Galectin-9 Enables HIV Infection of Resting CD4+ T Cells and Promotes Latent Reservoir Expansion

203

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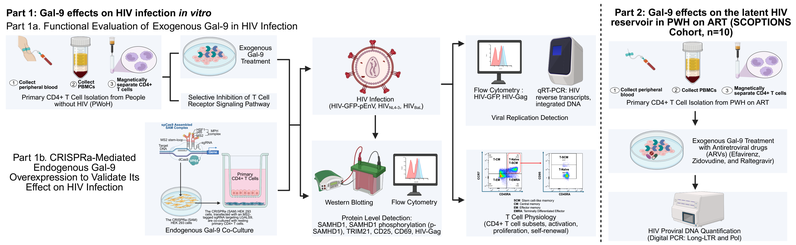
BACKGROUND

- The mechanisms underlying the establishment and maintenance of the latent HIV reservoir, the main hindrance to an HIV cure, remain poorly understood.
- Elevated plasma levels of galectin-9 (Gal-9), a β-galactoside-binding lectin, predict slower intact proviral DNA decay in people with HIV (PWH) on antiretroviral therapy (ART) and associate with increased gut mucosal SIV burden.

OBJECTIVE

- To investigate mechanisms underlying Gal-9-mediated reservoir establishment and expansion, and assess the potential of P4D2, an anti-Gal-9 monoclonal antibody (mAb), to target HIV persistence.

METHODS



RESULTS

1. Both Exogenous and Endogenous Gal-9 Render Resting Primary CD4+ T Cells Permissive to HIV Infection.

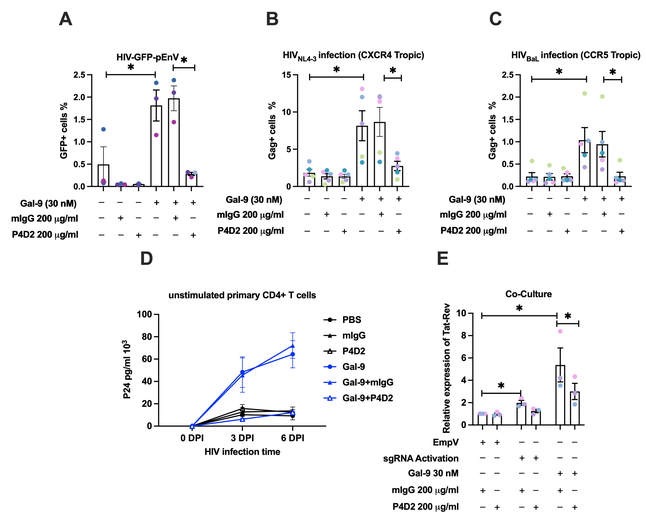


Figure 1. The P4D2 anti-Gal-9 monoclonal antibody prevents Gal-9-mediated HIV infection of resting CD4+ T cells. A-C. Percentage of HIV infection in resting CD4+ T cells stimulated with Gal-9, mouse IgG (mIgG), or P4D2 4 days before HIV infection with HIV-GFP-pEnV (A), HIV$_{NL4-3}$ (B), and HIV$_{BaL}$ (C). Infection was quantified 3 days post-infection by detecting GFP or by intracellular Gag staining using flow cytometry. D. p24 levels in culture supernatants collected at the indicated time points following HIV$_{NL4-3}$ infection under the indicated treatments. E. HIV *tat-rev* transcript levels measured by qRT-PCR in resting primary CD4+ T cells co-cultured for 7 days with HEK-293T cells engineered to overexpress endogenous Gal-9 via CRISPR activation (sgRNA Activation). Gene expression was normalized to 18S rRNA and presented as fold change relative to empty vector (EmpV) control. Gal-9 treatment served as the positive control. Bar, mean ± SEM. *, *p<0.05.*

Gal-9 promotes the establishment and expansion of a self-renewing HIV reservoir by facilitating infection and proliferation of stem cell-like memory CD4+ T cells. The P4D2 mAb shows potential as a therapeutic strategy to prevent Gal-9-mediated enhancement of HIV persistence.

