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 recurrence of clinical disease. Our long-term goal is 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 field type EIAVwyo. These viruses were then tested in neutralization assays against a panel of sera 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. 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.

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. Preliminary results indicate that genetic changes outside the PND contribute to antigenic variation and immune escape. Recombinant Viruses Differing in Variable Regions in EIAV SU

<table>
<thead>
<tr>
<th>Horse (Inoculum)</th>
<th>EIAV19</th>
<th>EIAV19/wyoV5-V6</th>
<th>EIAV19/wyoV5-V6-V7</th>
</tr>
</thead>
<tbody>
<tr>
<td>61B (Wyo)</td>
<td>389</td>
<td>nd</td>
<td>1721</td>
</tr>
<tr>
<td>2140 (EIAV19-like)</td>
<td>2520</td>
<td>256</td>
<td>321</td>
</tr>
<tr>
<td>Toby (Field infected)</td>
<td>380</td>
<td>+128</td>
<td>+10</td>
</tr>
<tr>
<td>2150 (EIAV19-like)</td>
<td>265</td>
<td>+128</td>
<td>+10</td>
</tr>
</tbody>
</table>

Figure 3. Neutralization of EIAV chimeric virus. Panel of antibodies collected from EIAV infected horses and tested in neutralization assays against EIAV chimeric virus. Results are reported as 50% NAb, the inverse dilution of serum that neutralized 50% of viral infectivity. Factor that horses Toby and 2150 are unable to neutralize EIAV19/wyoV5-V7.

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 synthetic nucleic acid 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. Transform 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.

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

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. These viruses were then tested in neutralization assays against a panel of sera 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.

5. Neutralization Data

To date, we have conducted virus neutralization tests on EIAV chimeric viruses substituted with either the V5, V6, or V7 region from EIAVwyo (V5s, V6s, or V7s). 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 V6s and V7s. V6s or V7s were not neutralized by sera from horse 2150, while V7s were not neutralized by Toby sera.

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 neutralization of neutralizing epitopes.

Figure 6. Method for construction of chimeric viruses.