Mapping Regions of *Env* Important in the Neutralization of Equine Infectious Anemia Virus

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1. Abstract

The development of neutralizing antibody (NAb) is important for the immunological control and clinical quiescence in horses infected with equine infectious anemia virus (EIAV). Over time, however, viral genotypes evolve that resist NAb, resulting in recrudescence of clinical disease. Our long-term goal to develop an effective vaccine for EIAV will be aided by identification of epitopes that facilitate viral immune escape from neutralizing antibody. There are eight variable regions, V1-V8, in the surface envelope glycoprotein (SU) of EIAV. Two neutralizing epitopes recognized by mouse monoclonal antibodies are found in V3, in a region termed the Principal Neutralizing Domain (PND). A third neutralization epitope is found in a conserved region of V5. Studies in our laboratory have shown that variation outside of these regions modify sensitivity to neutralization. In this study we investigated the role of variable regions V5, V6, and V7 in neutralization of EIAV. Six chimeric viruses that differed in V5, V6, and/or V7 sequences were created with the cell culture-adapted EIAV19 and the virulent wild-type EIAVwyo. These viruses were then tested in neutralization assays against a panel of serum collected from horses naturally or experimentally infected with EIAV. Preliminary results indicate the presence of an additional neutralization epitope in the V6 region. In addition, sensitivity to neutralization was modified by variation in V7 that added a potential N-linked glycosylation site. These results indicate that genetic changes outside the PND contribute to antigenic variation and immune escape. Further characterization of these epitopes could enhance design of safe and effective vaccines for EIAV.

2. Introduction

EIAV induces a persistent long-term infection characterized by recurrent febrile episodes. The onset of the clinical quiescence in the inapparent stage is associated with the development of broadly neutralizing antibody. Development of a safe and effective vaccine for EIAV is impeded by genetic and antigenic variation throughout the course of the disease.

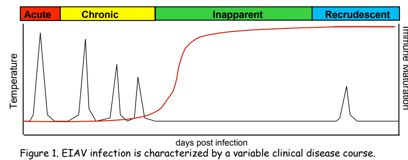

Figure 1. EIAV infection is characterized by a variable clinical disease course.

There are eight variable regions in the surface envelope glycoprotein (SU) of EIAV. Our long-term goal is to identify conserved EIAV epitopes that elicit broadly neutralizing antibody and limit immune escape. Previous studies have shown that variation in SU alters neutralization by virus neutralizing antibodies (NAb). Two neutralizing epitopes recognized by mouse monoclonal antibodies are found in V3, also known as the Principal Neutralizing Domain. A third neutralization epitope is found in a conserved region of V5. It is unknown how variation outside of these epitopes alter neutralization.

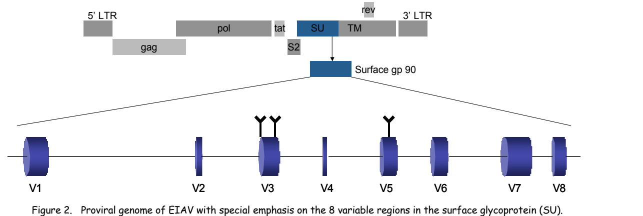

Figure 2. Proviral genome of EIAV with special emphasis on the 8 variable regions in the surface glycoprotein (SU).

3. V5-V6-V7 Play a Role in Neutralization

Previous studies have shown that variation in the PND (V3) alters neutralization of EIAV. To investigate the role of the variable regions outside of the PND, we created chimeric viruses using SU sequences from the cell-culture adapted EIAV19 and a virulent wild-type strain EIAVwyo. These were then tested in neutralization assays against a panel of sera collected from horses naturally and experimentally infected with EIAV. All horses were able to neutralize EIAV19 virus, as well as EIAV19 substituted with EIAVwyo sequences in the V2-V4 region. However, two horses, Toby and 2150, were unable to neutralize EIAV19 containing the EIAVwyo V5-V7 region. This suggests that genetic variation in the V5-V7 regions may contributes to antigenic variation and immune escape in vivo.

Recombinant Viruses Differing in Variable Regions in EIAV SU

Horse (Inoculum)	EIAV19	EIAV19/wyoV2-V4	EIAV19/wyoV5-V7
618 (Wyo)	389	nd	1721
2140 (EIAV19-like)	2520	256	321
Toby (Field infected)	380	>128	<10
2150 (EIAV19-like)	265	>128	<10

Figure 3. Neutralization of EIAv chimeric virus. Panel of antiserum collected from EIAV infected horses and tested in neutralization assays against EIAV chimeric virus. Results are reported as ID50, the inverse dilution of sera that neutralized 50% of virus infectivity. Notice that Horse Toby and 2150 are unable to neutralize EIAVwyo V5-V7.

