

Thymic pathogenicity of an HIV-1 envelope is associated with increased CXCR4 affinity and V5-gp41-dependent cytopathicity, but not V1/V2-associated CD4 affinity

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ABSTRACT

Background: We previously identified a primary HIV-1 envelope obtained at transmission from a rapid disease progressor that showed a distinct ability to allow replication and pathogenesis in the human thymus models (1). In this work, we dissect the envelope regions and elucidate the envelope properties which correlate with thymic depletion.

Methods: We determined the relative entry efficiency, receptor affinity, and coreceptor affinity of viruses expressing the uniquely pathogenic envelope (R3A) and a less pathogenic envelope (R3B) isolated from the same patient using *in vitro* T cell models. We created R3A and R3B recombinant envelopes to map the region(s) responsible for each *in vitro* phenotype. Finally, we created recombinant viruses to test the contribution of putative envelope domains to the pathogenic phenotype in the human fetal-thymus organ culture.

Results: The pathogenic R3A envelope allows enhanced virus entry into T cells and shows enhanced affinity for CD4 and CXCR4 relative to the NL4.3 or R3B envelopes. Furthermore, R3A is more cytopathic for T cells when expressed *in cis* or *in trans*. R3A and R3B expressing viruses show the same inherent stability and envelope incorporation. Interestingly, the enhanced entry and CD4 affinity of R3A map to the V1/V2 region, while both V1/V2 and V5-gp41 contributed to R3A envelope-mediated cytopathicity *in vitro*. Enhanced affinity for CXCR4 *in vitro* proved to be a complex phenotype not attributable to one region of envelope. Surprisingly, when the recombinant envelopes in the NL4-3 backbone were tested in the thymus, V1/V2 was not found to play a role in thymic replication or pathogenesis. Instead, V5-gp41 was able to partially account for the success of R3A, as both R3A (R3B V5-gp41) and R3B (R3A V5-gp41) showed intermediate levels of replication and pathogenesis.

Conclusions: The V5-gp41 region of the R3A envelope is partially responsible for the unique thymic pathogenesis of the R3A envelope. Furthermore, in spite of the clear contribution of V1/V2 to R3A entry and CD4 affinity *in vitro*, this property has no relevance for pathogenesis in the context of the thymic microenvironment. These data highlight the disconnection between *in vitro* and *ex vivo* pathogenesis and point to a unique contribution of V5-gp41 through an as yet unresolved mechanism.

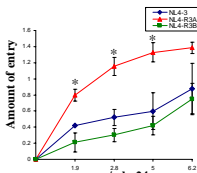


Figure 1: The R3A env enables enhanced virus entry into Sup-T1 cells. Beta-lactamase-vpr containing viruses were used to infect Sup-T1 cells. The amount of entry was calculated by measuring the ratio of cleaved to uncleaved fluorogenic beta-lactamase substrate added to Sup-T1 cells. (**p* < 0.05 by the student's *t* test for NL4-R3A vs. both NL4-R3B and NL4-3)

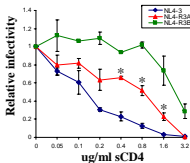


Figure 2: The R3A env shows enhanced sensitivity to sCD4. Virus was pre-incubated with the indicated dose of soluble CD4 (sCD4) prior to infection of TZM-bl cells, which express luciferase under control of the HIV-1 LTR. Infection in the presence of sCD4 was normalized to that achieved with no sCD4 for each virus. We take these data to indicate that R3A env has greater affinity for CD4 than R3B env. (**p* < 0.05 by the student's *t* test for NL4-R3A vs. both NL4-R3B and NL4-3)

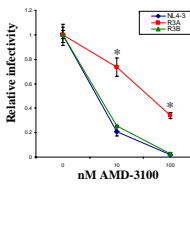


Figure 3: The R3A env shows enhanced resistance to AMD-3100. NL4-luciferase pseudotyped with each envelope was used to infect Sup-T1 cells which had been pre-incubated with the indicated dose of AMD-3100 prior to infection. Infection in the presence of AMD-3100 was normalized to that achieved with no drug for each virus. We take these data to indicate that R3A env has greater affinity for CXCR4 than R3B env. (**p* < 0.05 by the student's *t* test for R3A vs. both R3B and NL4-3)

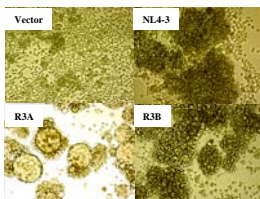


Figure 4: The R3A env enhances syncytia formation and cytopathicity when expressed in T cells. Retrovirus encoding an envelope expression vector was used to transduce Sup-T1 cells. Three days later, pictures were taken. Equivalent surface envelope expression was detected by FACS. Cytopathicity was quantitated by trypan blue exclusion and was significantly enhanced for the R3A env (data not shown). Cytopathicity could be blocked by either T20 or AMD-3100, showing it is fusion and CXCR4 dependent.

