Department of Medicine, Division of Infectious Diseases

Type I Interferons Drive Sustained Dominance of CCR5-Tropic Variants During HIV Infection *In Vivo*

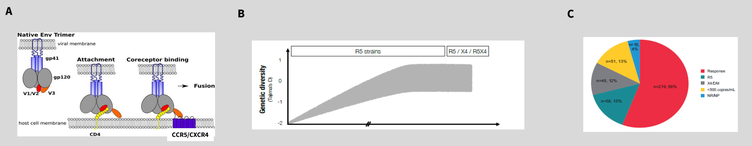
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Abstract

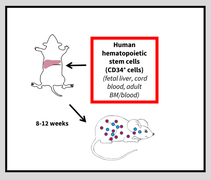
HIV invades host cells via a multistep process; the viral surface protein, gp120 engages with host CD4. This results in a conformation change that exposes the V3 loop of gp120. Many studies have shown that the charge of the V3 loop determines whether the virus will then subsequently bind CXCR4 or CCR5. The availability of CXCR4 in an individual is far greater than CCR5. CCR5 surface expression only occurs on activated CD4 T cells and not resting CD4 T cells. In contrast, all CD4 T cells express CXCR4. This might suggest that in individuals with HIV, CXCR4 tropic virus would be the predominant species. Surprisingly, in individuals with HIV the predominant circulating virus is CCR5 tropic virus. It is not until late stage disease when individuals develop significant immune compromise that CXCR4 tropic virus begins to emerge. Other work has shown that the tropism of the virus does not impact viral fitness as both grow equally well *in vitro*. This suggests that a component of the human immune system works to control CXCR4 tropic virus selectively. Our work is aimed at understanding what components of the immune system controls X4 tropic viral replication in patients. We hope to use this knowledge to design specific therapeutics to harness the immune system to control viral replication *in vivo*.

Viral Tropism Is Determined by the V3 loop of the Envelope protein



A) Gp120 binds to CD4 causing a conformational change which exposes the V3 loop of env, this V3 loop determines whether CCR5 or CXCR4 will be the coreceptor used. Only a few amino acids can change viral tropism from R5 to X4 and vice versa. (B) In a virus with an error prone RT, it is curious that X4 tropic virus is not observed until years into the clinical course. This suggests that X4 virus is actively restricted. (C) The best evidence of active restriction comes from looking at the clinical failures in patients in the MOTIVATE1/2 trials of Maraviroc, a CCR5 antagonist. Even when a selective advantage was given to emergence of X4 tropic virus, a majority of failures resulted from neo-binding to the Maraviroc bound CCR5 co-receptor

Humanized Mouse Model and Viral Constructs Used



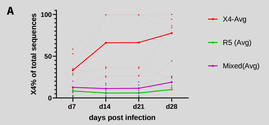


Using this powerful model we are able to probe the role innate immunity plays in restricting viral replication in the context of tropism.
In brief 1-3 day old pups are engrafted with human CD34+ cells purified from cord blood. Within 9 weeks we have reconstitution of a human immune system.

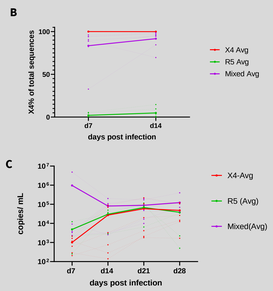
To probe the contribution of tropism, viral constructs using a consensus background sequences from the NL4-3 virus were used. An R5 virus using the envelope from BAL was used and further a construct with just the V3 loop from BAL was designed

