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The Role of CCR5 in HIV-induced CD4 T cell Depletion

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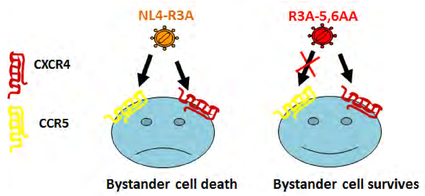
Abstract

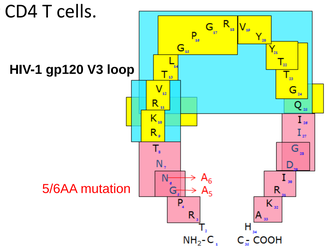
Bystander CD4 T cell depletion during HIV-1 infection plays an important role in AIDS disease progression, a process that is largely mediated by the HIV-1 Env protein. CCR5 is one of the two co-receptors required for HIV-1 binding and subsequent entry, but its involvement in Env-induced pathogenesis is poorly understood. In this report, we study the CD4 T cell pathogenesis caused by a dual-tropic HIV-1 virus R3A Env, focusing on the role of Env-CCR5 binding.

Using a cultured PBMC infection model or humanized mice in vivo, R3A can readily induce both infected CD4 T cell death and uninfected "bystander" CD4 T cell death. To study the involvement of Env-CCR5 binding, we utilized an Env-mutant of R3A, termed R3A-5/6AA, which has lost the potential for CCR5 binding.

We found loss of CCR5-binding by the mutant R3A-5/6AA resulted in reduced pathogenesis of bystander CD4 T cells. Importantly, R3A-5/6AA can replicate to the same level as wild type R3A by using CXCR4-binding for virus entry. Accordingly, treatment of CCR5 antagonist TAK-779 inhibited bystander CD4 T cell death in R3A infection without affecting viral replication. In addition, stimulation of CCR5 using MIP1-β during R3A-5/6AA infection increased caspase-3 activation in bystander CD4 T cells. We have further confirmed our finding in vivo using a humanized mice model, and we observed bystander CD4 T cell pathogenesis in the spleen and bone marrow is dependent on CCR5 usage by the HIV-1 Env.

We provide the first evidence in physiologically relevant in vivo models that shows CCR5 binding by HIV-1 Env plays an important role in Env-induced depletion of bystander CD4 T cells.





Methods

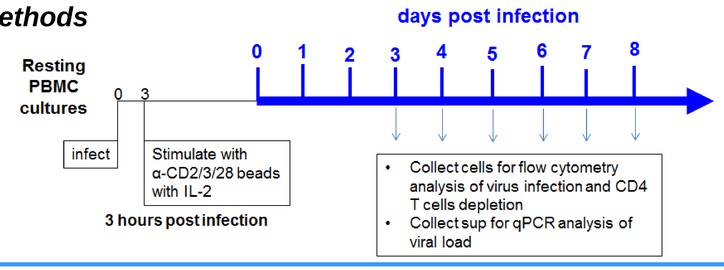
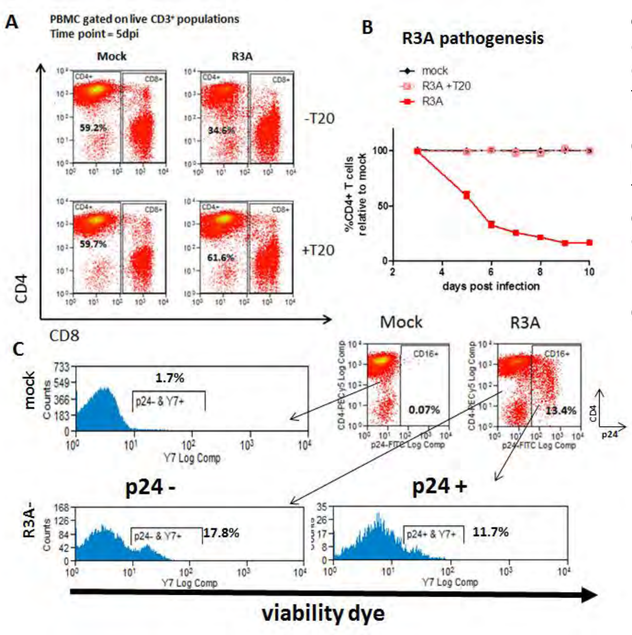


Fig 1. An in vitro primary cell culture system to study HIV-1 bystander pathogenesis



A). FACS analysis of R3A virus pathogenesis in CD4 T cells. %CD4 and %CD8 gated on CD3+ T cells are shown at 6dpi, with and without T20 fusion inhibitor.

