A Molecular Arms Race: The Immune System versus HIV
Pamela J. Bjorkman, Division of Biology and Biological Engineering, Caltech
HIV Lifecycle

Spike trimer subunits

gp120: receptor and co-receptor binding

gp41: membrane anchoring and target cell fusion
HIV gp120 binds to host cell receptor, CD4

Spike trimer subunits

gp120: receptor and co-receptor binding

gp41: membrane anchoring and target cell fusion
HAART
Highly-Active Anti-Retroviral Therapy
(given in combinations – usually 3 different drugs)

Reverse transcriptase inhibitors:
- Nucleoside analogs (AZT)
- Non-nucleoside analogs (nnRTIs) (Efavirenz)

Integrase inhibitors: (raltegravir)

Protease inhibitors: (crixivan, norvan)

Only ~37% of infected people are being treated with HAART.

Fusion and co-receptor inhibitors

Only ~37% of infected people are being treated with HAART.
Ideal way to protect against HIV/AIDS: A vaccine
Neutralizing antibodies (NAb) interfere with attachment of virus to host cell and/or fusion with host membrane.

Most NAbs neutralize only a subset of HIV-1 strains.
Viral diversity and antibody diversity during infection
Characterize antibodies from HIV-infected patients

Most antibodies are strain-specific, but rare antibodies from rare patients are broadly neutralizing.

We call these bNAbs for broadly Neutralizing Antibodies.
Broadly neutralizing anti-HIV antibodies

5-10% of HIV+ individuals develop broadly neutralizing serum antibodies

We can’t (yet) make a vaccine to elicit bNAbs in animal models or humans.

bNAbs could be used for passive delivery-based prophylaxis or therapy in humans.

bNAbs protect primates in virus challenge experiments

Structural studies of bNAb epitopes reveal mechanisms by which NAbs avoid or utilize the glycan shield on Env trimer

History of bNAbs against HIV-1

1990s – 2008:
- b12, 4E10, 2F5, 2G12

2009-13:
dozens of new antibodies
Including
- V1V2: PG9/PG16
- V3: PGTs
- CD4bs: VRC01 family
- MPER: 10e8 (anti-gp41)

2014:
gp120/gp41 interface Abs
- 8ANC195
- 35O22
- PGT151
HIV-1 spikes are heavily glycosylated (~50% of mass; ~30 PNGSs per monomer).

Mass spectroscopy has identified $N$-glycans at individual PNGSs in soluble HIV-1 Env trimer (BG505 SOSIP.664)

