Selection and Evolution of V1-V2 Length and Glycosylation of Transmitted HIV-1 Variants

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Background and Hypothesis

•A small number of viruses are transmitted to a newly infected subject from the diverse mixture of variants present in the chronically infected partner (1-3).

•In a recent study, a small number of newly infected subjects with HIV-1 subtype C viruses were shown to have significantly shorter envelope variable loops and a lower number of potential N-linked glycosylation sites (PNGS) as compared to viruses harbored by their long-term infected partners (3).

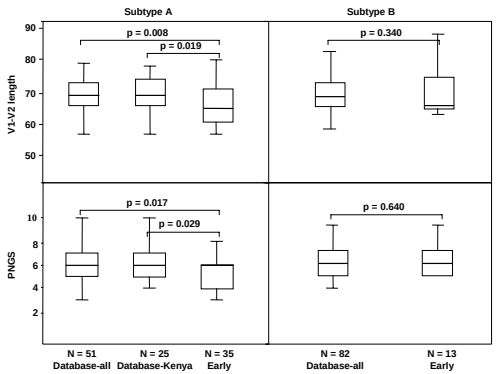
•We hypothesized that viruses with these characteristics are transmitted in other HIV-1 subtype infections.

Subjects and Methods

	HIV-1 Subtype A	HIV-1 Subtype B
Country of origin	Kenya	United States
# of subjects	27 women, 8 men	10 women, 3 men
Post negative serology (days)	70 (IQR 49-161)	142 (IQR 114-234)
Primary Risk Factors	Heterosexual contact	Homosexual contact, Injection drug use

•V1-V2 length and number of PNGS were compared to subtype specific sequences in the Los Alamos database. Sequences known to be within one year of infection were excluded from the dataset used for this comparison. Early subtype A sequences were also compared to Kenyan subtype A sequences in the Los Alamos database.

Results



Conclusions #1

•These studies indicate that similar to subtype C infections there is a selection for HIV-1 variants with fewer N-linked glycosylation sites and condensed V1-V2 loop sequences during heterosexual transmission of HIV-1 subtype A.

•There is no apparent selection for these signature envelope characteristics during HIV-1 subtype B transmission.

•These differences may be related to the differences in the mode of transmission. All subtype A viruses in this study and the subtype C viruses in the previous study (3) were all cases of heterosexual transmission. The reported risk factors for HIV-1 acquisition in the subjects who acquired subtype B were primarily homosexual contact and injection drug use.

•On the other hand, these differences may be subtype specific; Shorter less glycosylated variable loops are selected for transmission in subtype A and subtype C viruses but not in subtype B viruses.

•Understanding the signature characteristics of the heterosexually transmitted subtype A and subtype C viruses will be important in developing prevention strategies against these highly prevalent HIV-1 subtypes and the most common mode of transmission in the world.

Background and Hypothesis

•Studies of HIV-1 in humans and SIV/macaque model have shown that viruses evolve to increase the number and/or vary the position of the carbohydrates to shield them from the host immune response (4-7).

•We hypothesized that although viruses with shorter and less glycosylated envelope loops are selected for transmission, these signature characteristics are lost within 2 to 3 years after infection.

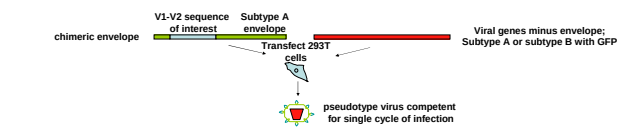
•We further hypothesized that V1-V2 changes over time lead to differences in antibody neutralization sensitivity and ability to infect cells with varying levels of receptors/coreceptors

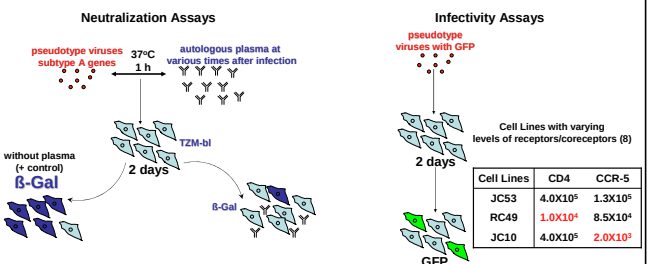
Subjects and Methods

•We examined V1-V2 envelope sequences from 9 women with HIV-1 subtype A early in infection and 2 - 3 years after infection. All these women acquired HIV-1 heterosexually and were not treated with antiretroviral medicines.

•A minimum of 10 V1-V2 sequences were analyzed from each subject both early and later in infection. These sequences were from 24 to 60 independent PCR reactions.

•Pseudotype viruses with chimeric envelopes





Cell Lines	CD4	CCR-5
JC53	$4.0X10^5$	$1.3X10^5$
RC49	$1.0X10^4$	$8.5X10^4$
JC10	$4.0X10^5$	$2.0X10^3$

Results

•6 subjects had a significantly higher number of PNGS in the later sequences compared to the transmitted variants. QA284 is an example.

```
      QA284 Day 36 after estimated infection
   CHH...NV..TKITQEMK GEIKNCSFNVTTELRDKKQKVYSLFYRLDVVPF DNSDDSSPYRLINC                15/15

      QA284 Day 1412 after estimated infection
   --DCHHN-..-N---D-T ----N---N----------------------L K---NN-S------                2/12
   -.........-N---D-- ----N---N----------------------L N---N---S------                2/12
   --Y...N-..-N------ ----N---N--------Q--------------N---N-N-S------                1/12
   ----...ND..-.----.. ----N---N----------------------  ---NN--L------                1/12
   -----..N-..-N---N-- ----N---N----------------------L N-NN---S------                1/12
   -R----.N-TN-N------ ----N---N----------------------L N---NN---------                1/12
   -----..N-..-N---D-- ----N---N----------------------  N---NN-S------                1/12
   -----..N-..-N---D-E ----N---N-----K----------------  S-N-NN-S------                1/12
   -----..N-..-N---D-T ----N---N----------------------  N---N--S------                1/12
   -R----.N-TN-N------ ----N---N----------------------  N---N---------                1/12
```

•3 subjects had significantly longer V1-V2 loops in the later sequences compared to the early viruses. QB424 is an example.

