Transmitter as Compared to Recipient Envelopes Confer Greater Replication and α4β7 Utilization

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BACKGROUND AND HYPOTHESIS

BACKGROUND: Newly infected individuals generally acquire only a limited number of viruses from chronically infected partners with diverse HIV-1 variants. The biological mechanism for this selection remains uncertain [1].

HYPOTHES: Variants with envelopes that have enhanced receptor usage, greater fusion capacity or confer higher replication preferentially establish infection in a naïve host

MATERIALS AND METHODS

- Compared envelope properties from 9 transmission pairs enrolled in the Rakai community cohort study in Uganda (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 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, Langerhans cells (LC) – CD4+ T cell co-cultures and CD4+ T cells expressing high levels of α4β7 integrin.
- Recipient to transmitter envelope characteristics were compared using the Wilcoxon rank-sum test and one-sample Wilcoxon signed-rank test respectively.

SUBJECTS

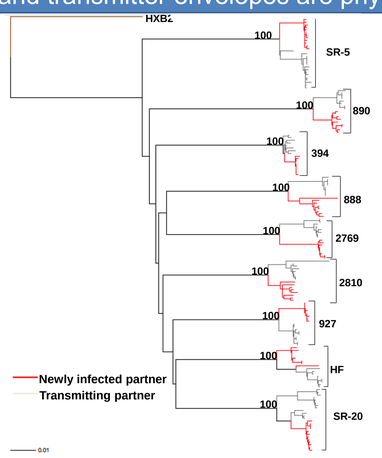
Table 1. Demographics, viral and coreceptor characteristics

Couple	Type[1]	Int. Days[2]	Partner Interval[3]	Recipient CCR5[4]	Recipient CXCR4[5]	Recipient Tropism[6]	Transmitter CCR5	Transmitter CXCR4	Transmitter Tropism
HF	FTM	17	3	7.24	<0.1	R5	8.27	<0.1	R5
888	MTF	74	19	10.39	<0.1	R5	11.91	<0.1	R5
890	MTF	138	12	3.79	<0.1	R5	2.27	<0.1	R5
394	MTF	93	2	7.79	<0.1	R5	10.85	<0.1	R5
927	MTF	324	46	12.55	<0.1	R5	13.37	<0.1	R5
2769	MTF	149	46	5.69	<0.1	R5	5.34	0.65	R5/X4
2810	MTF	161	23	5.49	<0.1	R5	6.12	<0.1	R5
SR-5	MTF	17	0	12.62	<0.1	R5	9.72	<0.1	R5
SR-20	MTF	91	34	6.70	<0.1	R5	7.24	<0.1	R5

[1]FTM: female to male; MTF: male to female
[2]Interval in days from the estimated date of acquisition to the day of sample collection for the newly infected partner. For seronegative individuals, interval was estimated as a maximum of 17 days.
[3]Interval in days between sampling of the two partners within a couple
[4]P24 (ug/ml) from U87/CD4/CCR5 cells at day 4 post-infection.
[5] P24 (ug/ml) from U87/CD4/CCR5 cells at day 4 post-infection.
[6]Tropism as determined on U87/CD4+/CCR5 and U87/CD4+/CXCR4 cells.

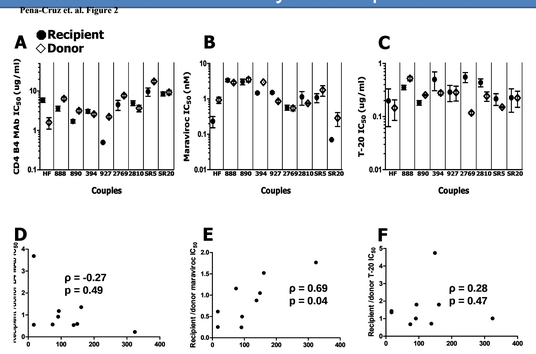
RESULTS

Figure 1: Recipient and transmitter envelopes are phylogenetically linked



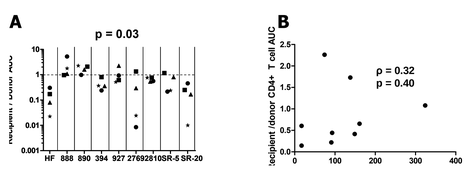
RESULTS

Figure 2: Recipient and transmitter envelope viruses have no significant differences in sensitivity to receptor and fusion blockers.



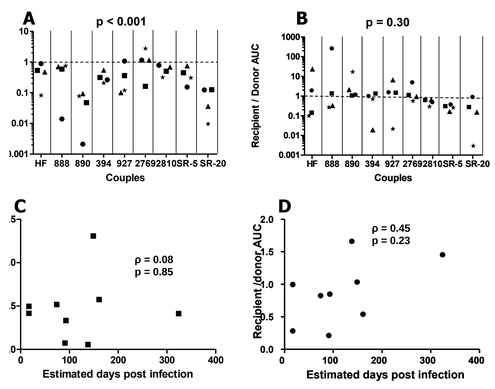
The recipient to donor Maraviroc IC_{50} ratio showed a significant direct correlation with estimated days post infection (Fig. 2E). Similar to previous publications, this suggests that the envelopes isolated earliest after acquisition either require higher CCR5 levels or structurally invariant CCR5 conformations [2, 3]

Figure 3: Transmitter envelope viruses replicate significantly better than recipient envelope viruses in CD4+ T cells.