B). Kinetics of CD4 T cells depletion by R3A from 3-10 dpi. Data presented as both %CD4 T cells in CD3+ population

C). Analysis of bystander p24- CD4 T cell death and direct p24+ CD4 T cell death in R3A infection at 6dpi. PBMCs were gated on CD3+ CD8- populations

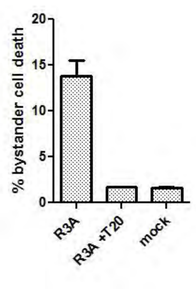
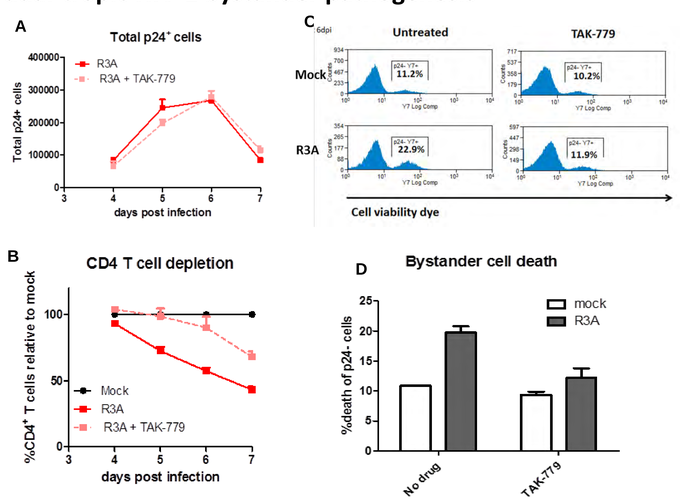


Fig 2. CCR5 antagonist TAK-779 protects CD4 T cells from dual-tropic HIV-1 bystander pathogenesis

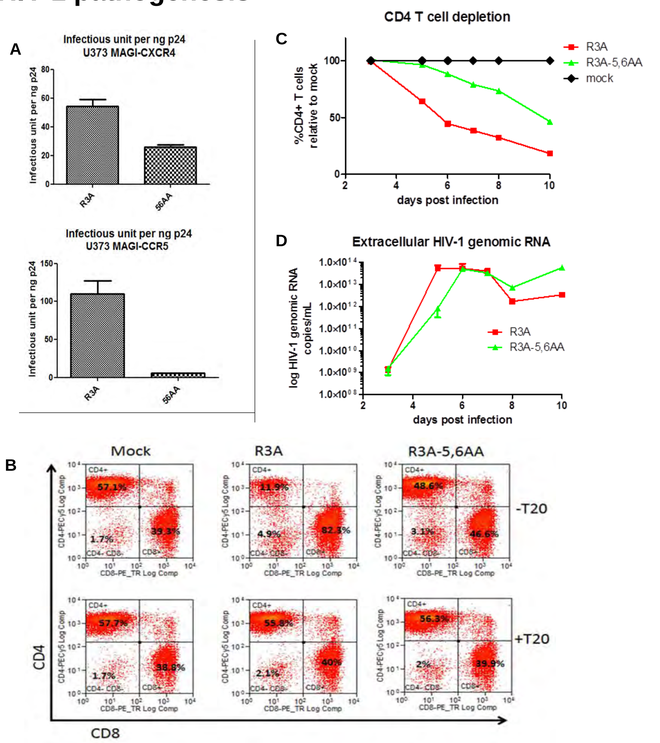


A). R3A replication in the presence or absence of TAK-779, presented as total p24+ cells over time

B). CD4+ T cell pathogenesis of R3A-WT in the presence or absence of TAK-779. CD4 T cells % are displayed relative to mock infected samples

C). Bystander pathogenesis analysis for R3A infection over time in the presence or absence of TAK-779

