Phagocytic uptake of HIV Infected CD4+ T Cells Enhances Macrophage Infection

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Background

Macrophages in HIV

- **Macrophages** remove apoptotic cells and pathogens by **phagocytosis**
- Together with **CD4+ T cells**, macrophages are the main cellular targets of HIV, and are central to pathogenesis
- HIV-1 infected macrophages survive longer than uninfected macrophages [1], and are an important reservoir of HIV-1 infection during antiretroviral therapy [2]
- Understanding macrophage infection is therefore highly relevant to HIV-1 eradication strategies [2]

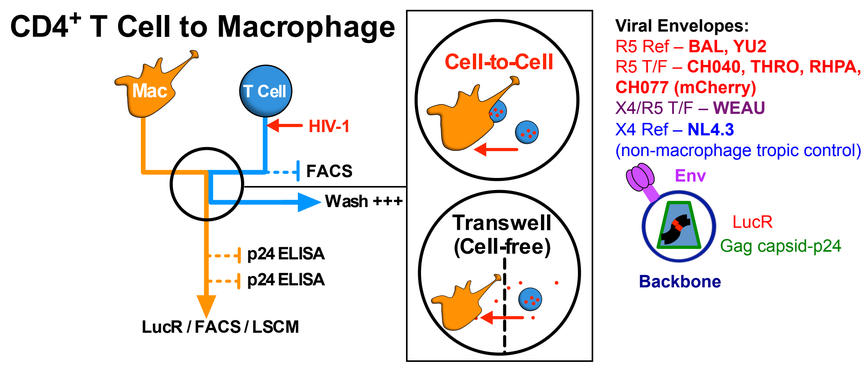
Importance of transmission

- HIV-1 transmission worldwide mainly occurs by the mucosal route, and 80% of infections are due to transmission of a single viral sequence, the **transmitted/ founder virus - T/F virus** [3]
- Therefore a critical **window of opportunity** exists for prophylactic vaccines [4]
- Cell-free T/F viruses efficiently infect CD4+ T cells, but not macrophages [3]
- However HIV-1 infected macrophages are observed *in vivo* during early stages of infection [5]

How are macrophages infected by T/F viruses?

Results

Human Primary Cell Model of HIV Spread



- **Cells**: Enriched autologous monocyte-derived macrophages and HIV-1 infected CD4+ T Cells
- **Viruses**: Chimeric Env_isogenic backbone_Luciferase reporter viruses
- **Co-culture**: 6h +/- virus permeable transwell membrane (cell-free infection)
- **Macrophage infection readouts**: FACS, Gag ELISA, microscopy, luciferase

