HIGH REPLICATION FITNESS AND POTENT INNATE IMMUNE EVASION FUNCTION OF A HIV-1 VB6 STRAIN

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BACKGROUND:

What are the molecular determinants of HIV-1 VB virulence?

In 2022, an exceptionally virulent HIV-1 clade B variant (VB) was identified in the Netherlands [1]. Since then, additional cases were discovered in multiple countries. However, the mechanism of its increased pathogenicity is unknown.

Clinical characteristics of HIV-1 VB:

- 1.5% of ATHENA HIV patient cohort
- AIDS within 2-3 years of diagnosis
- 3.5- 5.5-fold higher VL
- 2-fold faster CD4+ cell depletion
- Sensitive to cART

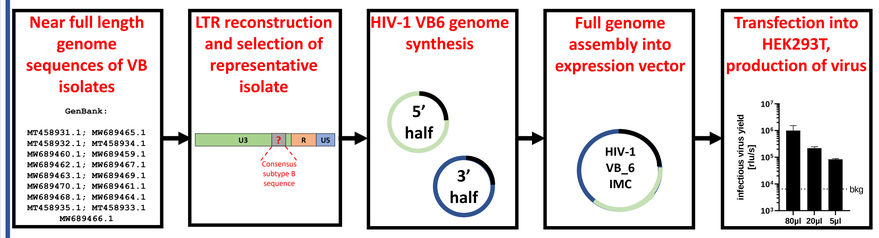
METHODS:

The sequence of the HIV-1 VB6 isolate (GenBank: MW689467) was used to synthesize a molecular clone. Following generation of virus, the ability to infect T cells, HLT and monocyte-derived macrophages was examined. Replication in PHA activated PBMCs +/- IFN were compared to other clade B strains. Resistance to IFI16 was tested by HEK293T co-transfection of the VB clone and infectivity reporter assay. Sensitivity to entry inhibitors, modulation of immune receptors and cytokine production were tested by flow cytometry.

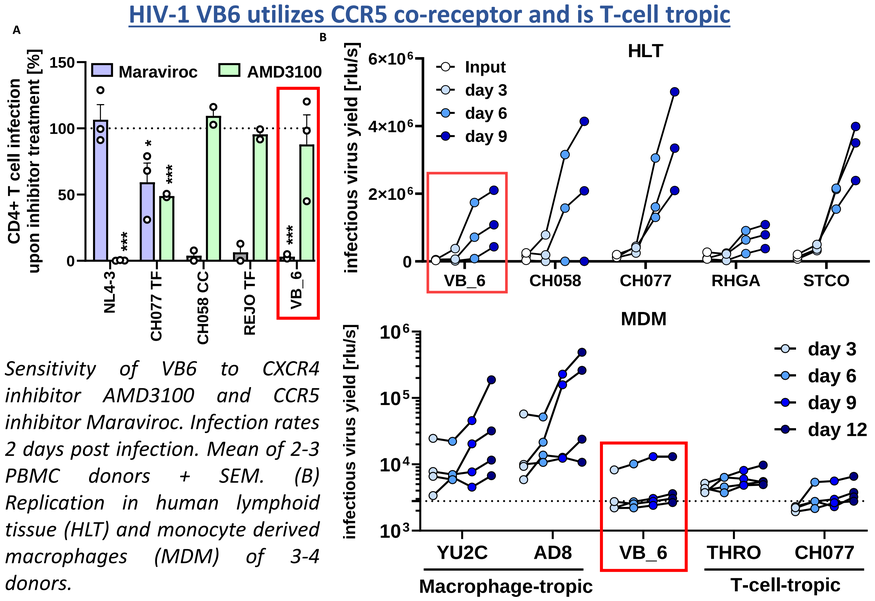
Hypervirulent VB6 HIV-1 strain shows enhanced replicative fitness and type I IFN resistance in activated primary T cells compared to other CCR5-tropic subtype B strains, potently downmodulates immune receptors, and efficiently evades tetherin and IFI16.

