# TREHALOSE INHIBITS HIV IN CD4+ LYMPHOCYTES AND MACROPHAGES BY TWO DISTINCT MECHANISMS

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## ABSTRACT

### BACKGROUND

We previously showed that induction of autophagy through the inhibition of mTOR inhibits HIV replication. However, inhibition of mTOR may have cellular effects other than autophagy that could affect HIV infection. Here, we examined trehalose, a naturally occurring glucose mTOR-independent inducer of autophagy, to determine the effects on HIV replication.

### METHODS

Human macrophages (MO) and CD4+ T lymphocytes (T-cells) treated with trehalose with or without HIV infection were assessed for cytotoxicity by LDH release assay and viral replication by p24 ELISA. Autophagy proteins were assessed by immunoblotting, qRT-PCR and fluorescence microscopy combined with assessment of LC3B lipidation. Viral entry was measured by intracellular p24. Data were analyzed using the Student paired T-*test* and one-way Anova.

### RESULTS

Pretreatment of T-cells and MO with trehalose resulted in a dose dependent inhibition of HIV reaching ~90% inhibition at 100mM in both cell types without cytotoxicity. Trehalose induced autophagic flux in T-cells and MO as indicated by increased LC3B lipidation and LC3B-II accumulation following treatment with the autophagic flux inhibitor bafilomycin. Inhibition of HIV was at least partially dependent on induction of autophagy since knockdown of ATG5 by RNAi significantly increased p24 release by 42% and 47% in trehalose-treated HIV-infected T-cells and MO. Surprisingly, trehalose also decreased HIV entry into T-cells and MO in a dose dependent manner reaching ~80% reduction of intracellular virus in both cell types. The inhibition of viral entry was associated with ~3-fold decrease in CD4 expression ($p<0.001$) and CCR5 expression ($p<0.001$) in T-cells, and a 4.6-fold decrease in CD4 expression ($p=0.002$) but no significant change in CCR5 expression in MO. CXCR4 expression was unaffected by trehalose.

## Trehalose is a non-cytotoxic inhibitor of HIV replication in human macrophages and CD4+ T cells

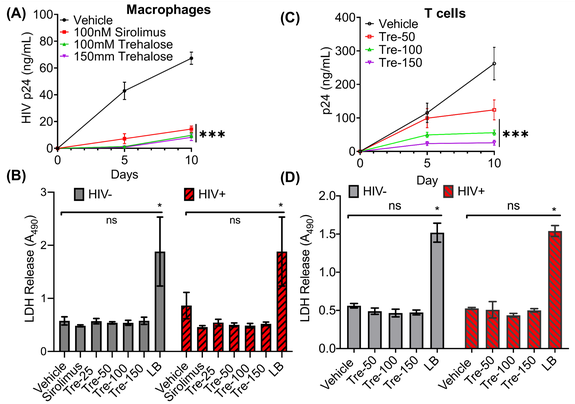


**FIGURE 1** (A) Macrophages were pre-treated with vehicle, 100 nM sirolimus, 100 mM or 150 mM trehalose for 12h prior to exposure to HIV. Cell culture supernatants were collected at days 5 and 10 p.i. for extracellular p24 antigen quantification by ELISA. (B) Uninfected and HIV infected macrophages were incubated with vehicle (V), 100 nM sirolimus or increasing concentrations of trehalose (Tre; 25-150mM) for 10 days. Cell culture supernatants were used to determine cellular toxicity by LDH assay. 1X lysis buffer (LB) treated cells were used as positive control. Data are derived from three independent donors and presented as means ± s.e.m. (C) PHA stimulated T cells (PHA-T cells) were pre-treated with vehicle and increasing concentrations of trehalose (50mM-150mM) for 6h prior to exposure to HIV. Trehalose pretreated HIV-infected T cells were incubated with vehicle or trehalose (Tre; 50mM-150mM) for 10 days and culture supernatants were collected at days 5 and 10 p.i. for extracellular p24 antigen quantification by ELISA. (D) Uninfected and HIV infected T cells were incubated with vehicle (V), and increasing concentrations of trehalose (Tre; 50-150mM) for 10 days and culture supernatants were used to determine cellular toxicity by LDH assay. Data are derived from three independent donors and presented as means ± s.e.m.