Figure 1 Macrophages Engulf HIV-1 Infected Apoptotic CD4+ T Cells

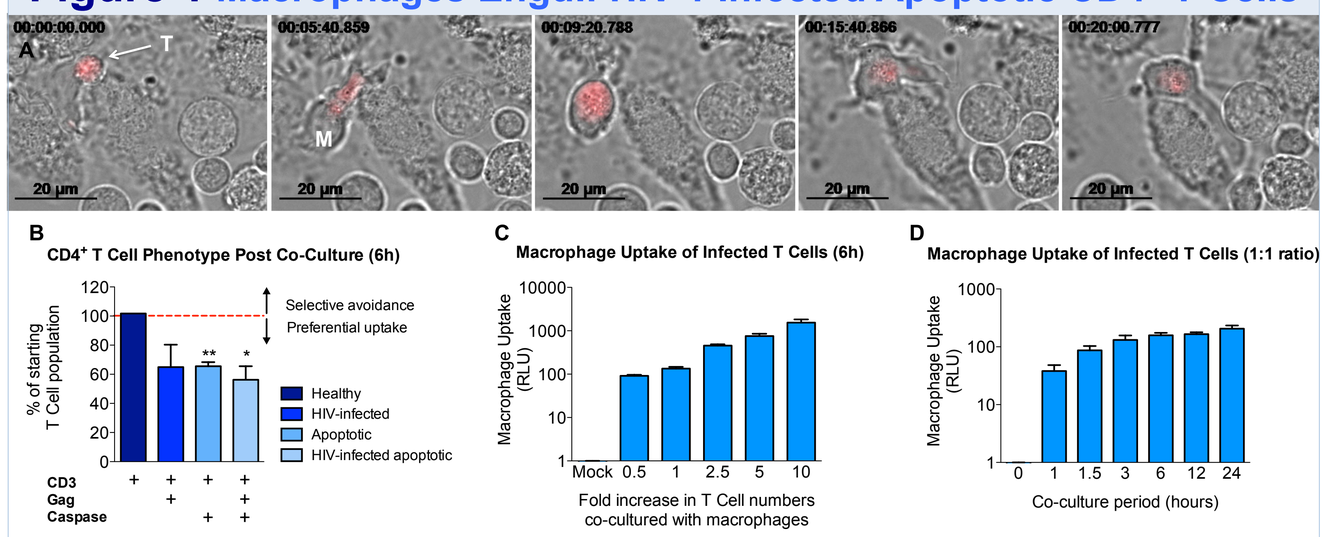


Figure 1. A, time-lapse live cell microscopy of uninfected macrophage (**M**) engulfing mCherry T/F virus infected CD4+ T cell (**T**). **B**, depletion of HIV+ apoptotic cells after 6h co-culture suggests preferential uptake. **C**, HIV+ T cell uptake by macrophages proportional to input T cell numbers. **D**, time-dependent increase in uptake. Mean ± s.e.m. of independent experiments in n=2 donors. *P<0.05, **P<0.01 ANOVA with Bonferonni post-test.

