Targeting TCF-1 to potentiate the functional capacity of HIV/SIV-specific CD8⁺ T cells

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BACKGROUND

The transcription factor T cell factor 1 (TCF-1) plays a critical role in maintaining functional memory CD8⁺ T cells and regulating their proliferative capacity. In HIV-specific CD8⁺ T cells, TCF-1 expression has been linked to viral control, suggesting its importance in promoting the functional activity of these cells. Glycogen synthase kinase-3 (GSK-3) inhibitors prevent the degradation of β-catenin, which in turn enhances TCF-1 expression and its transcriptional activity. In this study, we asked whether GSK-3 inhibitors can be used to induce TCF-1 expression in HIV/SIV-specific CD8⁺ T cells.

METHODS

TCF-1 expression in SIV-specific CD8⁺ T cells from 5 SIV-infected rhesus macaques (RM) with plasma viral load (pVL) between 1,700-170,000 SIV RNA copies/mL and 7 SIV controllers (pVL < 15 SIV RNA copies/mL) were assessed by flow cytometry. CD8⁺ T cells from humans (n=13) and RM (n=21) were treated with 5 different GSK-3 inhibitors (BIO, CHIR99021, TWS119, Li_2CO_3, and LY2090314) for 16 hours to evaluate their potential to induce TCF-1 expression. PBMC from 5 SIV-infected RM on ART and 5 individual living without HIV were treated with LY2090314 for 16 hours, followed by culture with IL-7 (10ng/mL) for 6 days to assess expansion of Mamu*A01 immunodominant Gag-CM9- and Tat-SL8-specific CD8⁺ T cells and total CD8⁺ T cells, respectively. Five SIV-infected RM on ART were treated with the GSK-3 inhibitor LY2090314 at 5mg/kg IV to assess TCF-1 expression in CD8⁺ T cells 5/6 days after the treatment.

RESULTS

SIV-specific CD8⁺ T cells from viral control animals expressed higher level of TCF-1 than viremic animals.

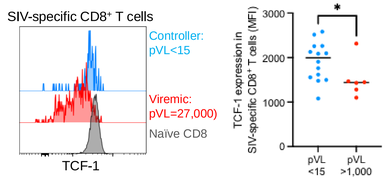

Figure 1. TCF-1 expression in SIV-specific CD8⁺ T cells. Comparison of TCF-1 expression level in Mamu*A01 Gag CM9- and Tat-SL8-specific pMHC Tetramer⁺ CD8⁺ T cells from SIV-infected rhesus macaques with plasma viral load (pVL) between 1,700-170,000 SIV RNA copies/mL and 7 SIV controllers (pVL < 15 SIV RNA copies/mL). Representative TCF-1 expression in $CD95^-CD28^+CCR7^+$ naïve CD8⁺ T cells was also shown. Differences between animal groups was analyzed by Mann-Whitney test. *P< 0.05.

Table 1. GSK-3 inhibitors tested in this study

Compound	Condition/ Disease	Clinical Trial Status	Clinical Trial ID	Administration	Dose
Li_2CO_3	Bipolar Disorder	FDA Approved	NCT01166425	Oral	300mg
9-ING-41	Cancer	Phase 2	NCT03678883	Intravenous	9.3 mg/kg
LY2090314	Leukemia	Phase 2	NCT01214603	Intravenous	40mg
CHIR99021	Type 2 diabetes	Animal (Rat)	NA	Oral	16 mg/kg
BIO	Melanoma	Animal (Mouse)	NA	Oral	50 mg/kg
TWS119	Stroke	Animal (Rat/Mouse)	NA	Intraperitoneal	30 mg/kg

GSK-3 inhibitor LY2090314 induced TCF-1 in CD8⁺ T cells in both human *in vitro* and SIV infected rhesus macaques *in vivo*.

GSK-3 inhibitors induced TCF-1 expression in human CD8⁺ T cells *in vitro*.