## Trehalose inhibits HIV in infected macrophages and CD4+ T cells by induction of autophagy

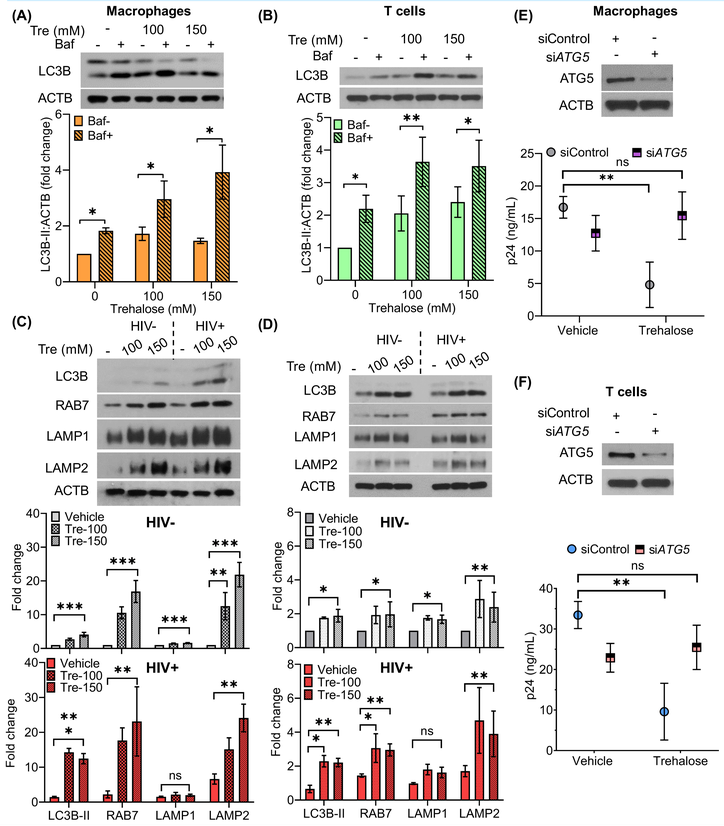


**FIGURE 2** (A, B) Human macrophages and T cells were treated with trehalose (Tre 100mM, 150mM) for 6-12h. Bafilomycin A1 (Baf) co-treatment was used to confirm the increase in autophagy flux in presence of trehalose. Cells were harvested, lysed and analyzed for LC3B lipidation by immunoblotting. *Top,* representative immunoblot showing expression of LC3B-II and ACTB in macrophages (A) and T cells (B). *Bottom*, relative fold change (densitometric analysis) in LC3B-II expression normalized to ACTB. (C, D) Uninfected and HIV infected macrophages and T cells were treated with vehicle or trehalose (Tre; 100mM, 150mM) on day 3 p.i. and incubated for 10 days. At day 10, cells were harvested and lysates were analyzed by immunoblotting with anti-LC3B, anti-RAB7, anti LAMP1, anti-LAMP2 and ACTB antibody. *Top*, representative immunoblots of LC3B, RAB7, LAMP1 and LAMP2. *Bottom,* densitometric analysis of immunoblots for uninfected (grey) and infected (red) cells. (E, F) HIV-infected macrophages and T cells were transfected with non-specific control siRNA (siControl) or *ATG5* siRNA. AT 48h post siRNA transfection cells were harvested and analyzed for ATG5 expression by immunoblotting (*Top*). At 72h post-trehalose treatment culture supernatant were collected and analyzed for p24 release by ELISA (*bottom*). Data are derived from three independent donors and presented as means ± s.e.m.

