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Capsid plays a distinct role in HIV infection of TCR or TLR2-activated CD4+ T cells.

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Background

HIV-1 capsid (CA) proteins play a crucial role in multiple stages of HIV infection including nuclear import. However, the function of the CA is not fully understood in primary resting CD4+ T cells. We have previously shown that TLR2 activation enhances HIV infection by promoting nuclear import in resting CD4+ T cells. In this study, we examined the role of the HIV CA protein and their analogs in TLR2-mediated enhancement of HIV infection in resting CD4+ T cells.

Methods

VSV-G pseudotyped luciferase or GFP reporter virus with wild-type (WT) CA or CA mutants were prepared by transfecting HEK293T cells. PBMCs were isolated from buffy coat of healthy donors by Histopaque gradient centrifugation. Primary resting CD4+ T cells were purified by negative selection from PBMCs, and exposed to pseudotyped virus for 2 h. Cells were stimulated with TLR2 ligand (Pam_3CSK_4) or immobilized anti-CD3 Ab in the presence of IL2. HIV infection was determined by measuring luciferase activity 3 days after infection. Cell pellets were collected 12 and 48 h after infection. Total DNA was isolated and analyzed for HIV late reverse-transcribed (RT) and 2LTR products by quantitative real-time PCR.

Results

E45A, E71A, and ΔcPPT mutants were infectious in TLR2- and TCR-activated CD4+ T cells. Conversely, P38A, K70A, and L136D mutants were not infectious in TLR2- or TCR-activated CD4+ T cells. T54A/N57A, Q63A/Q67A, E128A/R132A, R143A, and Q219A mutants were infectious in CD4+ T cells in response to TCR activation, but not TLR2. The levels of late RT products were reduced in TLR2-activated CD4+ T cells infected by N74D mutant, but not other mutants. The levels of 2LTR circles, markers for HIV nuclear import, were also reduced in N74D mutant-infected cells. 2LTR circles were diminished in TLR2-activated CD4+ T cells with infection by P38A,T54A/N57A, Q63A/Q67A, K70A, E128A/R132A, L136D, and R143A mutants, suggesting a crucial role for these residues in nuclear import. Infection by Q219A led to greater levels of 2LTR circles compared to WT in TLR2-activated cells, suggesting that Q219 may be important for viral integration.

Conclusions

Mutations in the CA have differential impacts on HIV infection of TLR2- and TCR-activated CD4+ T cells. CA mutations altered 2LTR production suggesting its role in HIV nuclear import in primary CD4+ T cells in response to TLR2 activation. Our study provides an insight for developing antiretroviral agents targeting HIV CA proteins.

Acknowledgement

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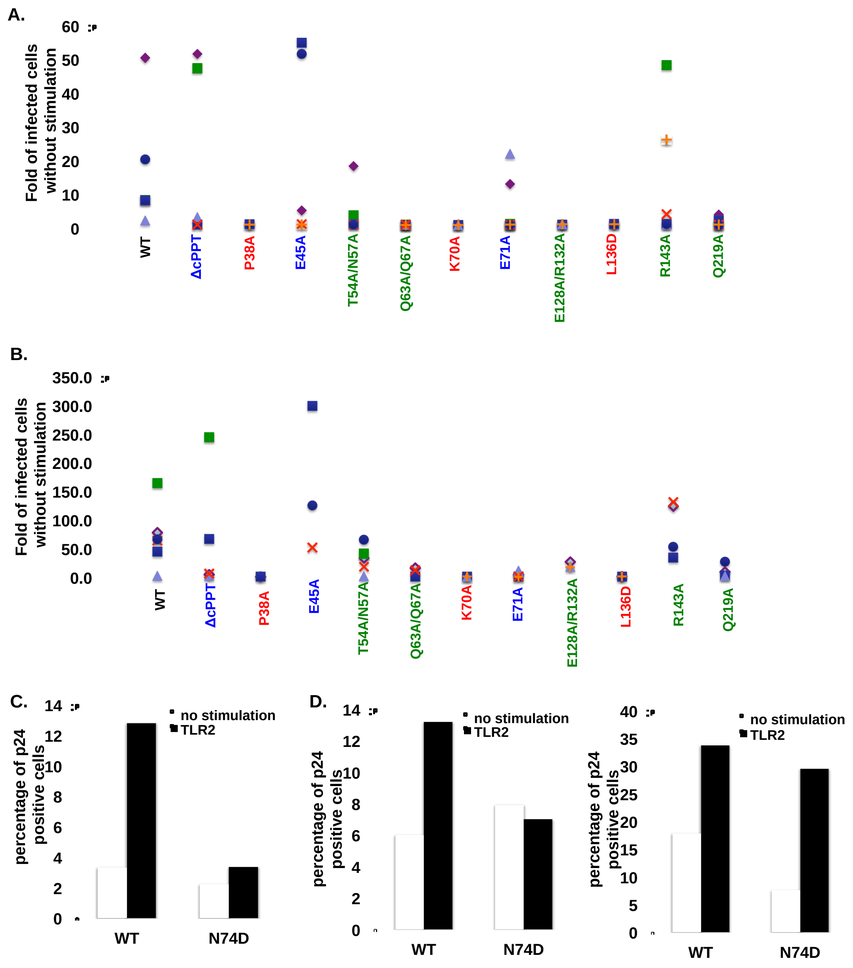


