Identification of Mutations in the HIV gp41 Subunit Associated with Neutralization Resistance

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What is HIV-1?

• HIV-1 is an epidemic that affects over 34 million people worldwide.

• HIV-1 causes disease by attacking CD4 T-cells, which are essential to a healthy immune system.

• Viral glycoproteins on the surface on the viral envelope are the proteins the virus uses to enter and infect these T-cells.

• These viral glycoproteins consists of two non-covalently bonded subunits: gp120 and gp41, which associate as a heterotrimer.

• gp120 binds to the CD4 cell receptor inducing a conformation change exposing the coreceptor binding site.
HIV-1 Fusion and Entry

• Once the gp120 subunit binds to the coreceptor, the gp41 subunit is exposed and undergoes a conformational change, unfolding and inserting into the host cell membrane.

• Once the gp41 subunit trimer is inserted, a cluster of two trimer prehairpins form.

• gp41 trimers then fold onto themselves creating a hairpin-like six helix bundle.

• Trimeric hairpins bring the membranes together, causing the outer membranes to fuse in hemifusion and the inner membrane to fuse, completely joining the two.

• The capsid is then free to enter the host cell
Why hasn’t a vaccine been developed yet?

- Because of the nature of these glycoproteins, it is very hard to establish a vaccine that will cause the production of broadly neutralizing antibodies (bnAb)
- Both gp120 and gp41 are highly variable
- Glycosylation on gp120 protects conserved epitopes on both gp120 and gp41
- The heavy glycosylation also forms a cage-like structure around the gp41 trimer, causing the gp41 subunit to be very difficult to access.
- This protection restricts antibody access to the slightly more conserved gp41 region, which provides a difficulty when producing a suitable vaccine.
Some strains can be neutralized!

- Even though it is difficult, several antibodies have been isolated from patients that can neutralize a broad range of HIV to a varying degree of success.

- Some of these antibodies are 2F5, 4e10, and 10e8, which all target the MPER in gp41.
  - Other regions in gp120 can also be targeted by different neutralizing antibodies.

- The most easily neutralized strains are tier 1 viruses. These viruses have typically been passaged in laboratories for easy use.

- Tier 2 and tier 3 viruses are harder to neutralize, but are the type of viruses that are found in most patients.
Why can only some strains be neutralized?

- Our lab focuses mainly on gp41 for several reasons:
  - gp41 is more highly conserved and the known bnAbs targeting gp41 are concentrated in a very small region (MPER).
  - This small region allows for a minimization of the vaccine candidate size, which can also limit the amount of non-neutralizing antibodies produced.

- We would like to understand why tier 2 viruses are more difficult to neutralize with gp41-targeting bnAbs than tier 1 viruses.

- The information that will be gathered from this study has the potential to shed light on the biological reasons that tier 2 viruses are more resistant than tier 1 viruses.

- If we are able to understand how these differences prevent neutralizing antibodies from binding, we may be able to produce better immunogens able to guide the immune system to overcome these resistance-inducing mutations

- For this study, we used MN (tier 1, sensitive) and 6535 (tier 2, resistant) as representative viral strains
Previous work identified a region of the gp41 envelope sequence that determined whether the strain was sensitive or resistant to neutralization.

This was accomplished by producing several chimera viruses using laboratory techniques such as digestion and ligation.

Digestion was done using appropriate restriction sites to cut the original sequences into the desired fragments.

Once the specific fragments were produced, ligation was used to create the original chimera viruses, which were used to narrow down the region in which the change(s) affecting neutralization resistance is located.
We originally hypothesized that gp120 region should block access to the gp41 region of interest.

However, we found that gp41 appeared to determine sensitivity and resistance.
Early Chimeras Produced

- We originally hypothesized that the gp120 region should block access to the gp41 region of interest.
- However, we found that gp41 appeared to determine sensitivity and resistance.
- Therefore, we made additional viruses to identify specific gp41 portions that may be involved.
Neutralization assay results

- These two graphs show the neutralization curve using the antibodies 2F5 and 10e8.

- The neutralization sensitive viral strains require a much lower amount of antibody for neutralization compared to the neutralization resistant strains.

- The viral strain MN.3 (dark blue), and chimeras 6535mMN (green) and 6535aMN (light blue) show neutralization sensitivity to both 2F5 and 10e8 antibodies
  - Because these two chimeras were sensitive, even though the gp120 region was from the resistant strain, means that gp120 had no effect on the neutralization.

- The viral strains 6535 (red) and the chimera 6535hMN (purple) both show neutralization resistance to both 2F5 and 10e8 antibodies.
What do these Results mean?

- There are seven possible differences within this region:

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MN: AVERYLKDQQLGGWGCSGKLICTTAGVPWNTSWSNKSLNYW
6535: AVERYLKDQQLGGWGCSGKLICTTAGVPWAWSNKLSDDIW
6535aMN: AVERYLKDQQLGGWGCSGKLICTTAGVPWAWSNKLSDDIW

AVrII: F595I T607A A612T D620N D621Y Q630E
HindIII: D621Y
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- These point mutants were produced via site directed mutagenesis (SDM) and tested but a single mutant was not enough to affect neutralization sensitivity.
In parallel with point mutant production via SDM, additional chimeras were made.

These additional chimeras were used to verify the hypothesis made by the original chimeras, that the region of interest is between the AvrII and HindIII sites.

- MNh6535 chimera has additional differences in the antibody binding sites that could prevent neutralization
Neutralization Assay results for Additional Chimeras

- Mm6hM was resistant as predicted, and Mh6 was sensitive.

- Ma6hM was predicted to be neutralization resistant, but is neutralization sensitive.
What do these Results mean?

![Diagram](image)

- Because of these results we can hypothesize that there are at least two mutations involved.
  - At least one mutation is located in the N-terminal and C-terminal sides of the AvrII site.

- To confirm this hypothesis we looked at the BG505 SOSIP structure from another paper.
BG505 SOSIP structure: 
gp120/gp41 region of interest

- We can use this structure because it includes the gp41 MPER region we are interested in.
  - It represents a resistant strain, so we can compare it to 6535
- N-terminal= yellow, differences marked in red
- C-terminal= green, differences marked in pink

- V535M (N-terminal)
  - May have an effect on interactions between env subunits
- L543Q (N-terminal)
  - May affect pre-to-post fusion conformation change
- F595I (C-terminal)
  - May affect conformational changes
Current Work

V535M and L543Q are both located between MfeI and AvrII (red region)
  - These two residues are located in the resistant region of the Mm6aM chimera

We are currently working on placing the F595I mutation within the Mm6aM chimera containing the V535M and L543Q changes.

After testing this mutant we will be able to determine whether or not the combination of these three point mutations is the cause of the change in neutralization sensitivity.
Future Applications: Why do we care?

- The information that will be gathered from this study has the potential to shed light on why tier 2 viruses are more difficult to neutralize than tier 1 viruses.

- If we are able to understand how these differences prevent neutralizing antibodies from binding, we may be able to produce better immunogens able to guide the immune system to overcome these resistance-inducing mutations.
Thank you!