## Trehalose treatment inhibits HIV entry into human macrophages and CD4+ T cells by modulating the CD4 and CCR5 expression

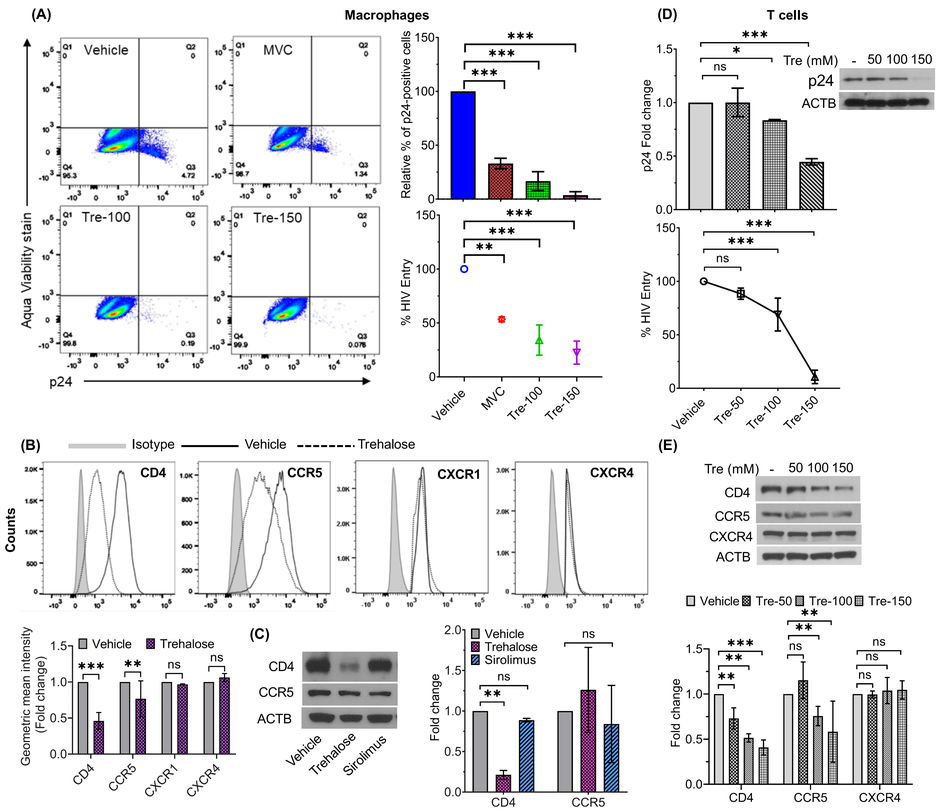


**FIGURE 3** (A) Macrophages pretreated with either 30nM maraviroc (MVC) or 100mM (Tre-100) or 150mM trehalose (Tre-150) were exposed to HIV for 8h. Following infection, cells were collected for detection of intracellular HIV p24 by flow cytometry (*top*), or lysates were prepared to detect HIV p24 antigen by ELISA *(bottom)* at 24h p.i. (B) Macrophages treated with vehicle or trehalose for 12h were analyzed for expression of CD4, CCR5, CXCR1 and CXCR4 by flow cytometry. *Top,* representative histogram showing surface expression of CD4, CCR5, CXCR1 and CXCR4 receptors. *Bottom,* geometric mean intensity fold change showing expression of CD4, CCR5, CXCR1 and CXCR4. (C) *Left,* representative immunoblot showing expression of CD4, CCR5 and ACTB in cell lysates from vehicle, trehalose (100mM) and sirolimus (100nM) treated macrophages. *Right*, relative fold change (densitometric analysis) in CD4 and CCR5 protein normalized to ACTB. (D) Untreated and trehalose (Tre) pretreated T cells were exposed to HIV (0.04) for 8h. At 24 p.i., cell lysates were prepared to detect HIV antigen by immunoblotting (*top*, representative immunoblot and densitometric analysis) and ELISA (*bottom*). (E) T cells were treated with vehicle or trehalose (50-150mM) for 6h and analyzed for expression of CD4, CCR5 and CXCR4 by immunoblotting. *Top*, representative immunoblot showing the expression of CD4, CCR5, CXCR4 and ACTB. *Bottom*, relative fold change (densitometric analysis) in CD4, CCR5 and CXCR4 protein normalized to ACTB.

## CONCLUSIONS

Trehalose, at doses safely achieved in humans inhibits HIV through two mechanisms: 1) decreased entry through the down-regulation of CCR5 in T-cells, and decreased CD4 expression in both T-cells and macrophages ; and 2) degradation of intracellular HIV through the induction of mTOR independent autophagy.

## ACKNOWLEDGEMENTS

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