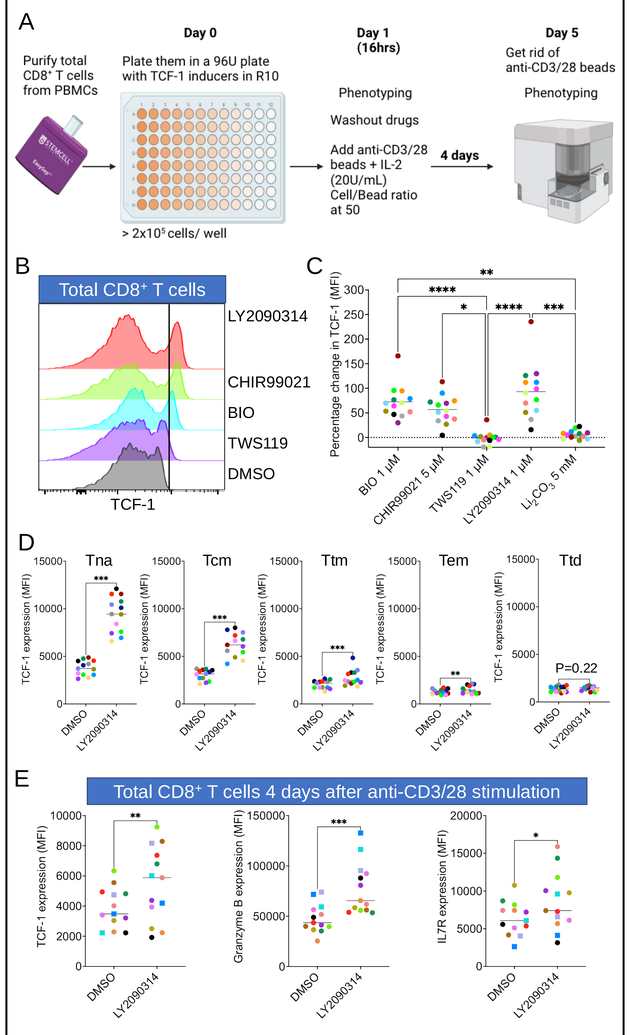

Figure 2. (A) Schematic of the *in vitro* treatment with the GSK-3 inhibitors. Isolated CD8⁺ T cells from people living without HIV were treated with GSK-3 inhibitors overnight followed by anti-CD3/28 beads stimulation for 4 days. (B, C) TCF-1 expression (B) and percentage increase in the expression (C) in CD8⁺ T cells after overnight treatment with GSK3 inhibitors. (D) TCF-1 expression in CD8⁺ T cell subsets after overnight LY2090314 treatment at 1μM. CD8⁺ T cells were classified into naïve (Tna), central memory (Tcm), transitional memory (Ttm), effector memory (Tem), and terminally differentiated (Ttd) CD8⁺ T cells. (E) Expression of TCF-1, Granzyme B, and IL7R in/on CD8⁺ T cells 4 days after anti-CD3/28 stimulation. Differences among GSK-3 inhibitors and between treated and control cells were analyzed by Friedman and Wilcoxon test, respectively. (*P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001)

GSK-3 inhibitors induced TCF-1 expression in total and SIV-specific rhesus CD8⁺ T cells *in vitro*.

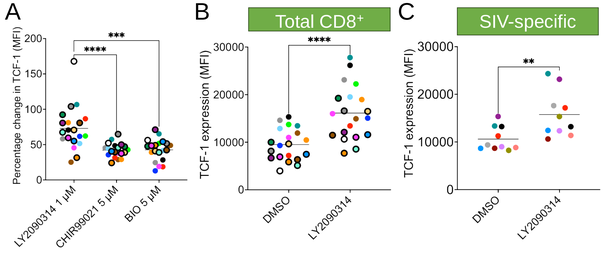

Figure 3. (A) Percentage increase in TCF-1 expression of CD8⁺ T cells in PBMC from SIVmac239-infected rhesus macaque on ART after overnight treatment with GSK3 inhibitors. (B, C) TCF-1 expression in LY2090314 treated CD8⁺ T cells (B) and Mamu-A*01 SIV Gag CM9-specific CD8⁺ T cells (C) after overnight treatment. Differences were analyzed by Friedman and Wilcoxon test. (**P< 0.01; ***P< 0.001; ****P< 0.0001)

Enrichment of central memory CD8⁺ T cells after IL-7 treatment following GSK3 inhibition.