Figure 2 Uptake Results in Efficient Macrophage Infection

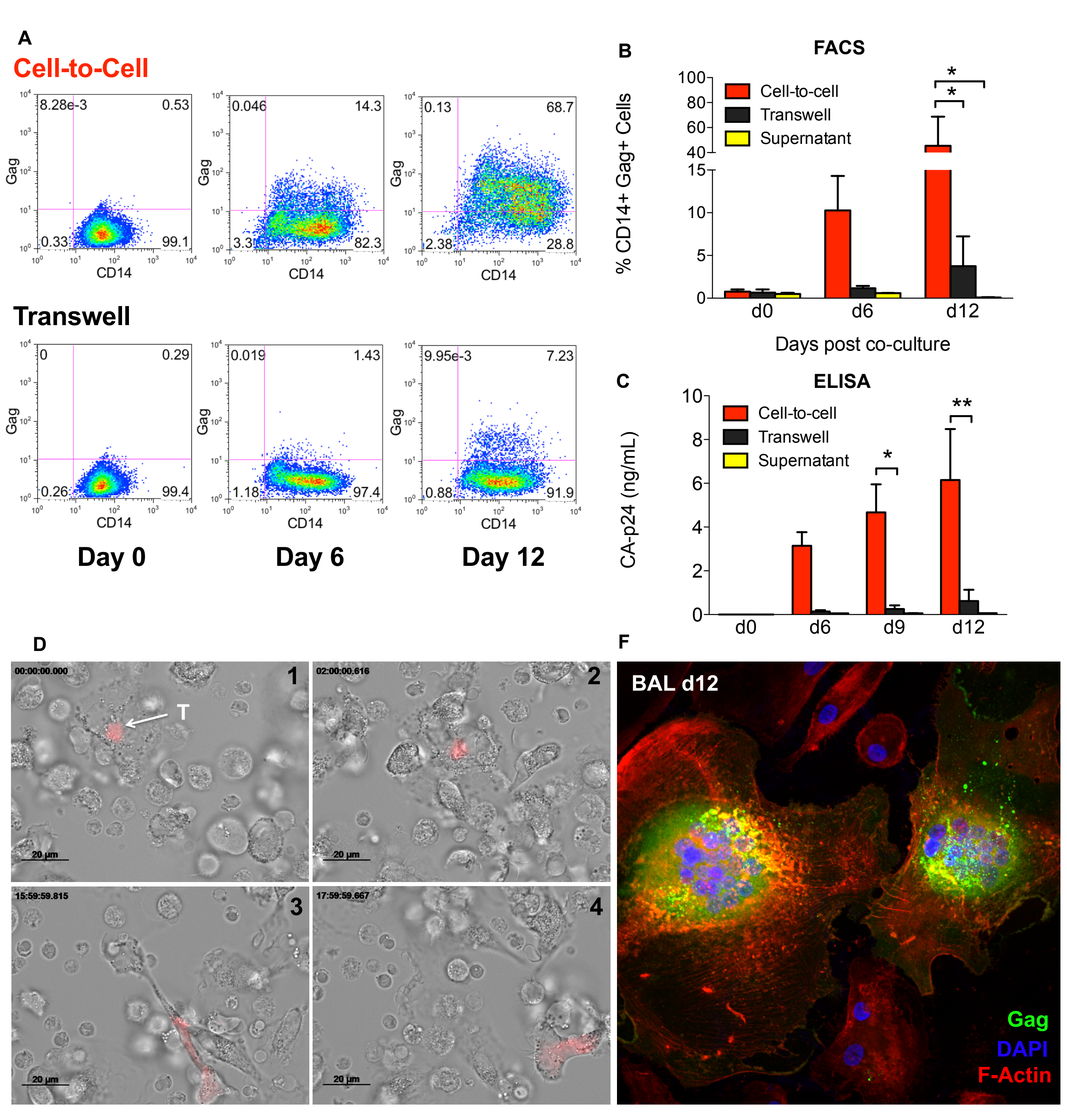


Figure 2 Macrophages co-cultured with HIV_{BAL} infected CD4+ T cells for 6h in the presence or absence of a 3.0um transwell membrane (TW) to prevent cell-contact but allow virus diffusion. Macrophages were washed extensively and cultured alone for days indicated. **A**, representative FACS plots. **B and C**, mean ± s.e.m. of independent experiments (FACS and ELISA) in n=2 donors **D**, mCherry T/F infected T cell (T) phagocytosed by a macrophage which subsequently develops diffuse cytoplasmic mCherry signal suggesting infection over 18h. **E**, LSCM image of d12 HIV_{BAL} infected macrophages following cell-to-cell spread from T cells (x40 oil immersion). *P<0.05, **P<0.01 ANOVA with Bonferonni post-test.

Figure 3 Cell-to-cell Spread Overcomes Restricted Macrophage Tropism of Transmitted/Founder Virus Envelopes