RESULTS:

Reconstruction and generation of hypervirulent HIV-1 molecular clone VB6

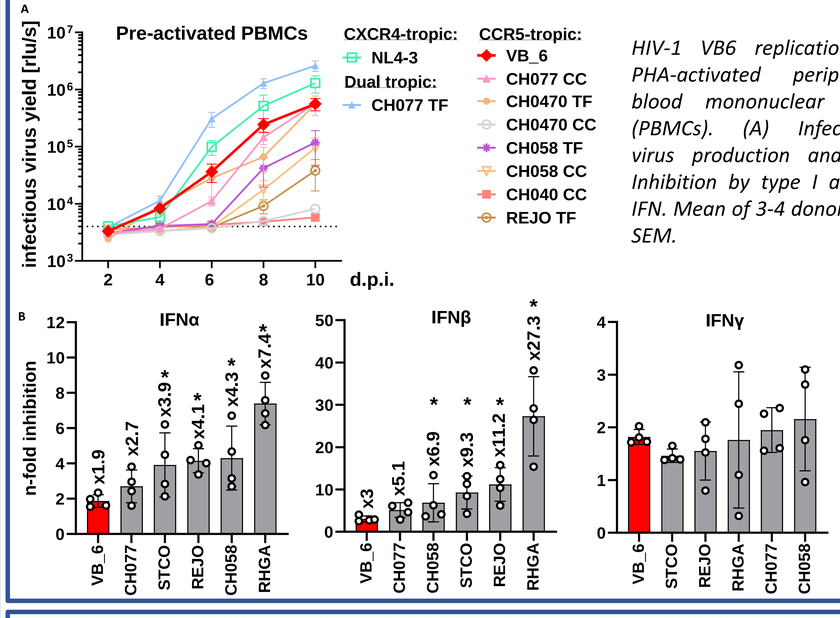


HIV-1 VB6 utilizes CCR5 co-receptor and is T-cell tropic



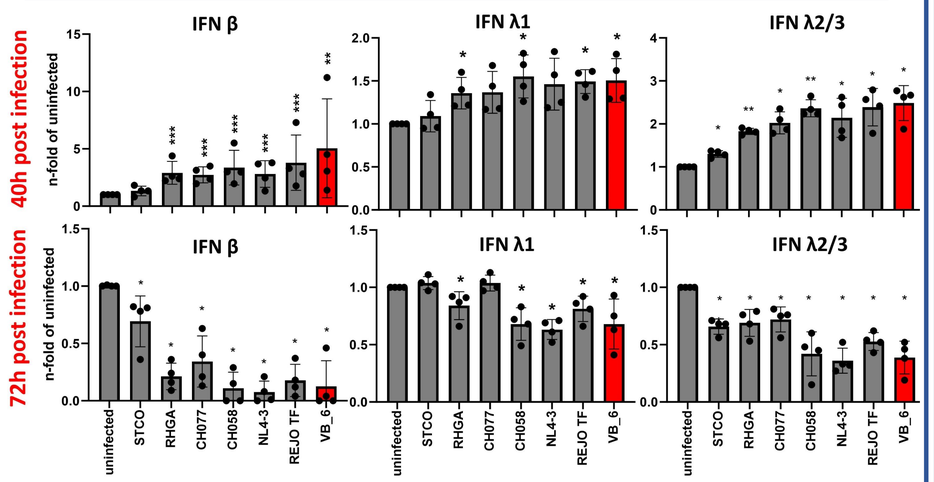
Sensitivity of VB6 to CXCR4 inhibitor AMD3100 and CCR5 inhibitor Maraviroc. Infection rates 2 days post infection. Mean of 2-3 PBMC donors + SEM. (B) Replication in human lymphoid tissue (HLT) and monocyte derived macrophages (MDM) of 3-4 donors.