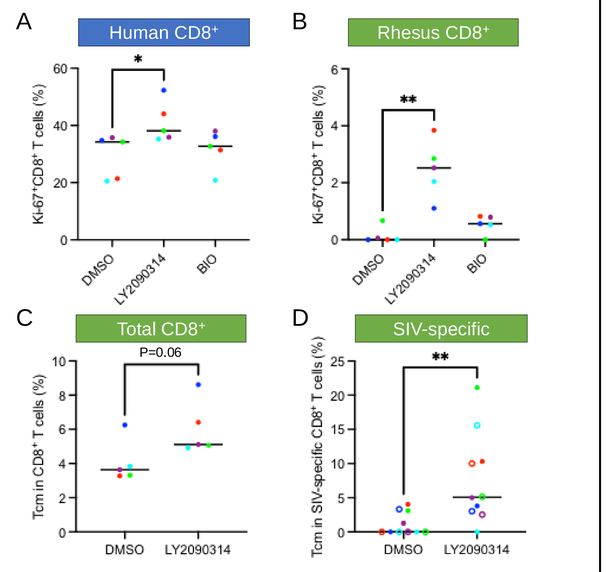

Figure 4. (A, B) PBMCs from human and SIVmac239-infected rhesus macaques on ART were treated with LY2090314 (1μM) or BIO (5μM) *in vitro* overnight. Washed treated cells were rested for 5 days followed by 6 day culture with recombinant human IL-7 at 10ng/mL. Percentage of Ki-67⁺ cells in total human (A) and rhesus CD8⁺ T cells (B) were analyzed. (C, D) Percentage of central memory cells (Tcm, $CD95^+CD28^+CCR7^+$) in total rhesus CD8⁺ T cells (C) and SIV-specific CD8⁺ T cells after the IL-7 culture. Closed and open symbols represent Mamu-A*01 SIV Gag CM9- and Tat SL8-specific CD8⁺ T cells, respectively. Differences were analyzed by Friedman and Wilcoxon test. (*P< 0.05; **P< 0.01.)

In vivo LY2090314 treatment induced TCF-1 expression in CD8⁺ T cells in blood of SIV infected rhesus macaques on ART.

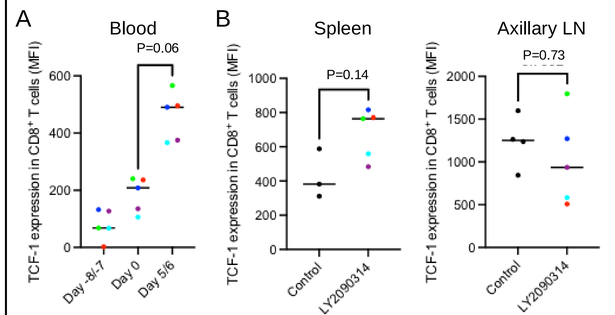

Figure 5. (A) Expression of TCF-1 in CD8⁺ T cells from 5 SIVmac239-infected rhesus macaques at -8\-7, 0, and 5/6 days after treatment with LY2090314 administered intravenously at 5mg/kg. (B) Expression of TCF-1 in CD8⁺ T cells from axillary lymph node and spleen of SIVmac239-infected rhesus macaques 5/6 days after LY2090314 treatment.

CONCLUSIONS

- GSK-3 inhibitors can increase TCF-1 expression in CD8⁺ T cells from both human and rhesus macaques.
- LY2090314 is the most potent inducer of TCF-1 expression in SIV-specific CD8⁺ T cells.
- LY2090314 enhanced IL-7-induced CD8⁺ T cell proliferation.
- Treatment with LY2090314 in SIV-infected rhesus macaques increased TCF-1 expression in CD8⁺ T cells in the blood, though formulation and dose optimization is needed.
- These data support further evaluation of LY2090314 in SIV-infected rhesus macaques on ART to assess whether TCF-1 induction can enhance CD8⁺ T cell functional activity and promote durable virologic control.

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