Behrens et al., 2016, *Cell Reports*

PNGS = Potential $N$-linked Glycosylation Site
Glycoproteins in crystal structures are rarely natively glycosylated because heterogeneous glycosylation usually impedes crystallization.
<table>
<thead>
<tr>
<th>Paper</th>
<th>Env trimer</th>
<th>Fab(s)</th>
<th>Endo H?</th>
<th>Resolution</th>
<th>Form of glycan</th>
<th>Schematic glycan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Julien et al., 2013, Science</td>
<td>BG505 from GnTI/- cells</td>
<td>PGT122</td>
<td>Yes</td>
<td>4.7 Å</td>
<td>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNAC</td>
<td><img src="image1" alt="Schematic glycan" /></td>
</tr>
<tr>
<td>Pancera et al., 2014, Nature</td>
<td>BG505 from GnTI/- cells</td>
<td>PGT122, 35O22</td>
<td>Yes</td>
<td>3.5 Å</td>
<td>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNAC</td>
<td><img src="image2" alt="Schematic glycan" /></td>
</tr>
<tr>
<td>Do Kwon et al., 2015, NSMB</td>
<td>BG505 from GnTI/- cells</td>
<td>None</td>
<td>Yes</td>
<td>3.72 Å</td>
<td>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNAC</td>
<td><img src="image3" alt="Schematic glycan" /></td>
</tr>
<tr>
<td>Scharf et al., 2015, Cell</td>
<td>BG505 from kif-treated cells</td>
<td>8ANC195</td>
<td>No</td>
<td>3.58 Å</td>
<td>High mannose; Man$_9$</td>
<td><img src="image4" alt="Schematic glycan" /></td>
</tr>
<tr>
<td>Kong et al., 2015, Acta Cryst D</td>
<td>BG505 from GnTI/- cells</td>
<td>8ANC195, PGT128</td>
<td>Yes</td>
<td>4.6 Å</td>
<td>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNAC</td>
<td><img src="image5" alt="Schematic glycan" /></td>
</tr>
<tr>
<td>Garces et al., 2015, Immunity</td>
<td>BG505-N137A from GnTI/- cells</td>
<td>3H/109L, 35022</td>
<td>Yes</td>
<td>3.0 Å</td>
<td>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNAC</td>
<td><img src="image6" alt="Schematic glycan" /></td>
</tr>
<tr>
<td>Stewart-Jones et al., 2016, Cell</td>
<td>SOSIPs from GnTI/- cells</td>
<td>scFv VRC01, PGT122, 35O22</td>
<td>No</td>
<td>3.4 Å – 3.7 Å</td>
<td>High mannose; Man$_{5-9}$ described as “fully glycosylated”</td>
<td><img src="image7" alt="Schematic glycan" /></td>
</tr>
<tr>
<td>Paper</td>
<td>Env trimer</td>
<td>Fab(s)</td>
<td>Endo H?</td>
<td>Resolution</td>
<td>Form of glycan</td>
<td>Schematic glycan</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------</td>
<td>----------------------------------</td>
<td>---------</td>
<td>------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Julien et al., 2013, <em>Science</em></td>
<td>BG505 from GnTI/- cells</td>
<td>PGT122</td>
<td>Yes</td>
<td>4.7 Å</td>
<td><strong>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac</strong></td>
<td><img src="image" alt="High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac" /></td>
</tr>
<tr>
<td>Pancera et al., 2014, <em>Nature</em></td>
<td>BG505 from GnTI/- cells</td>
<td>PGT122, 35O22</td>
<td>Yes</td>
<td>3.5 Å</td>
<td><strong>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac</strong></td>
<td><img src="image" alt="High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac" /></td>
</tr>
<tr>
<td>Do Kwon et al., 2015, <em>NSMB</em></td>
<td>BG505 from GnTI/- cells</td>
<td>None</td>
<td>Yes</td>
<td>3.72 Å</td>
<td><strong>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac</strong></td>
<td><img src="image" alt="High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac" /></td>
</tr>
<tr>
<td>Scharf et al., 2015, <em>Cell</em></td>
<td>BG505 from kif-treated cells</td>
<td>8ANC195</td>
<td>No</td>
<td>3.58 Å</td>
<td><strong>High mannose; Man$_9$</strong></td>
<td><img src="image" alt="High mannose; Man$_9$" /></td>
</tr>
<tr>
<td>Kong et al., 2015, <em>Acta Cryst D</em></td>
<td>BG505 from GnTI/- cells</td>
<td>8ANC195, PGT128</td>
<td>Yes</td>
<td>4.6 Å</td>
<td><strong>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac</strong></td>
<td><img src="image" alt="High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac" /></td>
</tr>
<tr>
<td>Garces et al., 2015, <em>Immunity</em></td>
<td>BG505-N137A from GnTI/- cells</td>
<td>3H/109L, 35022</td>
<td>Yes</td>
<td>3.0 Å</td>
<td><strong>High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac</strong></td>
<td><img src="image" alt="High mannose; Man$_{5-9}$ where protected from Endo H; otherwise core GlcNac" /></td>
</tr>
<tr>
<td>Stewart-Jones et al., 2016, <em>Cell</em></td>
<td>SOSIPs from GnTI/- cells</td>
<td>scFv VRC01, PGT122, 35O22</td>
<td>No</td>
<td>3.4 Å – 3.7 Å</td>
<td><strong>High mannose; Man$_{5-9}$ described as “fully glycosylated”</strong></td>
<td><img src="image" alt="High mannose; Man$_{5-9}$ described as “fully glycosylated”" /></td>
</tr>
<tr>
<td>Gristick, von Boehmser et al., 2016, <em>NSMB</em></td>
<td>BG505 from wt HEK cells</td>
<td>10-1074, IOAMA</td>
<td>No</td>
<td>3.5 Å and 3.9 Å</td>
<td>Fully AND natively glycosylated; Complex and high mannose glycans</td>
<td><img src="image" alt="Fully AND natively glycosylated; Complex and high mannose glycans" /></td>
</tr>
</tbody>
</table>
10-1074: Asn332\textsubscript{gp120} glycan-directed V3 loop bNAb in clinical trials