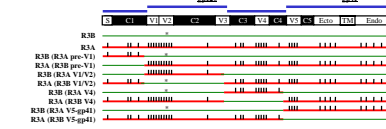


Figure 5: Generation of recombinant envelope genes to map the pathogenic determinants in the R3A env. Schematic map of the eight recombinant envelopes made using conserved restriction sites. Vertical bars indicate specific amino acid differences between R3A and R3B. The asterisk indicates the additional putative glycosylation site in the R3B envelope. The V1/V2 and V5-gp41 recombinants were also cloned into the backbone of the NL4-3 virus, resulting in the deletion of nef, as previously described (1).

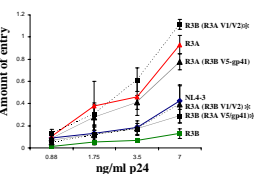


Figure 6: The V1/V2 region mediates enhanced entry and sCD4 resistance. The Blam-vpr assay was performed with the four recombinant viruses shown, as in Figure 1. The data indicate a predominant contribution of the V1/V2 region to viral entry. Similar mapping data was obtained for sCD4 sensitivity (data not shown). (**p* < 0.05 by the student's *t* test for each recombinant relative to the parental virus)

METHODS and RESULTS

CONCLUSIONS

In vitro correlates of thymic pathogenesis for the R3A envelope

- Increased viral entry into Sup-T1 cells
- Increased soluble CD4 sensitivity (increased CD4 affinity)
- Increased AMD-3100 resistance (increased CXCR4 affinity)
- Increased cytopathicity when expressed in T cells

Envelope region responsible for each phenotype

- Viral entry and CD4 affinity map to the V1/V2 region
- CXCR4 affinity is complex and does not map to one particular region
- T cell cytopathicity receives contributions from both the V1/V2 and the V5-gp41 regions

Env regions involved in thymus

- The V1/V2 region makes no contribution in the thymus
- The V5-gp41 is partially responsible for thymic replication and pathogenesis

Conclusions and Implications

- CD4 affinity and increased viral-cell entry do not correlate with replication or pathogenesis in the thymus organ, suggesting they may not contribute to pathogenesis *in vivo*
- The V5-gp41 region, and perhaps CXCR4 affinity, can account for replication and pathogenesis of the R3A env in the thymus
- The data are largely correlative and await mechanistic confirmation

REFERENCES

1. Meissner, E.G., K.M. Duus, F. Gao, X.F. Yu, and L. Su. 2004. Characterization of a thymus-tropic HIV-1 isolate from a rapid progressor: role of the envelope. *Virology* 328:74-88.

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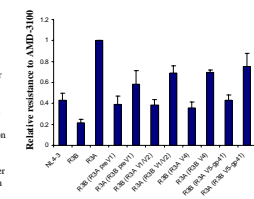


Figure 7: AMD-3100 resistance does not map to one particular region. NL4-luciferase pseudotyped with each envelope was used to infect Sup-T1 cells in the presence or absence of 200 nM AMD-3100. Resistance to AMD-3100 was set as 1 for R3A and relative resistance for each envelope was determined as follows: [1 - (Infection env X w/ amd-3100 w/ amd)/Infection R3A w/o amd-3100 w/ amd]. Shown are the combined results from five experiments.

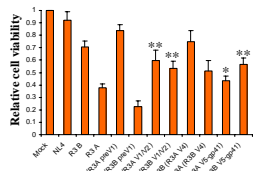


Figure 8: The V1/V2 and V5-gp41 regions contribute to cytopathicity in Sup-T1 cells. Sup-T1 cells were transfected with envelope expression vectors. Cytopathicity was quantitated by trypan blue exclusion. * = significantly different from the parental envelope. ** = significantly different from both R3A and R3B (p < 0.05 by the student's *t* test). Shown are the combined results of 3-6 independent experiments with normalization to mock transfected cells within each experiment.

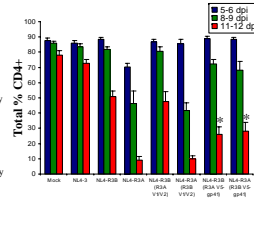


Figure 9: V5-gp41, but not V1/V2, is uniquely involved in R3A-mediated pathogenesis in the thymus model. NL4-3 recombinant viruses expressing the indicated envelope were used to infect human fetal-thymus organ culture fragments. Pathogenesis was assessed by depletion of total CD4+ thymocytes using flow cytometry. Shown are the combined results from five experiments. * = indicates significant differences in depletion relative to both NL4-R3B and NL4-R3A at day 11-12 (p < 0.05 by the student's *t* test). Replication correlated directly with pathogenesis (data not shown).