Evaluating the influences of glycosylation on the antigenicity and immunogenicity of Ebola virus glycoprotein


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Evaluating the influences of glycosylation on the antigenicity and immunogenicity of Ebola virus glycoprotein
Ebola Virus

- Causative agent of severe hemorrhagic fever
- Identified in Zaire in 1976
- Sporadic outbreaks in Africa
- High mortality rate - 50-88%
- Animal reservoir unknown
- Lack of preventative vaccine or effective antiviral treatments
- Potential bioterrorism agent
Ebola Virus Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>#Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayinga</td>
<td>Zaire 1976</td>
<td>318 (88%)</td>
</tr>
<tr>
<td>Zaire 95</td>
<td>Zaire 1995, Gabon 94-96, Gabon/Congo 2002, Congo 2003</td>
<td>315 (77%), ~100 (50%), 92 (75%), 143 (89%)</td>
</tr>
<tr>
<td>Ivory Coast</td>
<td>Ivory Coast 1994</td>
<td>1 (0%)</td>
</tr>
<tr>
<td>Reston Phil</td>
<td>Philippines 1989</td>
<td>284 (53%)</td>
</tr>
<tr>
<td>Reston Siena</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reston Res</td>
<td>Sudan 1976</td>
<td>34 (65%)</td>
</tr>
<tr>
<td>Sudan Bon</td>
<td>Sudan 1979</td>
<td>26 (65%)</td>
</tr>
<tr>
<td>Sudan Maleo</td>
<td>Uganda 2000-01</td>
<td>~400 (62%)</td>
</tr>
</tbody>
</table>

Phylogenetic Relationships of GP gene sequence
Ebola virus Structure

GP Transmembrane Glycoprotein
VP40 Matrix Protein
VP24 Matrix Protein (Minor)
NP Nucleoprotein
VP35 Phosphoprotein (Transcription Factor)
VP30 Ribonucleoprotein Associated (Minor)
L RNA-Dependent RNA Polymerase
Synthesis of Ebola virus glycoproteins

NP 35 40 GP 30 24 L

RNA edit

sGP $\Delta$
(soluble)

GP$_0$

furin cleavage

GP1 GP2
(virion)

Secreted from cell
Immunologic decoy?
Bind neutrophils?

Transported to cell surface
On surface of virus

Disulfide bond
N-linked glycosylation

• Co-translational modification
• Eukaryotic cells in the endoplasmic reticulum
• Occurs at a triplet amino acid sequence (SEQUON):
  Asn-X-Ser/Thr

• Not all sequons are glycosylated
• Influences protein folding and transport
• Changes in viral glycoproteins can lead to altered immunogenicity (HIV, CAEV, influenza, rabies) and virulence (influenza, NDV, MHV, PERV)
Ebola GP: N-linked Glycosylation sites

- Zaire GP - 17 potential sites
- Other strains:
  - Reston - 15
  - Sudan - 12
  - Ivory Coast - 12
- Conserved sites - 5 sites in GP1, 2 in GP2. Those in GP2 are the only sites identical at all 4 amino acid positions.
N-linked mutation set 1

N-linked glycosylation sites around known protective epitopes

- Set of 5 monoclonal antibodies
- Protect mice against EBOV infection
- “Protective” epitopes mapped for 3 of the antibodies
- Groups 1 and 2 have overlapping epitope in GP1
- N-linked glycosylation sites FLANK either side of this epitope

**QUESTION:**
Do glycans at either or both of these sites shield or enhance this epitope?
N-linked glycosylation sites in known immunogenic regions

- GP2 shown to be protective for EBOV and MBGV
- Only two sites for N-linked glycosylation in GP2
- Highly conserved among different strains/subtypes of EBOV

QUESTION:
Do glycans in GP2 affect the immunogenicity of this region?
DNA vaccines for EBOV

pWRG7077

CMV intron A

KanR

BGH pA

Not I

BamH I

GP1

GP2

PROTECT

PROJECT

-SU
In vitro expression of EBO-Z GP N-linked glycosylation mutants
DNA Vaccination

