17β-estradiol Protects Primary Macrophages Against HIV Through Induction of IFNα and APOBEC3A

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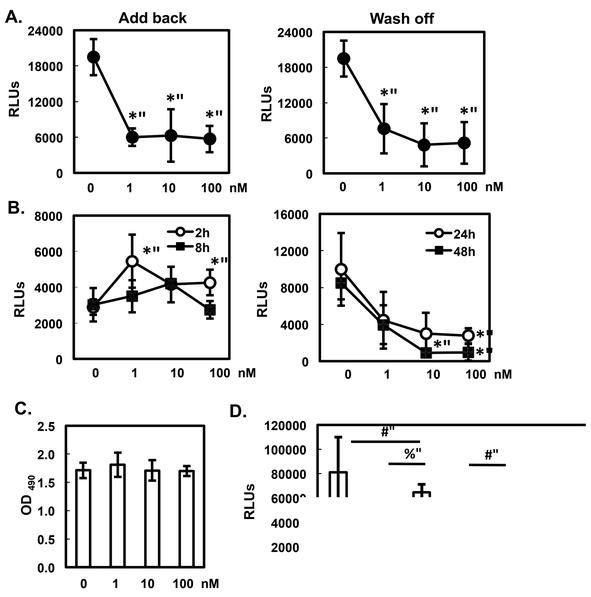
Background: Estrogen has been shown to display a protective role against HIV/SIV transmission. The effect of estrogen on HIV infection of primary cells has recently been reported, but the mechanism underlying estrogen-mediated protection is not well defined. In this study, we examined the mechanism by which estrogen inhibits HIV infection in primary macrophages.

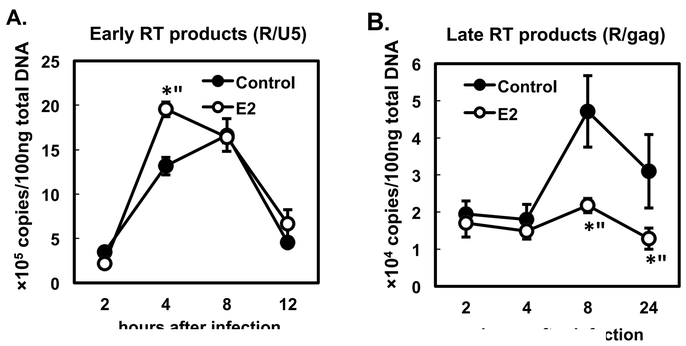
Methods: Monocyte-derived macrophages (MDMs) were isolated from PBMCs of healthy human donors, and then treated with 17β-estradiol (E2) at different time points during HIV infection. Cells were infected with either replication-defective HIV pseudotyped luciferase reporter viruses for single-cycle infection assays or replication-competent viruses for multiple round infection assays. Level of viral infection was determined by measuring luciferase activity or by quantitating levels of HIV p24 in culture media by ELISA. E2-induced changes in gene expression of IFNs, SAMHD1, and the APOBEC family proteins were examined by RT-PCR analysis.

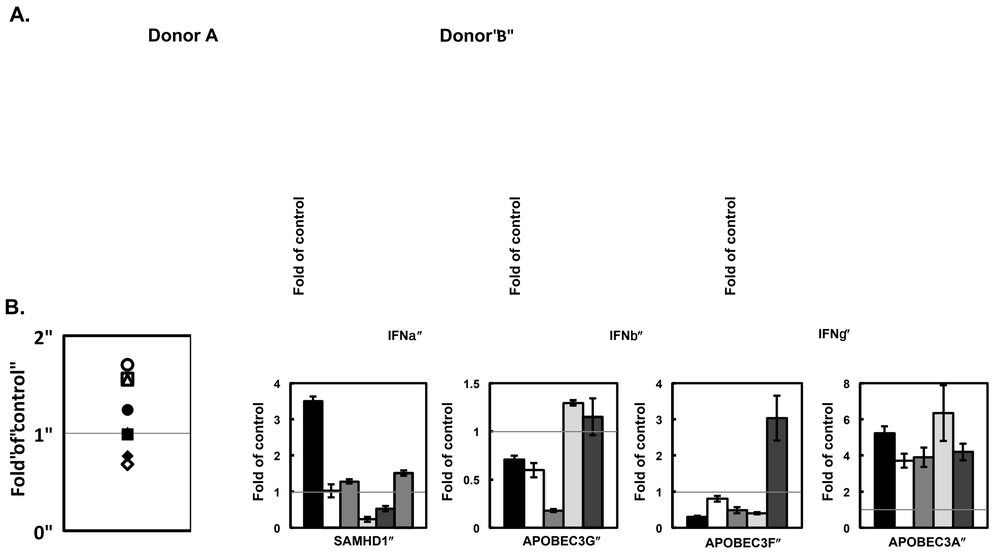
Results: E2 inhibited HIV replication in MDMs but not activated PBMCs or CD4+ T cells. Pretreatment of MDMs with E2 for 24 h was required to block HIV infection. E2 had no effect on HIV inhibition when MDMs were exposed to virus prior to E2 treatment. Investigation of the mechanism of E2-mediated HIV inhibition revealed E2 did not affect surface expression of CD4 and HIV co-receptors nor HIV attachment. E2 pretreatment also protected MDMs from infection by HIV re[illegible] VSV Env, which can enter cells indepenc Quantitative PCR analysis of HIV reverse showed E2 blocked the synthesis of late the involvement of host restriction factors indicated that E2 induced gene expressic in MDMs from multiple donors. Important abolished in the presence of IFNα neutra bone marrow-derived macrophages from Interestingly, HIV exposure suppressed E suggesting that HIV could antagonize E2 responses.