The innate immune system appears to restrict X4 tropic virus

Transfusion

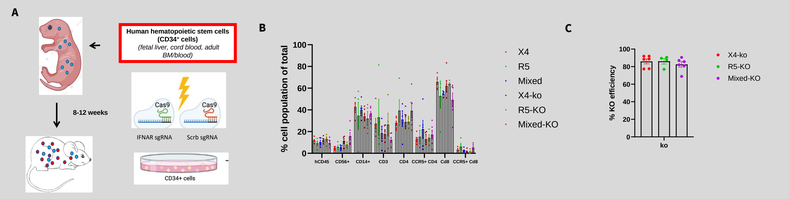


Engrafted

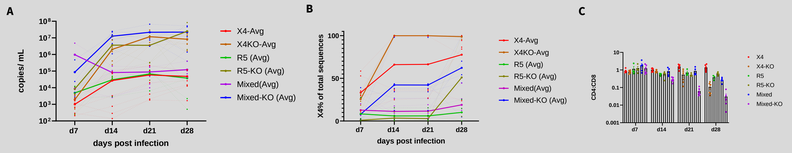


In (A) hu-mice that have not been engrafted with CD34+ hematopoietic stem cells are infused with primary CD4+ T cells purified from healthy donors. Mice are subsequently infected with viral constructs shown in prior panel containing either the X4 envelope, R5 envelope or an equal mixture of X4 and R5 envelope viruses. In this transfusion model X4 outcompetes R5 highlighting that X4 tropic virus is not less fit than R5 virus in the absence of any restriction factors. In (B) and (C) mice engrafted with human CD34+ cells are used. Here we can see that R5 virus outcompetes X4 virus in a competition experiment. Panel (B) highlights the X4 percentage of circulating variants using deep sequencing of circulating variants and panel (C) highlights the X4 viral load using qPCR for gag and deep sequencing.

Type I Interferons restrict X4 viral replication

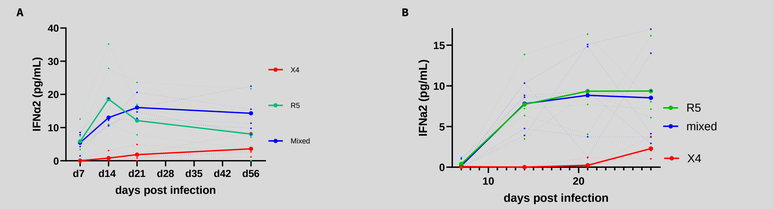


Interferon-α/β receptor knockout (*IFNAR*-KO) humanized mice were generated as follows. (A) Human CD34+ cells were transduced with Cas9 protein and guiding RNAs targeting the IFNAR coding gene or scrambled controls by electroporation before injection into 1-3 day old pups. Nine weeks post engraftment, these mice were assessed for appropriate engraftment. (B) Percentages of various cell subsets and level of activation of T cells by CCR5 is demonstrated. No differences were noted by 2-way ANOVA comparisons. (C) Knockout efficiency is assessed by sequencing the region the guide RNAs target. Editing efficiency at the target gene is routinely >80%.



To better understand tropism specific immune control of HIV, an interferon-α/β receptor knockout (*IFNAR*-KO) human immune system was reconstituted in mice. Briefly, human CD34+ cells were transduced with Cas9 protein and guiding RNAs targeting the IFNAR coding gene or scrambled controls by electroporation before injection into 1-3 day old pups. Nine weeks post engraftment, these mice were infected with X4- or R5-tropic HIV isolates.
(A) Shows X4 viral load calculated using gag qPCR from plasma and deep sequencing of the envelope to determine tropism. (B) highlights the percentage of circulating viral variants which are X4 tropic based on deep sequencing. Both (A) and (B) demonstrate that R5 tropic variants dominate in an intact human immune system. However if the role of type I IFN is removed, X4 tropic viruses dominate. (C) CD4 T cell decline is more pronounced in mice infected with X4 tropic virus. Notably we can see that in IFNAR KO mice infected with both X4 and R5 viruses where X4 virus is dominant (purple), CD4 T cell decline is more pronounced than in Scrb mice infected with both viruses (blue).

X4 virus infection results in limited detectable plasma type I IFN



Plasma collected over time after infection with NL4-3, VL4-3 BAL virus or an equal mixture of NL4-3 and NL4-3 BAL. In (A) each group has between 4-6 mice. In (B) each group has between 5-7 mice per group. Not shown, but no plasma IFNa2 is detectable in uninfected mice.

Conclusions

1.) In the absence of any other human immune cells, X4 tropic virus replicates in CD4+ T cells more efficiently than R5 tropic viruses

2.) As evidenced by looking at engrafted mice, with an intact innate immune system, X4 tropic virus replication Is restricted compared to R5 tropic virus highlighting a role of innate immunity in restricting X4 viral replication.

3.) Type I Interferons selectively restrict X4 tropic viruses more than R5 tropic viruses

4.) R5 tropic virus replication elicits higher plasma IFN in humanized mice than X4 tropic virus replication

Future Directions

1.) Does this pattern of X4 tropic Interferon restriction hold for other X4 and R5 tropic strains?

2.) Why does X4 tropic virus elicit a weaker type I IFN response than R5 tropic virus?

3.) Can understanding this selective restriction of X4 tropic viruses be used to also control R5 tropic virus replication?

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