M48U1 CD4 mimetic has a memory effect on cell-associated HIV-1 by severely reducing virion infectivity through gp120 shedding

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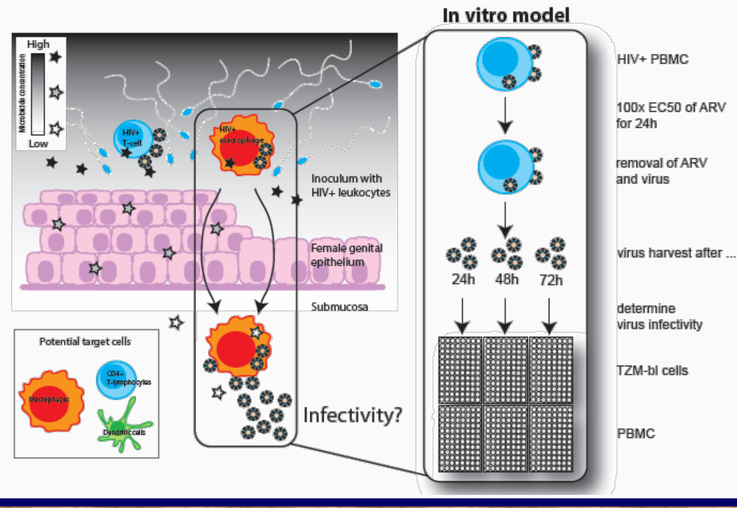
BACKGROUND

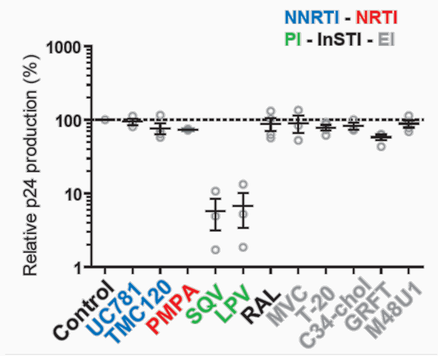
Although vaginal microbicides should inactivate both cell-free and cell-associated virus (CAV) in semen of HIV-positive men, several studies have shown that **infected seminal leucocytes are able to cross intact vaginal epithelia to reach the submucosa and even the draining lymph nodes**. These 'Trojan Horse' leucocytes may establish infection by escaping high microbicide concentrations in the vaginal lumen. However, **it is not known whether the initial vaginal drug exposure exerts a sustained inhibitory or 'memory' effect on these cells by attenuating virion production or infectivity**, even after their migration to deeper tissues.

METHODS

Here we investigated the potential memory effect of several antiretrovirals (ARVs) which are currently under development as candidate microbicides. The Trojan Horse transmission concept was modeled *in vitro* using HIV BaL-infected PBMCs which were treated for 24h with ARVs from different classes at 100x their EC50 concentrations. Subsequently, culture supernatant was harvested to determine virus production using a Gag p24 ELISA. To mimic escape from vaginal drug pressure, the infected cells were then incubated in ARV-free medium for three consecutive periods of 24h. To measure infectivity of newly produced virions, supernatant was harvested after each period and titrated in TZM-bl cells and PBMCs using equal amounts of p24. Among the tested compounds is the **CD4-binding site inhibitor M48U1 (3kDa) which inhibits the gp120-CD4 interaction in the nanomolar range** by specifically targeting the highly conserved and vulnerable Phe43-cavity in the HIV envelope.

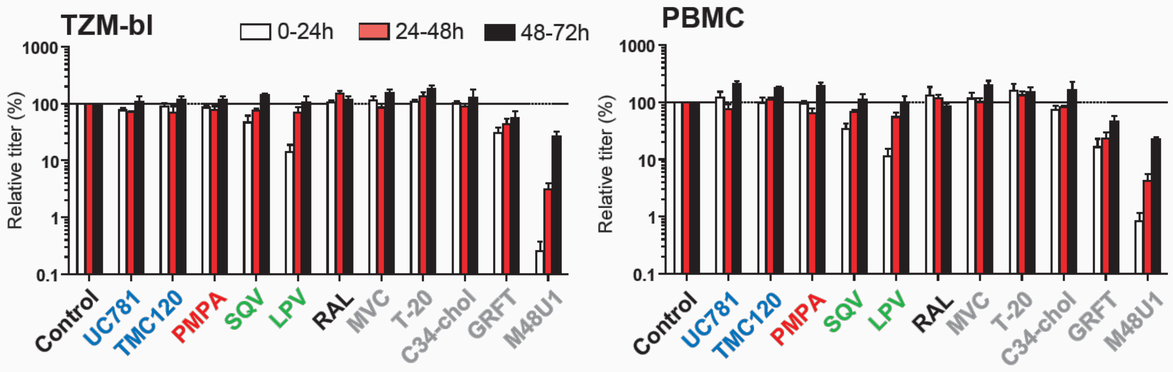


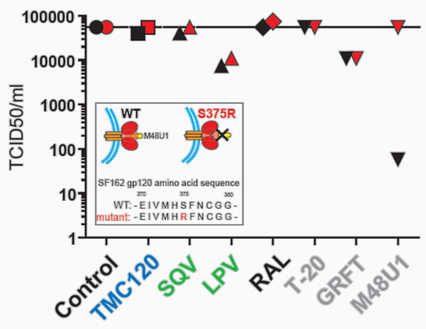
RESULTS



Pretreatment with most ARVs did not inhibit virus production by infected cells as compared to the untreated control cultures. However, as expected, in the supernatant of cultures treated with the protease inhibitors (PIs) lopinavir (LPV) and saquinavir (SQV), a significantly lower (i.e., 0.5-1 log) Gag p24 concentration was observed.