Fig. 1. HIV infectivity of CA mutants in TLR2- or TCR-activated CD4+ T cells. Primary CD4+ T cells were exposed to HIV-1 WT and CA mutants expressing luciferase or GFP (N74D) reporter gene for 2h. Infected cells were then stimulated with TLR2 ligand, Pam_3CSK_4 at 5 μg/mL (panel **A**), or immobilized anti-CD3 Ab (panel **B**) in the presence of IL-2. HIV infection (relative light units, RLUs) was determined by measuring luciferase activity at day 3 after infection. The degree of HIV infection of each mutant in stimulated cells was compared to that in the non-stimulated control. Data from various donors are shown. (**C and D**) HIV infection by WT and N74D mutant was determined at day 5 after infection by intracellular HIV-1 p24 staining using FACs analysis. Results from two different donors are shown. Panel D represents infection with (right panel) or without spinoculation (left panel). CD4+ T cells were spinoculated with WT and N74D for 2h at 1000 xg, then stimulated with TLR2 ligand, Pam_3CSK_4 at 5 μg/mL, in the presence of IL-2. Results showed that there was donor variability in the degree of HIV infection when the mutation was not lethal. E45A, E71A, and ΔcPPT mutants were infectious in TLR2- and TCR-activated CD4+ T cells (in blue). T54A/N57A, Q63A/Q67A, E128A/R132A, R143A, or Q219A mutant were infectious in CD4+ T cells in response to TCR activation, but not TLR2. These mutants were grouped as “differential mutants” (in green). P38A, K70A, or L136D mutants were not infectious in TCR- or TLR2-activated CD4+ T cells. These mutants were grouped as “lethal mutants” (in red).

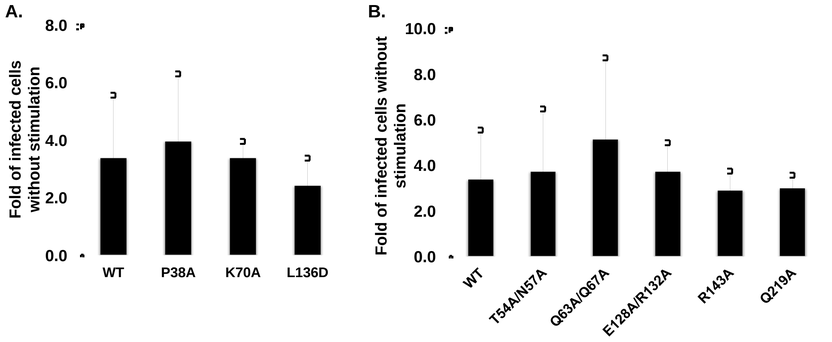


Fig. 2. The decrease in HIV infection by CA mutants is not due to the block of the synthesis of late RT products in TLR2-activated CD4+ T cells. Primary CD4+ T cells were exposed to WT or CA mutants for 2h, and then stimulated with TLR2 ligand (Pam_3CSK_4, 5 μg/mL) in the presence of IL-2. Total DNA was prepared from HIV-infected CD4+ T cells 12h after infection for the analysis of late RT products. The levels of HIV RT products were determined by quantitative PCR analysis. Late RT products of lethal (panel **A**) and differential mutants (panel **B**) were induced in response to TLR2. Data represents the fold of late RT products produced in stimulated (Pam_3CSK_4) cells compared to that in non-stimulated cells (control). The results are presented as mean±SD of three independent experiments.