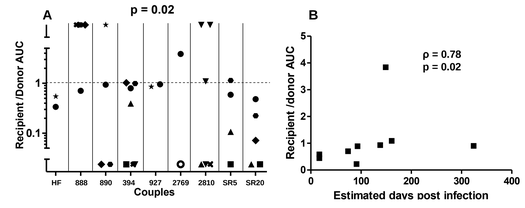
(A) Recipient relative to transmitter envelope virus replication area under the curve (AUC) among CD4+ T cells from 4 different blood donation volunteer 1 (circle), 2 (square), 3 (triangle), and 4 (star). The dotted line at a ratio of 1 separates instances when the recipient envelope viruses replicated to a greater extent than the transmitter envelope viruses (> 1) or vice-versa (< 1).
(B) Graph B shows correlation between interval from estimated infection to sampling and recipient to transmitter CD4+ T cell replication AUC ratio.

Figure 4: Transmitter envelope viruses have higher replication compared to recipient envelope viruses in monocyte derived-dendritic cells (MDDC) – autologous CD4+ T cell cultures.



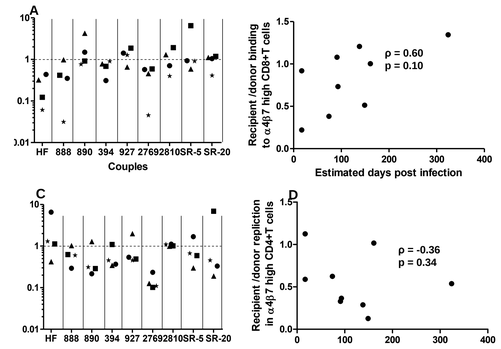
Recipient relative to transmitter envelope virus replication AUC among mature MDDCs – autologous CD4+ T cells (A) and immature MDDCs – autologous CD4+ T cells (B). Cells were from 4 different blood donation volunteer s (see Fig. 3). The dotted line at a ratio of 1 separates instances when the recipient envelope viruses replicated to a greater extent than the transmitter envelope viruses (> 1) or vice-versa (< 1).
The bottom graphs show correlation between interval from estimated infection to sampling and recipient to transmitter replication AUC ratio in mature MDDC (C) and immature MDDC (D).

Figure 5: Transmitter as compared to recipient envelope viruses replicate to higher levels in skin Langerhans cells (LCs) – heterologous activated CD4+ T cell cultures.



Recipient relative to transmitter envelope virus replication AUC among the 10 different skin donation volunteers LCs. Each symbol represents LCs from a different subject. Dotted line shows where ratio is greater or less than 1. Symbols in the bottom and top third of the y-axis depict instances where replication was observed either only in the recipient or transmitter envelope virus within a partnership respectively.
Graph B show correlation between interval from estimated infection to sampling and recipient to transmitter replication AUC ratio.

Figure 6: Transmitter envelope viruses demonstrate higher α4β7 usage.



Binding to α4β7 was estimated by recipient versus transmitter HIV-1 RNA copies recovered from α4β7 high / CD8+ T cells (top graphs). Replication was measured in α4β7 high / CD4+ T cells (bottom graphs). Methods for estimating of α4β7 utilization have been described previously (4).

The recipient to donor replication in α4β7 high / CD4+ T cells is the only phenotype to show an inverse correlation to estimated days post-infection, although not statistically significant (Fig. 6D). This potentially suggests infecting strains isolated early after acquisition replicate efficiently in α4β7 high / CD4+ T cells.

CONCLUSIONS

- **Envelopes found in recently infected subjects as compared to the corresponding transmitting partner have significantly lower replication capacity in primary cells and decreased binding to the α4β7 integrin.**
- **Even though the majority of recently infected subjects were not sampled soon after estimated acquisition, our results suggest that if enhanced infectivity and/or greater α4β7 binding are transmission phenotypes then viruses with these characteristics are selected against early after acquisition and subsequently enriched during the chronic phase of disease.**

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REFERENCES

1. Sagar M: HIV-1 transmission biology: selection and characteristics of infecting viruses. *J Infect Dis* 2010, 202 Suppl 2:S289-296.
2. Etemad, B., et. al., 2009. Human immunodeficiency virus type 1 V1-to-V5 envelope variants from the chronic phase of infection use CCR5 and fuse more efficiently than those from early after infection. J Virol **83:**9694-708.
3. Parker, Z. F., S, et. al., 2013. Transmitted/Founder and Chronic HIV-1 Envelope Proteins Are Distinguished by Differential Utilization of CCR5. J Virol **87:**2401-11.
4. Etemad B, et. al., Early Infection HIV-1 Envelope V1-V2 Genotypes Do Not Enhance Binding or Replication in Cells Expressing High Levels of alpha4beta7 Integrin. J Acquir Immune Defic Syndr 2013.