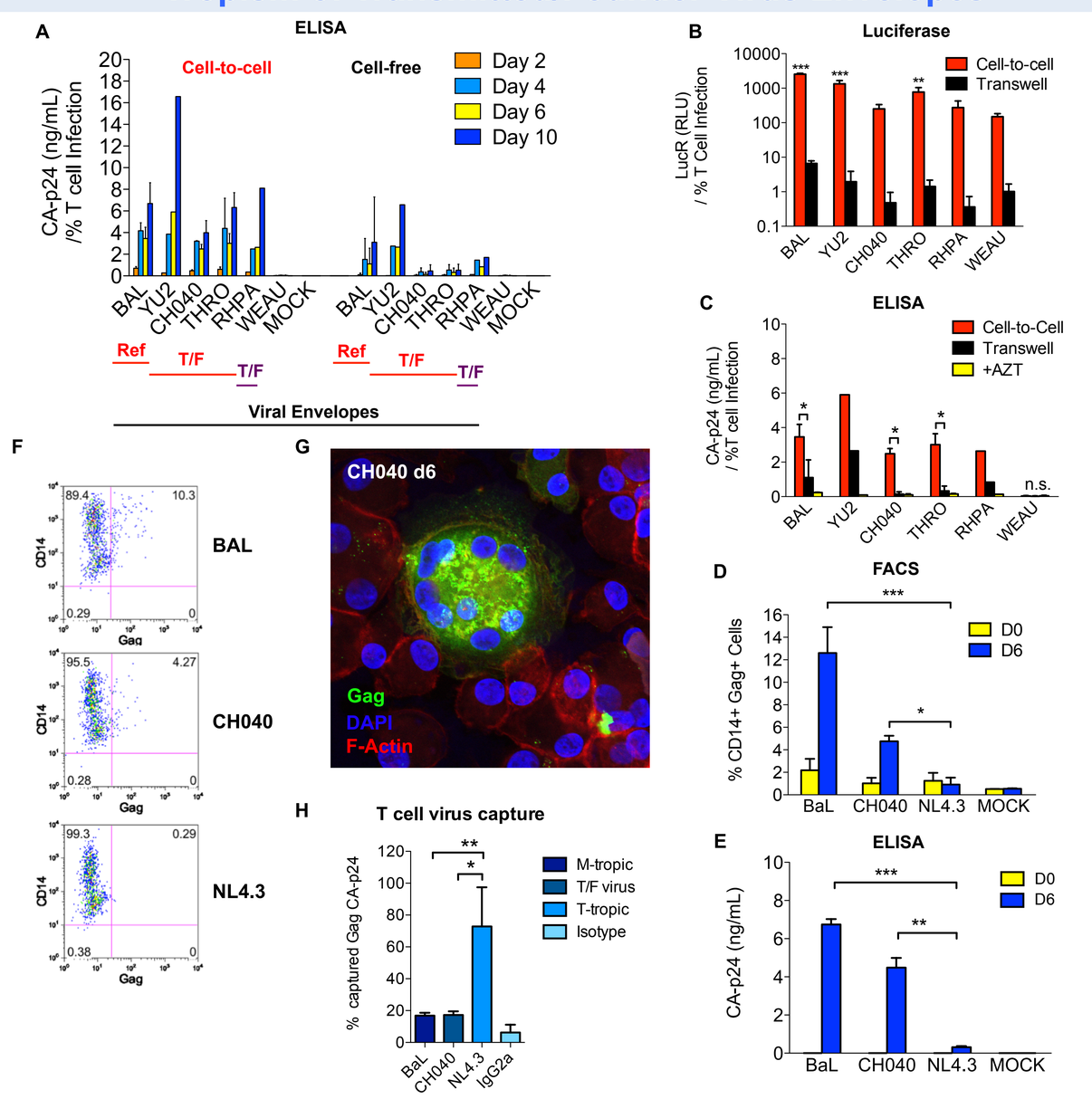


Figure 3. A, ELISA time-course of infection in macrophages by cell-to-cell spread versus cell-free spread through a transwell membrane. **B**, Enhanced macrophage infection efficiency of T/F Envelopes by cell-to-cell spread assayed with luciferase (**C**) and ELISA (**D**) at d6. Mean ± SD of independent experiments in n=2 donors (n=1 for YU2/RHPA). Infection is not due to ongoing T cell replication since replication of the non-macrophage tropic reference Env NL4.3 is significantly impaired, and direct evidence of macrophage infection is demonstrated (**D-G**) using several techniques, and a T cell virion capture assay does not detect virus of T cell origin (**H**). *P<0.05, **P<0.01, ***P<0.001 ANOVA with Bonferonni post-test.

Conclusions

- Our observations of macrophage infection by phagocytic uptake of HIV-1 infected apoptotic T cells represent the first evidence of a 'Trojan Horse' model of viral infection
- Cell-to-cell transfer of T/F virus Envelopes from infected CD4+ T cells appears to overcome the restricted macrophage tropism phenotype
- This mode of macrophage infection may be highly relevant to the establishment of the macrophage reservoir during acute HIV-1 infection
- Further work is required to identify signaling events leading to recognition and the downstream pathways leading to phagolysosome escape.

Future Priorities

1. Expand the panel of Envs and test full-length T/F infectious molecular clones for macrophage infection capacity
2. Address the implications for immunoprophylaxis of this route of spread, particularly with reference to recently identified and highly promising broadly-neutralizing antibodies
3. Assess this mechanism in primary mucosal macrophages

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