VB6 shows high replication fitness and type I IFN resistance in activated PBMCs



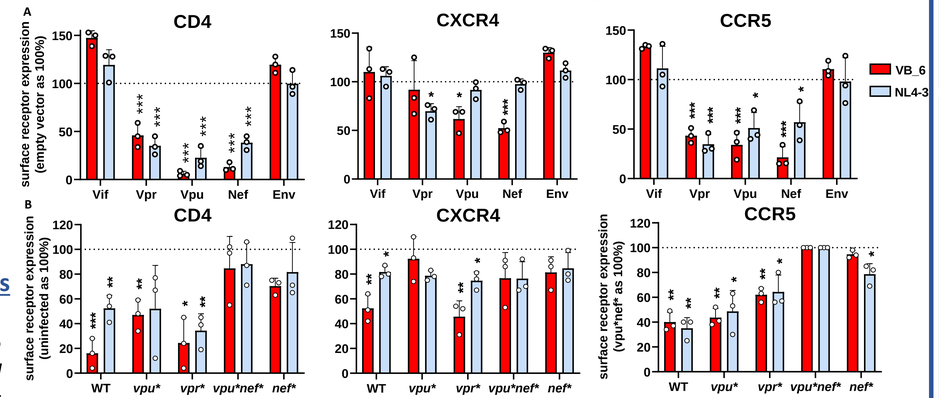
HIV-1 VB6 replication in PHA-activated peripheral blood mononuclear cells (PBMCs). (A) Infectious virus production and (B) Inhibition by type I and II IFN. Mean of 3-4 donors +/- SEM.

VB6 induces IFN response but effectively controls it at a late stage of infection



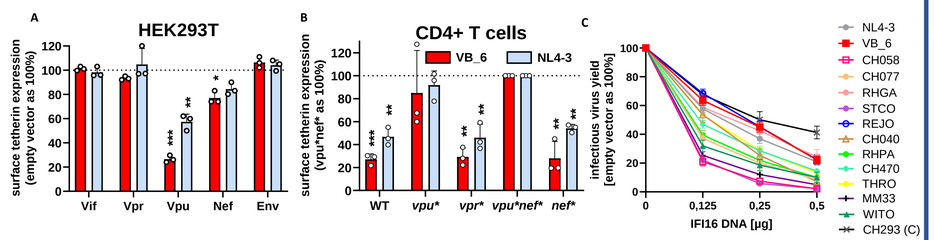
Production of IFN 40h or 72h after HIV-1 infection in primary human CD4+ T cells (4 donors +/- SEM) measured using Legendplex. Values were normalized to uninfected control for each donor.

HIV-1 VB6 accessory proteins efficiently downregulate viral entry receptors



Downmodulation of HIV-1 entry receptor CD4 and co-receptors CXCR4 and CCR5 by VB_6 accessory proteins. (A) Surface expression of indicated receptors measured by flow cytometry in HEK293T cells co-transfected with accessory proteins of NL4-3 or VB_6. (B) Receptor expression in primary T cells infected with accessory protein deficient virus. Mean of n=3 + SD.

HIV-1 VB6 downregulates tetherin and is relatively resistant to IFI16



Downmodulation of tetherin by VB6 accessory proteins (A) Surface tetherin downmodulation in transfected HEK293T cells and (B) infected CD4+ T cells 3 days post infection, measured by flow cytometry. (C) Sensitivity to human IFI16 overexpression in HEK293T cells. N=3-4 +/- SD.

CONCLUSIONS:

We generated an infectious molecular clone of HIV-1 (VB6) and found that it:

- Is CCR5-tropic and efficiently replicates in primary human T cells and lymphoid tissues but not in macrophages
- Has superior replicative fitness and IFN resistance in activated T cells compared to other primary CCR5-tropic clade B strains
- Induces IFN production but controls it at a late stage of infection
- Potently modulates entry receptors and tetherin
- Shows higher level of resistance to IFI16 than other subtype B strains

OPEN QUESTIONS:

- Are the determinants of VB_6 fitness shared by other VB isolates?
- Is VB lineage more resistant to other restriction factors?
- Is the VB variant more efficiently transmitted than other clade B strains?
- Is VB prevalence going to increase in the future?

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REFERENCES: [1] Wymant C. et al., A highly virulent variant of HIV-1 circulating in the Netherlands. Science. 2022 Feb 4;375 (6580):540-545.