Fig 3. Ablation of CCR5 usage reduces dual-tropic HIV-1 pathogenesis

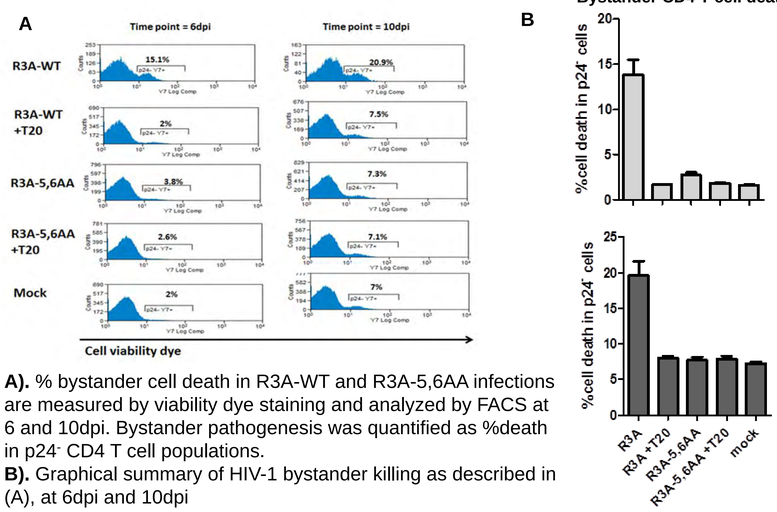


A). Infectious units per ng p24 of R3A-WT and R3A-5/6AA virus measured in CXCR4-expressing or CCR5-expressing U373-MAGI cells.

B & C). CD4 T cell pathogenesis in R3A-WT and R3A-5,6AA infected samples, analyzed as %CD4+ cells in CD3+ T cells and as total CD4+ T cells number over time

D). Kinetics of R3A-WT and R3A-5,6AA virus replication was analyzed by measuring extracellular viral genomic RNA over time

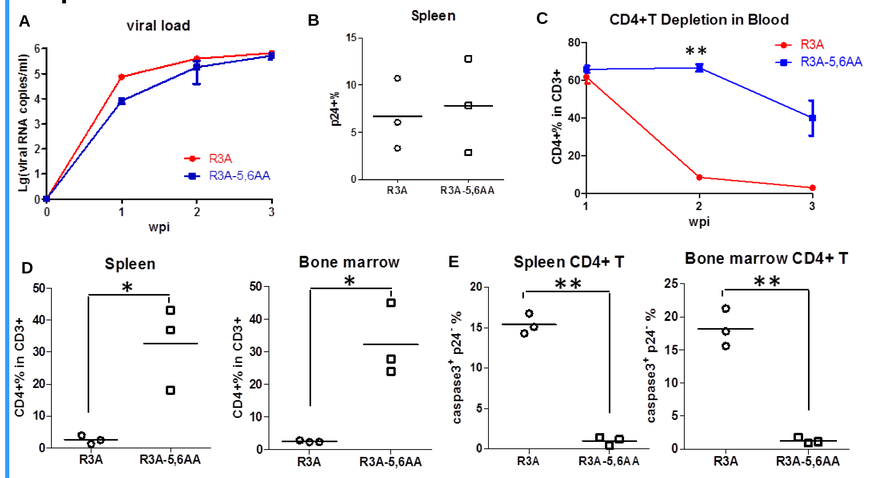
Fig 4. Dual-tropic HIV-1 requires CCR5 usage to induce bystander cell death



A). % bystander cell death in R3A-WT and R3A-5,6AA infections are measured by viability dye staining and analyzed by FACS at 6 and 10dpi. Bystander pathogenesis was quantified as %death in p24- CD4 T cell populations.

B). Graphical summary of HIV-1 bystander killing as described in (A), at 6dpi and 10dpi

Fig 5. Ablation of CCR5 usage reduced pathogenesis of dual tropic HIV-1 in a humanized mouse model



A). qPCR measurement of HIV-1 genomic RNA in the blood of humanized mice infected with R3A-WT or R3A-5,6AA

B). Percent infected CD4+ T cells in the spleen of mice infected by R3A-WT and R3A-5,6AA at 3 weeks post infection

C & D). Percent CD4 T cells of mice infected by R3A-WT or R3A-5,6AA analyzed in the blood (C), Spleen and Bone Marrow.

E). R3A vs R3A-5,6AA bystander apoptosis of CD4 T cells analyzed by Caspase 3 staining in p24- population

Summary

- ❖ Dual-tropic HIV-1 induces uninfected bystander CD4+ T cell death
- ❖ Ablation of CCR5 usage delays HIV replication, but reach to similar viremia
- ❖ Bystander pathogenesis requires viral *Env* usage/binding of CCR5 co-receptor

Take home message

- ❖ CCR5 plays a critical role in HIV-1 induced bystander killing

Acknowledgement

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