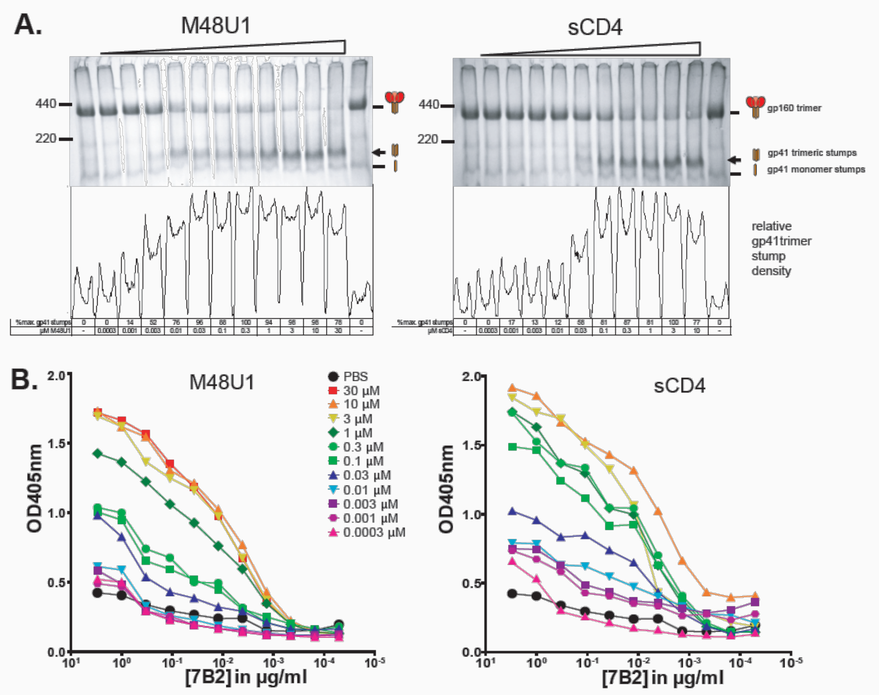
Infected cells exposed to the CD4 mimetic M48U1 produced largely defective viral particles during the first 24 hours after treatment. Virus infectivity was gradually, but never fully restored at later time points. Although modest in comparison to the infectivity reduction caused by M48U1, we also observed a decline in infectivity of virus budding from cells that were pre-treated with the PIs LPV and SQV or the gp120-glycan binder griffithsin (GRFT).



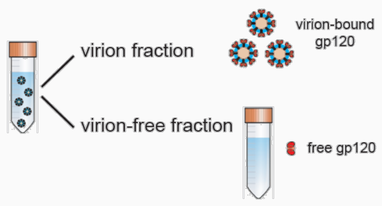


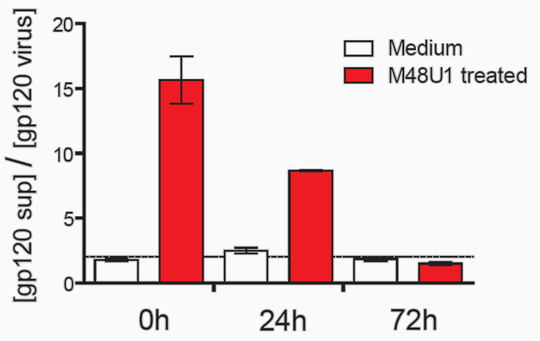
The memory effect of M48U1 depends on direct interaction between M48U1 and gp120 as in contrast to the low infectious titer (<1%) of wild type virus after M48U1 treatment, a normal titer (100%) was found for M48U1 resistant virus (S375R mutant).

M48U1 induces gp120 shedding as confirmed by two different approaches in comparison to the reference compound sCD4. First, using BN-PAGE, we saw an increase in gp41 stumps together with a decrease in native Env trimer following M48U1 treatment of virus-like particles (VLPs) (A). Second, in an ELISA detecting the cryptic 7B2 epitope on gp41 stumps of VLPs, M48U1 caused an increase in 7B2 binding titers (B).



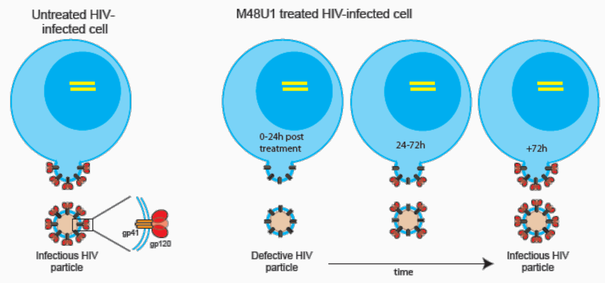
Memory effect of M48U1 is linked to gp120 shedding as significantly larger amounts of gp120 were detected by ELISA in virus-depleted supernatant of M48U1-treated cultures. Moreover, coincident with the time-dependent recovery of virus infectivity, the ratio of virion-free and virion-bound gp120 gradually decreased to reach equilibrium levels 72h post treatment.





CONCLUSIONS

Our observations strongly suggest that **M48U1 induces gp120 shedding at the cell membrane of infected PBMCs, resulting in defective nascent virions after drug removal.** The continuous recruitment of new freshly synthesized envelope proteins from the endoplasmic reticulum to the cell membrane can explain why infectivity is restored over-time. From a microbicide perspective, **entry inhibitors with a memory effect on CAV are desirable as they might provide a window of opportunity to eliminate Trojan Horse leucocytes before they establish local infection.** Therefore, together with its physico-chemical characteristics such as its small size, stable conformation in denaturing conditions and relative resistance towards proteases, **M48U1 has an extremely favorable profile as potential microbicide**.



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