- 9 plasmids: GP wt, pWRG7077, A, B, C, D, AB, CD, ABCD
- 4-6 wk balb/c female mice
- 3 gene-gun vaccinations, 4 wk apart
- Challenge - with mouse-adapted Ebola virus
- ELISA- test sera using irradiated ZEBOV and pools of overlapping peptides as antigens
- ELISPOTs - stimulate with pools of overlapping peptides
## Survival Data

<table>
<thead>
<tr>
<th>pWRG7077</th>
<th>0/7 (0%)</th>
<th>0/10 (0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP (wt)</td>
<td>7/7 (100%)</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>GPmutA</td>
<td>7/7 (100%)</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>GPmutB</td>
<td>7/7 (100%)</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td>GPmutC</td>
<td>3/6 (50%)</td>
<td>6/10 (60%)</td>
</tr>
<tr>
<td>GPmutD</td>
<td>7/7 (100%)</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td>GPmutAB</td>
<td>ND</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>GPmutCD</td>
<td>7/7 (100%)</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>GPmutABCD</td>
<td>4/6 (67%)</td>
<td>8/10 (80%)</td>
</tr>
</tbody>
</table>
Virus-specific T-cell responses in mice vaccinated with DNA vectors expressing the Ebola GP

Deletion of N-linked glycosylation sites alters T cell specificities and frequencies.

Ebola GP overlapping peptide pools (n=8 peptides/pool)
Summary

- All N-linked mutants express when transfected into mammalian cells, as shown by IFA and RIPA. Those containing the “C” mutation at position 586 (C, CD, ABCD) demonstrate a normal level of GP1 but a reduced amount of GP2 by RIP assay.

- Vaccination with wild-type GP or with mutants A, B, D and AB protected mice from challenge with mouse-adapted Ebola Zaire. Mutant C and ABCD afforded partial protection in both sets of experiments while CD was completely protective in the first experiment but only partially protective in the second experiment.

- ELISPOT results showed a decrease in breadth and intensity of T-cell responses from mice vaccinated with mutants C, CD and ABCD. Mutants A, B,D and AB had similar responses to wild type.

- ELISA titers were decreased in mutants C, CD and ABCD compared to wild type and elevated slightly in mutants A, B, and D.
Future Directions

- Assess GP2 expression of C-containing mutants by IFA, RIPA and Western Blot
- Peptide ELISA
- Repeat of original 9 groups to test conflicting results (in progress)
- Other mutants: O-linked glycosylation mutants, new N-linked mutants
Acknowledgements

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**FDA**
Dr. Jenny Riemneschneider
Lab Animal Usage

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Disclaimer

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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**In vitro expression of ZEBO GP N-linked glycosylation mutants**

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>GP^C</th>
<th>GP^D</th>
<th>GP^CD</th>
<th>GP^WT</th>
<th>Mock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

EAIVNAQPKCNPNLHYWTQTDEGAAIGLAWIPYFGPAAEGITYTEGLMHNQDGLFCGLRQLA**NET**TQALQLFLRATTELRTFSILNRKAI**D**FLLQRWGGTCHILGPDCCIEPHDWTK**NIT**DKIDQIHDFVDKTLPDQGDNDNWWTGWWRQ**WIPAGIGVTGVIIAVIALFCICKFVF.
Ebola Virus Vaccines

• GP has been tested as a vaccine candidate in the DNA, VEE replicon, VLP, recombinant VSV and adenovirus vector systems.
• GP protects mice from challenge in all of these systems.
• Both the humoral and cellular immune responses play important roles in protection.
Survival Data Exp. 2

% survival

A  B  C  D  AB  CD  ABCD  GP  pWRG7077
Ebola Virus

Unknown reservoir

Monkey

Human

Fever, Malaise, Diarrhea, Hemorrhage

Filoviridae
enveloped, filamentous
ssRNA, (-) >19 kilobases
Ebola Virus Glycoprotein (GP)

- 676 amino acids, approx. 140 kDa
- Post-translation cleavage by furin at AA 501, forms GP1 and GP2 domains
- GP2 has a transmembrane domain and a small intracellular domain
- GP1 remains linked to GP2 by disulfide bonding
- Glycosylation accounts for about half of the molecular weight