs. Through serological comparisons we were able to identify 12 different antigenic variants among 16 virus strains.

In comparison studies with our panel of 18 MAbs, we observed that both neutralizing and nonneutralizing MAbs could differentiate between EIAV isolates. MAbs that reacted with conserved epitopes on gp90 and gp45 failed to neutralize EIAV. On the other hand, the variable regions of the envelope gp90 were often associated with neutralizing epitopes. With our battery of neutralizing MAbs, a minimum of three neutralization epitopes simultaneously present on the prototype EIAV were detected. The possible coexistence of these different neutralization epitopes on a single virion was also noted for other isolates. Therefore, after in vivo virus replication an epitope may lose its function in virus neutralization but may retain its capacity to bind antibody in the in vitro assays employed. For example, isolates P2.1-1 or P2.1-6 and P3.1-1 through P3.1-4, which were demonstrably distinct in cross-neutralization studies with homologous serum (25), possessed epitopes that reacted with prototype-neutralizing MAbs without inactivation of the isolate. Thus, antibody binding to epitopes and neutralization of infectivity are apparently discrete functions. Based on these results, a mutation can affect a neutralization site in two ways: either by modifying its antigenic conformation or by abolishing its function in virus neutralization. These observations suggest that mutations involving residues outside the antigenic site can effect conformational alterations of the whole antigen. Recently, studies on type 1 poliovirus reported that variants resistant to neutralization which were selected in the presence of neutralizing MAb always had mutations situated in VP3, outside the antibody-binding site for the MAb (3).

The results described here confirmed that antigenic variation can occur in EIAV isolates recovered from disease episodes separated by only 14 to 16 days (P3.2-1 and P3.2-2; P3.3-1 and P3.3-2). Thus, the time required for apparent antigenic variation in EIAV is considerably shorter than that reported for visna virus, which requires 1 to 2 years for the emergence of antigenic variants in the persistently infected animal (18). The spectrum of EIAV variants which arose in the three animals infected in parallel were distinguished antigenically on the basis of their immunoreactivity pattern. Each isolate from the same animal was different antigenically from the preceding isolates. However, the emergence of these variants appears to be random, as no predictable pattern of immunoreactivity was observed in the three animals infected in parallel with the same virus inoculum. These findings differ from those with visna virus, for which a similar pattern of variants evolved in parallel persistent infections (5). Our findings paralleled those of HIV, in which genetic variants appear to have evolved randomly (10). Thus, our documentation of rapid epitope alteration in the virion surface glycoproteins among sequential virus isolates demonstrated that each disease episode is associated with a unique virus strain.

The use of MAbs to define epitopes important in stimulation of neutralizing antibody and their relevance in constructing an effective vaccine for EIAV are important. Our studies suggest that gp90 is the major glycoprotein that acts as a target for neutralizing antibodies. However, polyclonal equine immune sera which neutralize EIAV infectivity contain antibodies to both gp90 and gp45, and the functions of the different antibody response are yet to be determined. Immune factors to gp45 may participate in "cooperative neutralization," a synergistic phenomenon which results when two antibodies cause a greater loss of infectivity than the sum of their effects individually (8). Alternatively, gp45 may carry determinants that interact with specifically sensitized cytotoxic T cells. Further clarification of protective immune factors to EIAV and other lentiviruses should provide important information relative to effective immunologic management of these viral infections.

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