4. Generation of Chimeric Viruses That Differ in V5, V6, and V7

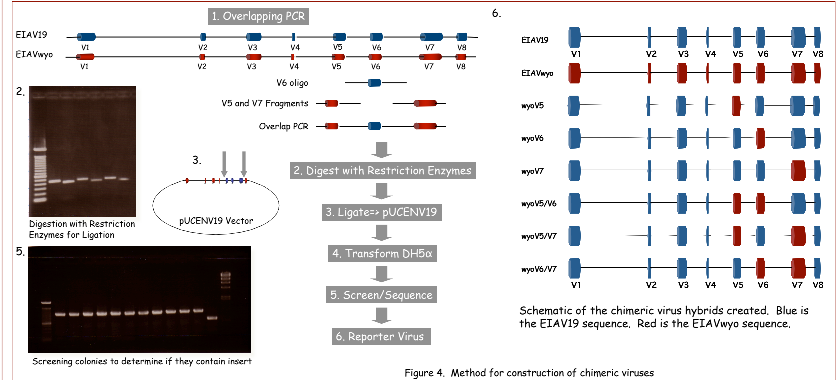

Figure 4. Method for construction of chimeric viruses

In order to investigate the role of the V5, V6, and V7 regions in neutralization, we generated chimeric viruses that differed only in the V5, V6, and/or V7 region. The V5, V6, or V7 fragments were generated by PCR or oligonucleotide synthesis and the V5-V6-V7 chimeric fragment was made by overlapping PCR amplification using primers specific to each region and EIAV strain. These chimeric fragments were then gel purified, digested, and ligated into pUCENV19 vectors. Transformed DH5α E.coli cells were then screened by PCR and positive clones were sequenced and used to produce chimeric viruses with variable V5, V6 and/or V7 regions.

	V5	V6	V7
EIAV19	LVQEKGIADTSR	GDYTACNVRRLN	TNQVQYLLCNNNNSNNYNC
EIAVwyo	LVQEKGIANNSR	GNSTICKVNITE	TNQVQYLLCNNNSSNNYNC

Figure 5. Amino acid sequences of EIAV19 and EIAVWyo in the V5, V6, and V7 regions. The amino acid differences between the two viruses are indicated in red The conserved V5 epitope is located just upstream of the V5 sequence.

5. Neutralization Data

To date, we have conducted virus neutralization tests on EIAV19 chimeric viruses substituted with either the V5, V6 or V7 region from EIAVwyo (WV5, WV6, or WV7). In all horses, the replacement of EIAV19 V5 with EIAVwyo V5 resulted in a moderate increase or decrease in neutralization. More striking results were observed with WV6 and WV7. WV6 or WV7 were not neutralized by sera from horse 2150, while WV7 was not neutralized by Toby sera.

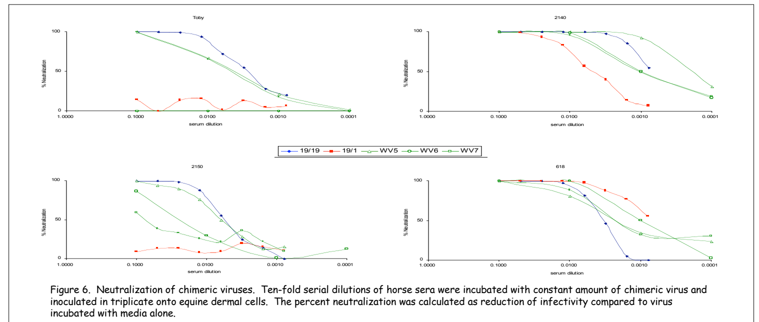

Figure 6. Neutralization of chimeric viruses. Ten-fold serial dilutions of horse sera were incubated with constant amount of chimeric virus and inoculated in triplicate onto equine dermal cells. The percent neutralization was calculated as reduction of infectivity compared to virus incubated with media alone.

The neutralization results are consistent with the presence of a conserved neutralization epitope in V5, which is shared between EIAV19 and EIAVwyo. The data also suggest that a novel neutralization epitope is present in V6. This is most clearly demonstrated with horse 2150 serum, as neutralization is lost with the replacement of 19V6 by WV6. The loss of neutralization is not surprising considering the sequence variation in this region (Figure 5). Virus sensitivity to neutralization can be altered by changes in glycosylation, which can mask neutralizing epitopes in other regions of SU. The single amino acid difference in WV7 resulted in an additional glycosylation site (Figure 7), and the presence of WV7 may mask a neutralizing epitope recognized by sera from Toby and horse 2150. Sera from horses 618 and 2140 were still able to neutralize WV6 and WV7, but at lower levels than homologous virus. These horses may recognize additional or different epitopes than those recognized by 2150 and Toby.

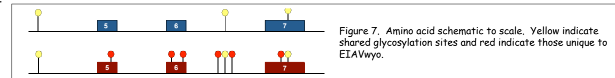

Figure 7. Amino acid schematic to scale. Yellow indicate shared glycosylation sites and red indicate those unique to EIAVwyo.

6. Summary and Conclusion

- The EIAV SU contains multiple neutralizing epitopes recognized by sera from experimentally infected and field-infected horses.
- Genetic variation in V5, V6, and or V7 regions of EIAV SU can alter sensitivity to neutralization.
- The V6 region contains a novel neutralizing epitope.
- Genetic changes in V7 that increase potential N-linked glycosylation sites may mask recognition of neutralizing epitopes.