Characterization of HIV-1 Envelopes in Acutely and Chronically Infected Injection Drug Users

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BACKGROUND AND GOAL

BACKGROUND: Mucosally acquired HIV-1 infection results from a limited number of variants, and these infecting strains potentially have unique properties, such as increased susceptibility to entry blockers, relative interferon-alpha (IFN-α) resistance, and replication differences in some primary cells [1-3].
GOAL: There is no data about the phenotypic properties of HIV-1 envelope variants found early after acquisition among subjects infected through injection drug use (IDU).

MATERIALS AND METHODS

- Compared the characteristics of virus envelopes among injection drug users sampled prior to seroconversion (HIV RNA+/Ab-), within 1 year (early), and more than 2 years (chronic) after estimated acquisition (Table 1).
- Full-length HIV-1 envelopes generated from pooling multiple bulk PCRs were incorporated into a NL4-3 backbone to create replication competent recombinant viruses .
- Viruses were examined for receptor usage, gut homing receptor, α4β7, binding, sensitivity to entry inhibitors and IFN-α, and replication capacity in various primary cell cultures, such as CD4+ T cells, immature and mature monocyte derived dendritic cell (MDDC) – CD4+ T cell co-cultures, and CD4+ T cells expressing high levels of α4β7 integrin.
- Summary phenotypic characteristics were compared using the Wilcoxon rank-sum test.

SUBJECTS

Table 1. Demographics, viral and coreceptor characteristics

Subject	Estimated PI (months)[1]	Plasma virus level	CCR5[2]	CXCR4[3]	Tropism[4]	Estimated PI (months)[1]	Plasma virus level	CCR5[2]	CXCR4[3]	Tropism[4]
HIV RNA+/Ab-										
A31	Ab-/VL+	154,381	12.7	13.83	R5/X4					
A33	Ab-/VL+	1,031,929	9.7	<0.03	R5					
A38	Ab-/VL+	31,174	10.8	<0.03	R5					
A40	Ab-/VL+	14,422	14.9	<0.03	R5					
A41	Ab-/VL+	1,289,549	13.3	<0.03	R5					
A42	Ab-/VL+	2,285,517	12.6	<0.03	R5					
A43	Ab-/VL+	499,713	12.3	<0.03	R5					
EARLY						CHRONIC				
A2	3.0	85,439	11.5	<0.03	R5	21.4	30,996	19.1	<0.03	R5
A4	5.0	2,158,680	1.9	14.3	R5/X4	24.5	79,094	0.3	12.2	R5/X4
A5	4.1	279,745	8.7	<0.03	R5	27.2	137,661	11.1	<0.03	R5
A17	4.9	40,634	14.6	<0.03	R5	20.9	20,995	13.0	<0.03	R5
A18	3.2	188,258	12.1	<0.03	R5	27	12,299	11.6	<0.03	R5
A23	4.9	56,189	10.6	<0.03	R5	27.7	79,490	12.1	<0.03	R5
A27	4.1	152,472	10.4	<0.03	R5	27.1	17,668	9.8	11.91	R5/X4

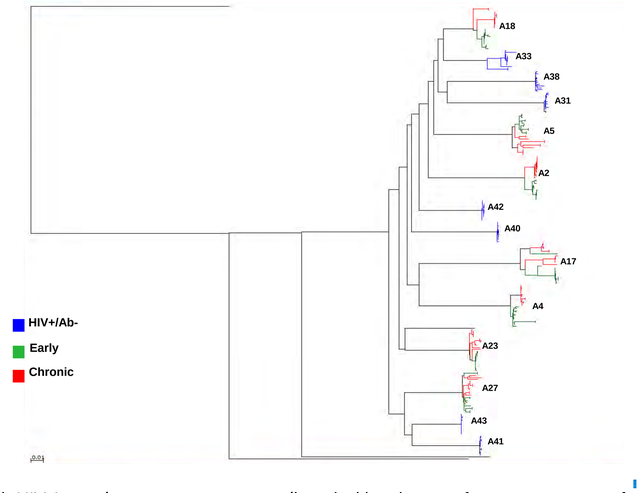
[1]Interval in months from the estimated date of seroconversion to the day of sample collection. Ab-/VL+: Sampled prior to seroconversion
[2]P24 (ug/ml) from U87/CD4/CCR5 cells at day 4 post-infection.
[3]P24 (ug/ml) from U87/CD4/CXCR4 cells at day 4 post-infection.
[4]Tropism as determined on U87/CD4+/CCR5 and U87/CD4+/CXCR4 cells.

REFERENCES

1. Fenton-May, A.E., *et al.* Relative resistance of HIV-1 founder viruses to control by interferon-alpha. *Retrovirology* **10**, 146 (2013).
2. Pena-Cruz, V., *et al.* HIV-1 envelope replication and α4β7 utilization among newly infected subjects and their corresponding heterosexual partners. *Retrovirology* **10**, 162 (2013).
3. Parrish, N.F., *et al.* Phenotypic properties of transmitted founder HIV-1. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 6626-6633 (2013).

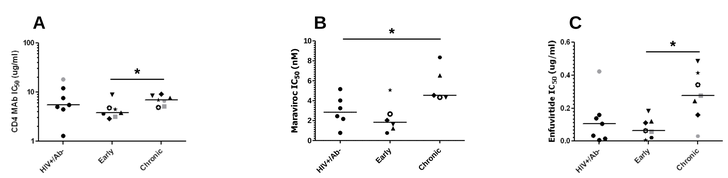
RESULTS

Figure 1: The HIV RNA+/Ab- envelopes are not related to the virologically linked early and chronic infection variants.