IOMA: New CD4bs bNAb with unusual CD4-mimetic properties

BG505: soluble native-like gp140 Env trimer (SOSIP)
Structures of IOMA–10-1074-BG505 prepared from higher and lower MW SEC fractions show differences in glycosylation.
Natively-/fully-glycosylated Env trimer in IOMA–10-1074–BG505 crystal structure reveals most complete view of ordered glycans yet obtained.

Natively-/fully-glycosylated structures

IOMA–10-1074
BG505
PGT151 (PDB 5FUU)

4.2 Å (EM)
JR-FL

3.0 Å
PGT121_{INT}–35O22 (PDB 5CEZ)

3.7 Å
PGT122–35O22–VRC01 (PDB 5FYL)

HM-only structures

Natively-/fully-glycosylated structures

High Mannose

Harry Gristick
10-1074 Fab

IOMA Fab

BG505 gp120

BG505 gp41

Complex glycans

High mannose glycans

Exposed protein surface area that is not conserved
We compared ordered N-glycans at each PNGS in our two structures, the cryo-EM structure (5FUU), a BG505 HM crystal structure (5FYL), and mass spectroscopy assignments.
We resolved the complete epitope of 10-1074 in the context of native glycosylation.
10-1074 shows maturation towards increased electropositivity.

Germline

Scharf et al., 2016, *Elife*
IOMA is not as potent as more heavily mutated VRC01-class bNAbs, but is more potent than CD4bs bNAbs with similar levels of SHM.
IOMA is framed by complex-type N-glycans attached to $N_{197}^{\text{gp120}}$ and $N_{276}^{\text{gp120}}$. 

[Diagram showing molecular structures and glycans with labels for N-Acetylglucosamine (GlcNAc), Mannose (Man), Galactose (Gal), Fucose (Fuc), and Sialic Acid.]
Mapping accessible areas onto natively-glycosylated BG505 reveals antibody-vulnerable glycan holes that can be targeted by strain-specific antibodies.

Areas of contiguous red (left) that are white/light purple (right) are sites of low sequence conservation potentially accessible to antibodies. Can find one such site on BG505 adjacent to N241$_{gp120}$.

See McCoy et al., 2016, “Holes in the Glycan Shield of the Native HIV Envelope Are a Target of Trimer-Elicited Neutralizing Antibodies, Cell Reports.”
The HIV-1 Env utilizes multiple strategies to avoid antibodies

- Glycan shield
- Rapid mutation
- Hide conserved regions in interfaces
- Low density of Env spikes
Few and far between: how HIV may be evading antibody avidity
Klein and Bjorkman, 2010, *PLoS Pathogens*

For most viruses, two identical Fabs in IgGs permit bivalent binding through inter-spike crosslinking.
For most viruses, two identical Fabs in IgGs permit bivalent binding through inter-spike crosslinking.

Unlike other viruses, HIV has very few spikes, and the spikes are far apart – most antibodies can’t bind with both Fabs.

Zhu et al., 2006, *Nature*

HIV spikes are relatively immobile in virus membrane
HIV’s low spike density plus its rapid mutation – a deadly combination

Intra-spike cross-linking would allow bivalent binding (avidity) despite low spike density.