Conclusions: IFNα contributed to E2-m E2 blocked HIV infection at the step of la of IFNα by E2 was associated with an inc APOBEC3G, APOBEC3F or SAMHD1. H mediated type I IFN induction. Our study the interplay between HIV and E2-media provide insight into an effective strategy f



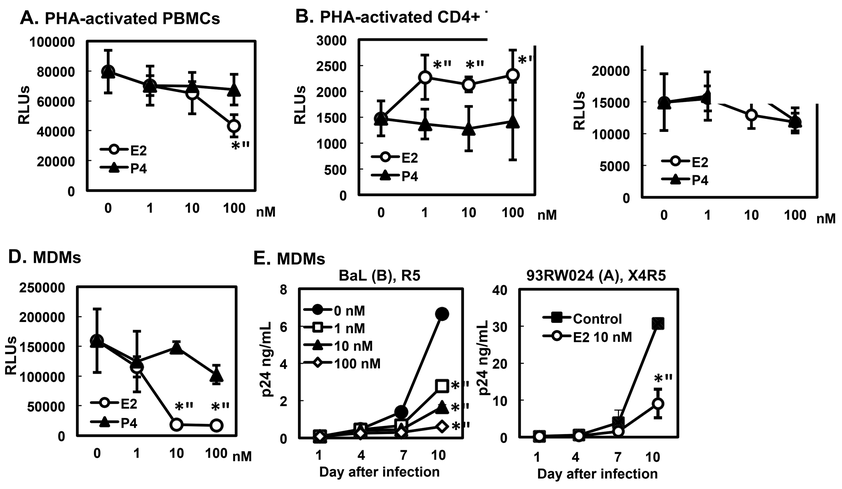
were then treated with (add back infection. **(B)** MDMs were pre-treate HIV infection. **(C)** The cytotoxicity of determined by MTS assay. **(D)** MD

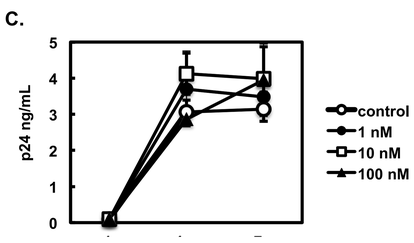


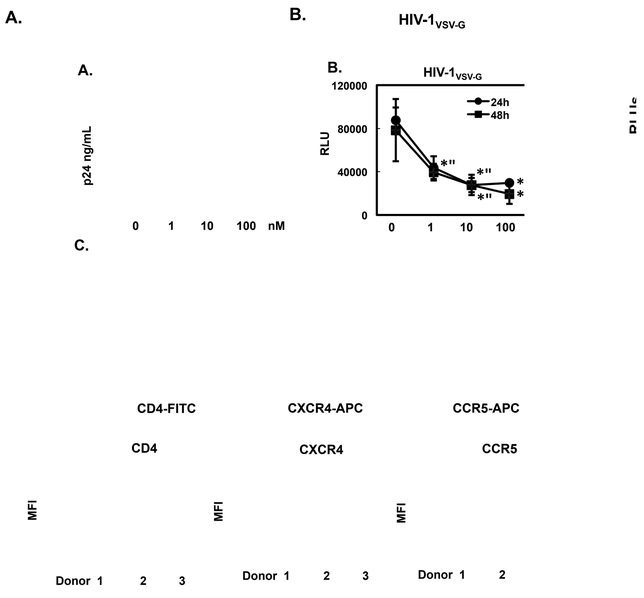


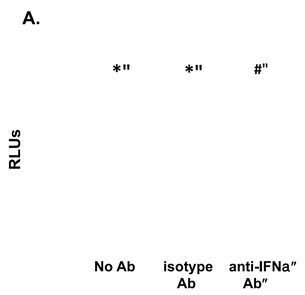
ıors. Additionally, a consistent induction of APOBEC3A was

Figure









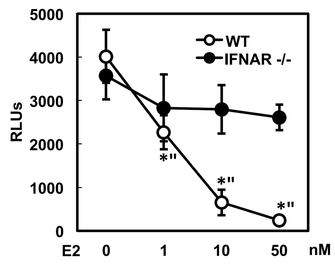


Fig 7. IFNα contributes to E2-mediated HIV inhibition in primary macrophages. (A) MDMs were treated with E2 for 24h along with media alone (no Ab), isotype control antibody or anti-IFNα neutralization antibody. MDMs were exposed to pseudotyped HIV-1_{JR-FL} for 2 h.

Fig

Figure 1 [illegible]/-1vsv luciferase reporter virus. E2 was added back during infection. **(B)** The effect of E2 on HIV attachment was assessed by incubation of E2-pretreated MDMs with R5 pseudotyped HIV-1_{JR-FL} luciferase reporter virus for 2h. Cells were washed and lysed with 1% Triton X-100. The level of cell-associated HIV p24 was determined by ELISA. **(C)** Surface expression of CD4, CXCR4, and CCR5 on MDMs with or without E2 treatment was determined by FACS analysis.

1vsv luciferase reporter virus. E2 was added back after viral exposure and the luciferase activity was determined at day 2 after infection. **(C)** MDMs were exposed to HIV-1_{JR-FL} or to virus particles without Env (HIV Env(-)) for 2 h before treatment with or without E2 at 10 nM for 6 h. Uninfected MDMs were included as a control. Total RNA was prepared and gene expression of type I IFNs was analyzed by quantitative real-time PCR. Result for IFNα expression are shown.

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