2. Gal-9 Promotes HIV Reverse Transcription and Integration.

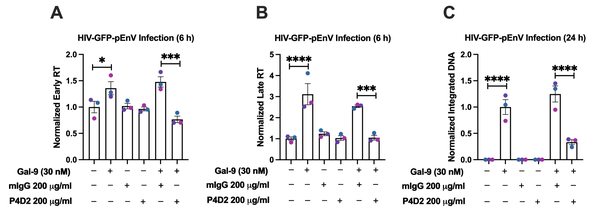


Figure 2. P4D2 impairs Gal-9-mediated HIV infection of resting CD4+ T cells at the reverse transcription stage. A-B. Early (A) and late (B) reverse transcription products were quantified by qRT-PCR in primary CD4+ T cells treated as indicated for 5 days and subsequently infected with HIV-GFP-pEnV for 6 hours. C. Following infection, cells were further cultured for an additional 2 days, and proviral integration was quantified by qRT-PCR. Viral DNA levels were normalized to CCR5 DNA and presented as fold change relative to untreated control. Bar, mean ± SEM. *, *p<0.05*; **, *p<0.01*; ***, *p<0.001*; ****, *p<0.0001*.

3. Gal-9 Deactivates SAMHD1 Restriction of HIV in Resting CD4+ T Cells.

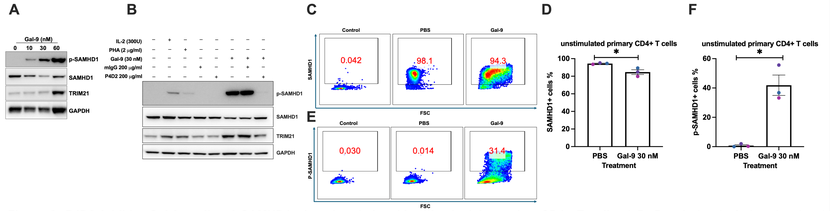


Figure 3. P4D2 inhibits Gal-9-mediated SAMHD1 phosphorylation (deactivation) in resting CD4+ T cells. A-B. Protein level of phosphorylated SAMHD1 (p-SAMHD1), total SAMHD1, TRIM21, and GAPDH were analyzed by immunoblotting in whole-cell lysates from resting CD4+ T cells treated with Gal-9 at the indicated concentrations for 5 days (A) or treated with the indicated reagents (B). C. Representative plot showing total SAMHD1 expression in primary CD4+ T cells treated with PBS or Gal-9. D. Percentage of SAMHD1-positive cells determined by flow cytometry. E. Representative plot showing p-SAMHD1 expression in primary CD4+ T cells treated with PBS or Gal-9. F. Percentage of p-SAMHD1-positive cells determined by flow cytometry. Bar, mean ± SEM. *, *p<0.05.*

4. Gal-9 Can Deactivate SAMHD1 in the Absence of T Cell Activation.

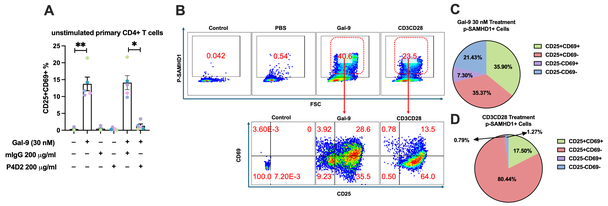


Figure 4. A subset of SAMHD1-deactivated cells remain activation marker-negative after Gal-9 treatment. A. Percentage of CD25 and CD69 co-expressing cells on the cell surface after indicated treatments. B. Representative plot of p-SAMHD1 expression in primary CD4+ T cells treated with PBS, Gal-9, or CD3/CD28 activator. Cells were gated on FSC and p-SAMHD1, and then analyzed for CD25 and CD69 expression. C. Percentage of p-SAMHD1-positive cells expressing CD25 and CD69 after Gal-9 treatment. D. Percentage of p-SAMHD1-positive cells expressing CD25 and CD69 after CD3/CD28 activator treatment. Bar, mean ± SEM. *, *p<0.05*; **, *p<0.01*.

PLAIN LANGUAGE SUMMARY

Galectin-9 enhances HIV infection and persistence in long-lived cells. Blocking Galectin-9 activity using the therapeutic antibody P4D2 is a potential strategy to target HIV reservoir and achieve a cure.