Engineer Abs that can crosslink between spikes?

Can’t make inter-spike crosslinking reagents that consistently crosslink because inter-spike distances vary even on a single virion.

How to make bivalent reagents that bind to ≥2 epitopes within a single spike trimer? Modeling based on Env trimer structures?
HIV-1 Env exists in multiple conformations on virions

We use dsDNA as a ruler to measure distances on Env spike trimers on virions.

Persistance length \(~500\, \text{Å}\); 3.4 Å/bp increments


Galimidi et al., 2015, *Cell*
Compare neutralization potencies of CD4bs homo-diFab as a function of dsDNA linker length

Most potent neutralization seen with a 62 bp DNA linker.

Galimidi et al., 2015, *Cell*
Best separation distance for CD4bs homo-diFabs: ~210 Å

62 bp x 3.4 Å/bp = 211 Å
Optimal 3BNC60 homo-diFab is much more potent than 3BNC60 IgG when compared against many HIV strains.
Optimal homo-diFab and hetero-diFab reagents show there is enormous potential for improving bNAb by using intra-spike cross-linking to achieve synergistic neutralization.

Galimidi et al., 2015, *Cell*
What about using avidity to suppress viral escapes?

Preliminary *in vitro* evolution data suggest suppression of HIV-1 escape mutations by PG16-50bp-3BNC60 hetero-diFab
Intra-spike cross-linking can overcome HIV evasion of avidity

- Measuring method reveals dynamic information about Env conformations during neutralization
  - Compare optimal distances for Tier 1 versus Tier 2; CD4-dependent versus CD4-independent strains
- Up to 100-fold increases in geometric mean potency achieved with first generation intra-spike crosslinking reagents (homo- and hetero-diFabs)
  - Supports hypothesis that low spike density contributes to vulnerability of anti-HIV antibodies to spike mutations
- Ideal anti-HIV therapeutic for passive delivery would utilize avidity to achieve intra-spike crosslinking (using protein-based linkers, not DNA)
  - Reduce the concentration required for sterilizing immunity
  - Render HIV’s low spike density irrelevant
  - Hopefully would be resistant to Env mutations (in vitro evolution results)
- Analogous to using several drugs or antibodies during ART, simultaneous binding to different epitopes would also reduce/abrogate sensitivity to Env mutations
Understanding structural changes in Env induced by CD4 and coreceptor binding could facilitate design of new therapeutic targets

Adapted from Didigu and Doms, *Viruses* 2012, 4, 309-324
HIV-1 Env exists in different conformations on virions

Liu et al., 2008, Nature; Merk & Subramaniam, 2013, Curr Opin in Struct Biol
Another conformational state of HIV-1 Env

3.58 Å crystal structure of 8ANC195-Env trimer complex

~17Å single particle EM structure of 17b-CD4-8ANC195-Env complex

Env conformation is mid-way between partially open and open

Scharf et al., 2015, Cell
Env trimer in CD4-bound partially-open 8.9 Å cryo-EM structure: V1V2 undergoes ~40 Å conformational change and interacts with CD4
Env trimer in CD4-bound partially-open 8.9 Å cryo-EM structure: V1V2 undergoes ~40 Å conformational change and interacts with CD4
Natively-glycosylated Env

Harry Gristick
Lotta von Boehmer (RU)
Anthony West

Michel Nussenzweig (RU)

Single particle EM
Haoqing Wang

Michael Schamber
Michael Seaman, CAVD Neutralization Facility
Donor samples: Florian Klein, Gerd Fätkenheuer (U of Cologne)

Funding: HIVRAD P01, BMGF
Funding for EM: NIH P50 Cheetah center
Intra-spike crosslinking reagents

Rachel Galimidi
Anthony West
(Joshua Klein → Google)

Devashish Joshi, Maria Politzer,
Shiyu Bai, Pri Gnanapragasam
Caltech Protein Expression Center
Michael Seaman, CAVD Neutralization Facility

Funding: BMGF, NIH Director’s Pioneer Award