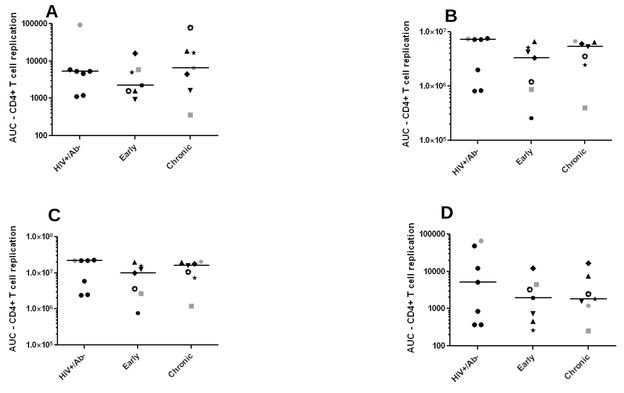
Full-length HIV-1 envelope sequences were aligned with subtype reference sequences from the Los Alamos database using Clustal X. Maximum likelihood phylogenies were generated using Paup with parameters from FindModel best fit evolutionary model as described previously. Subject IDs are noted with the different color nodes representing sequences from the 3 groups.

Figure 2: The HIV RNA+/Ab- envelopes have greater sensitivity to CCR5 and fusion inhibitors.



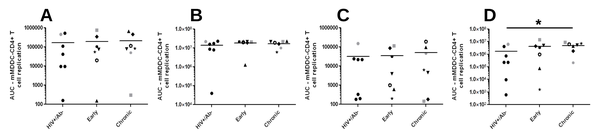
Scatter plots show IC_{50}s to CD4 antibody (A), Maraviroc (B), and Enfuvirtide (C) with a line denoting the median. Each individual symbol represents a subject's mean from a minimum of 3 independent experiments. Circles denote the estimates for the HIV RNA+/Ab- subjects. The longitudinal sampling is denoted by a unique symbol (♦ A2, ■ A4, ○ A5, ● A17, ▲ A18, ▼ A23, ●A27) for each subject. Symbols in gray denote virus stocks shown to be able to utilize the CXCR4 receptor. Significant differences (p 0.05) are indicated with a star in the graphs. Four envelope quasispecies with the documented ability to use the CXCR4 receptor were omitted from the examination of Maraviroc sensitivity.

Figure 3: Viruses from different phases of infection display similar replication capacity in CD4+ T cells.



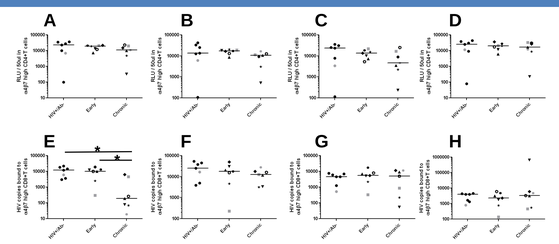
Scatter plots show replication area under the curve (AUC) in activated CD4+ T cells from 4 different HIV-1 seronegative donors (A-D). The AUC was calculated over 10 days post infection. Each individual symbol represents a subject's AUC. Similar to figure 2, in the early and chronic group unique symbols are used to show the longitudinal changes within an injection drug user. Symbols in gray denote virus stocks shown to be able to utilize the CXCR4 receptor.

Figure 4: Viruses with HIV RNA+/Ab- compared to chronic envelopes have lower replication in mature MDDC – CD4+ T cells.



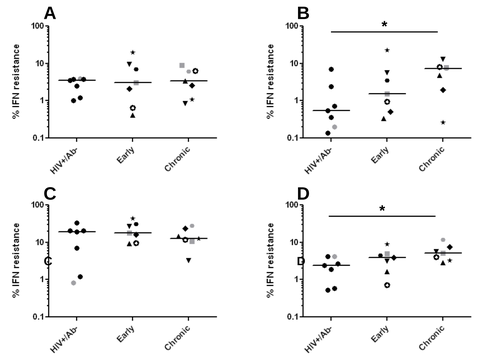
Scatter plots show replication area under the curve in mature (A – D) MDDC – CD4+ T cell co-cultures from 4 different HIV-1 seronegative donors. Significant differences (p 0.05) are indicated with a star in the graphs. Symbols in gray denote virus stocks shown to be able to utilize the CXCR4 receptor. For symbols, see Figure 2.

Figure 5: Viruses with envelopes from different phases of infection demonstrate similar replication in α4β7 high CD4+ T cells.



Scatter plots show relative light units in TZM-bl cells infected with 50 ul of virus supernatants recovered from virus exposed FACS confirmed α4β7 high CD4+ T cells (A – D). Each symbol represents mean of 3 independent RLU measurements. Scatter plots show number of RNA copies recovered from virus exposed FACS confirmed α4β7 high CD8+ T cells from 4 different HIV-1 seronegative donors (E - H). Each symbol represents mean of 2 independent RNA copy quantifications. Significant differences (p 0.05) are indicated with a star in the graphs. Symbols in gray denote virus stocks shown to be able to utilize the CXCR4 receptor. Symbols, see Fig. 2

Figure 6: The HIV RNA+/Ab- compared to the chronic envelopes have greater sensitivity to interferon-α



Scatter plots show % interferon resistance (replication in the presence as compared to the absence of IFN- α) in CD4+ T cells from 4 different HIV-1 seronegative donors (A – D). Significant differences (p 0.05) are indicated with a star in the graphs. Symbols in gray denote virus stocks shown to be able to utilize the CXCR4 receptor. Symbols, see Fig. 2

CONCLUSIONS

- **Similar to mucosal acquisition, HIV-1 envelope quasispecies present in injection drug users prior to seroconversion have unique phenotypic properties compared to those circulating during the chronic phase of disease.**
- **This suggests that there is selection for specific HIV-1 variants during injection drug use.**

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