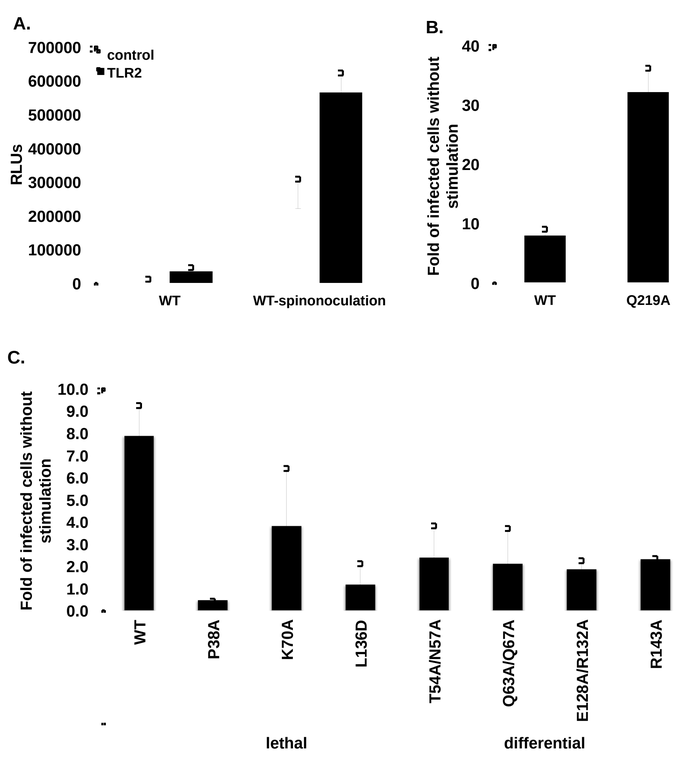


Fig. 3. CA mutants have reduced levels of 2LTR circles in TLR2-activated primary CD4+ T cells, suggesting its role in HIV nuclear import. Primary CD4+ T cells were spinoculated with WT and CA mutants for 2h at 1000 xg, then stimulated with TLR2 ligand, Pam_3CSK_4 at 5 μg/mL, in the presence of IL-2. Total DNA was prepared from HIV-infected CD4+ T cells at 48h post-infection, and the levels of 2LTR circles were determined by quantitative PCR analysis. **(A)** Infectivity of WT HIV-1 with and without spinoculation. **(B)** Infection by Q219A led to greater levels of 2LTR circles compared to WT in TLR2-activated cells. **(C)** The levels of 2LTR circles were diminished in TLR2-activated CD4+ T cells with infection by P38A,T54A/N57A, Q63A/Q67A, K70A, E128A/R132A, L136D, or R143A mutant, suggesting a crucial role for these residues in nuclear import. 2LTR data represents the fold of viral products produced in stimulated (Pam_3CSK_4) cells compared to that in non-stimulated cells (control). Data is presented as mean±SD of 2 or more independent experiments.

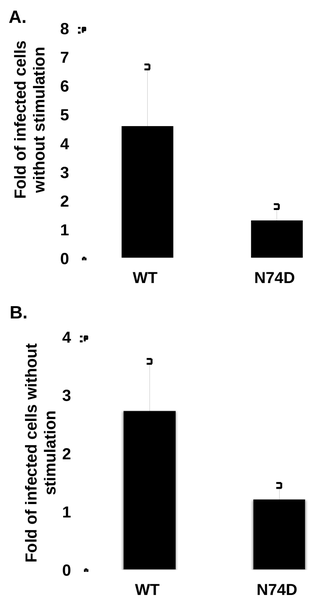


Fig. 4. N74D mutant reduced late reverse transcription and nuclear import of HIV in TLR2-activated CD4+ T cells. Primary CD4+ T cells were exposed to WT-GFP and N74D-GFP mutant, then stimulated with TLR2 ligand (Pam_3CSK_4, 5 μg/mL) in the presence of IL-2. **(A)** Late RT products were reduced in TLR2-activated CD4+ T cells infected by N74D mutant. **(B)** The levels of 2LTR circles, markers for HIV nuclear import, were also reduced in N74D mutant-infected cells. Late RT and 2LTR data represents the fold of viral products produced in stimulated (Pam_3CSK_4) cells compared to that in non-stimulated cells (control). Data is presented as average fold of control in 2 or more independent experiments.