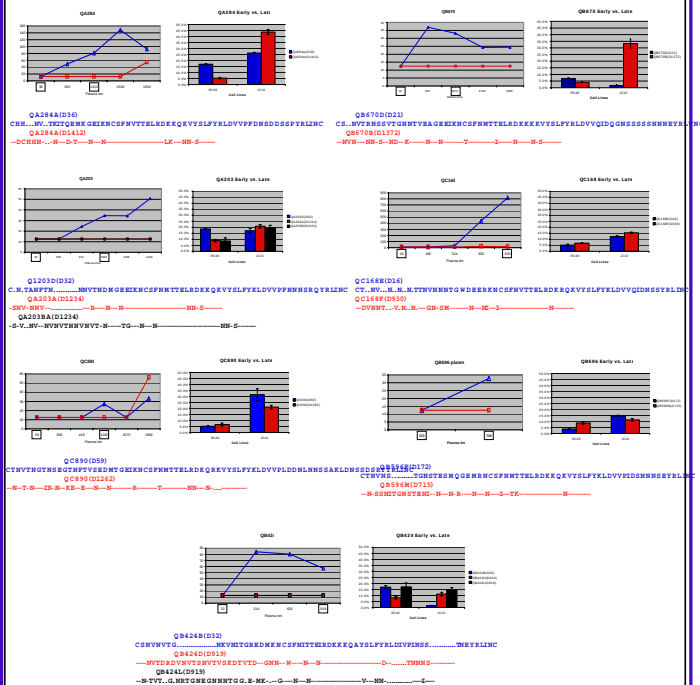
```
      QB424 Day 36 after infection
CSNVNVTG.................NKVNIT GREDMKNCSFNITTEIRDKKKQAYSLFYRLDIVPI NSS............TNEYRLINC 12/21
----N---.....................N-- ------N---N---------------------- N-.............I--------  7/21
----N---.....................N-- ------N---NV--------------------- N-.............I--------  1/21
-C--N---.....................N-- ------N---N---------------------- N-.............I--------  1/21

      QB424 Day 919 after infection
--N-TV..S..NDTAP.........D-EGN-- -G----N---N---------------------- D-N.......TKDSS-K-------  1/12
----NVTDS..NGTVP.........D--GNN- -W----N---N---------------------- D-.........TNDSS-S-------  1/12
----RV.....NDTGNH.......TDNQTN-- -G----N---N---------------------- --DIVPIDSSTNDSR---------  1/12
----NVTDRDVNVTSNVTVSKDTVTD--GNN- -W----N---N---------------------- D-........TNNNS---------  1/12
----NVTDS..NVNVTDSN....V..TVTNN- -W----N---N---------------------- D-........TNDNS-K-------  1/12
-----VNDTG.NH...........TDNQTN-- -G----N---N---------------------- --DIGPNDSS.....-K-------  1/12
----NVTDS..NGTDK.........D--GNN- -W----N---N---------------------- D-.........TNDNS-K-------  1/12
--N-TV..S..NDTA.........TD-EGN-- -G----N---N---------------------- D-N.......TKDSS-K-------  1/12
--N-TV..S..NNVT........VTD-EGN-- -G----N---N---------------------- D-.........TNDRS---------  1/12
----NVTDS..NVNVTDSNVH..VTD-QGN-- -G----N---N---------------------- D-........TNDNR---------  1/12
----IVNDTG.NH...........TDNQTN-- -G----N---N---------------------- -NK.............N--------  1/12
--N-TVT..G.NRTGNEGNNNTGG.E-MK-.- -G----N---N----------------------V--- NN-.............----I----  1/12
```

•In aggregate (when sequences from all 9 subjects were pooled), sequences 2 - 3 years after infection had significantly higher number of PNGS (P<0.0001) but no significant change in V1-V2 length compared to the transmitted sequences.

Neutralization and Infectivity Results

•Blue = pseudotype virus with early V1-V2 sequence; Red = pseudotype virus with late V1-V2 sequence

•Boxed plasma time indicates the time the V1-V2 early and late sequence variants were isolated



Conclusions #2

•Viruses isolated late in infection had significantly more potential glycosylation sites compared to transmitted variants. There was no significant difference in V1V2 length over 2-3 years after infection.

•V1-V2 sequences influence antibody neutralization. Insertion of V1-V2 sequences from HIV-1 variants around 2-3 years after infection rendered pseudotype viruses resistant to concurrent or earlier autologous plasma.

•V1-V2 sequences have minor influences on infectivity. The majority of pseudotype viruses with V1-V2 sequences from early in infection were slightly better at infecting cell lines with low CD4 concentrations compared to pseudotype viruses with V1-V2 sequences later in infection. The majority of viruses with late V1-V2 sequences were subtly more efficient at infecting cell lines with low CCR5 levels compared to variants with early V1-V2 sequences.

•These phenotypic differences may be important in understanding why certain HIV-1 variants are selected during heterosexual transmission. Designing an effective HIV-1 vaccine will require a better understanding of the genotypic and phenotypic properties of the transmitted variants.

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