5. Gal-9-Mediated SAMHD1 Deactivation and T Cell Activation Require Glycan-Glycan Interaction.

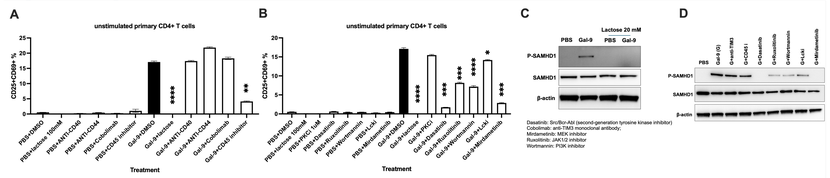


Figure 5. Inhibition of CD45 and JAK signaling pathways preventes Gal-9-mediated SAMHD1 deactivation. A-B. Percentage of CD25 and CD69 co-expressing cells on the cell surface after indicated treatments. C-D. Protein level of p-SAMHD1, total SAMHD1, and GAPDH were analyzed by immunoblotting in whole-cell lysates from resting CD4+ T cells treated with indicated reagents. Bar, mean ± SEM. *, *p<0.05*; **, *p<0.01*; ***, *p<0.001*; ****, *p<0.0001*.

6. Gal-9 Treatment Leads to Increased Infection and Expansion of the T-SCM Compartment (Stem Cell-Like Memory Cells).

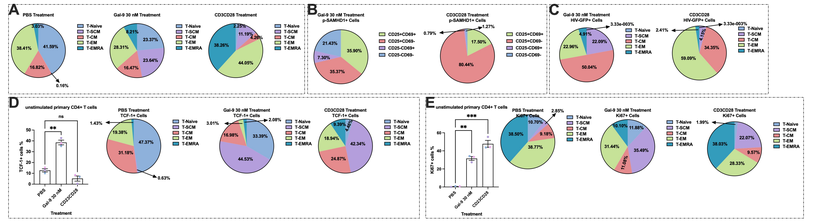


Figure 6. Gal-9 induces SAMHD1 phosphorylation in HIV-susceptible T-SCM, T-CM, and T-EM Subsets. A. Mean distribution of CD4+ T cell subsets from three donors after 5 days of treatment with the indicated reagents, measured by flow cytometry. B. Percentage of p-SAMHD1-positive cells expressing CD25 and CD69 after Gal-9 or CD3/CD28 activator treatment. C. Mean distribution of CD4+ T cell subsets from HIV-infected cells after Gal-9 or CD3/CD28 activator treatment. D. Percentage TCF-1-positive cells after indicated treatments. Mean distribution of CD4+ T cell subsets among TCF-1-positive cells after indicated treatments. E. Percentage of Ki-67-positive cells after indicated treatments. Mean distribution of CD4+ T cell subsets among Ki-67-positive cells after indicated treatments. Bar, mean ± SEM. *, *p<0.05*; **, *p<0.01*; ***, *p<0.001*.

7. Gal-9 Increases HIV Proviral DNA Frequencies in CD4+ T Cells from PLWH on ART.

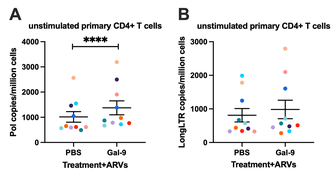


Figure 7. Gal-9 promotes accumulation of HIV proviral DNA in CD4⁺ T cells from PLWH on ART. A-B. Absolute copies of HIV Pol (A) and Long-LTR (B) DNA per million CD4⁺ T cells. CD4⁺ T cells were isolated from 10 PWH receiving ART and cultured for 5 days with PBS or Gal-9 in the presence of ARVs (efavirenz, zidovudine, and raltegravir). Genomic DNA was extracted, and digital PCR was performed to quantify HIV *pol* and Long-LTR DNA copies. RPP30 served as the reference control. Bar, mean ± SEM. *, *p<0.05*; **, *p<0.01*; ***, *p<0.001*; ****, *p<0.0001*.

CONCLUSIONS

1. Gal-9 promotes establishment and expansion of a self-renewing HIV reservoir by facilitating infection and proliferation of stem cell-like memory CD4+ T cells. Signaling via CD45 and JAK mediates these effects.
2. Gal-9 may render CD4+ T cells permissive to HIV infection in the absence of cellular activation, contributing to the long-lived reservoir.
3. The P4D2 mAb shows potential as a therapeutic strategy to prevent Gal-9-mediated enhancement of HIV persistence.

ADDITIONAL KEY INFORMATION

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