School of Medicine & Health Sciences
THE GEORGE WASHINGTON UNIVERSITY
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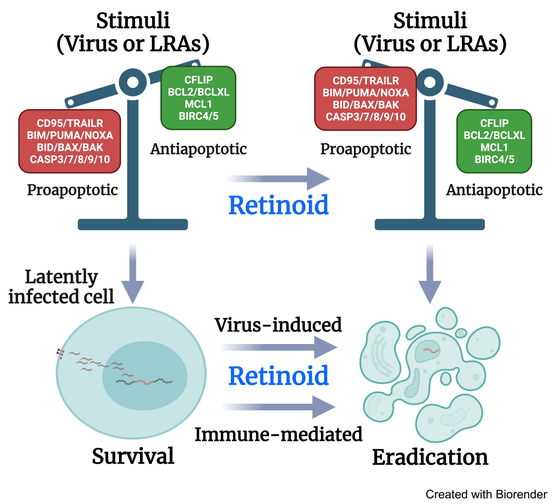
Retinoids Enhance NK Natural and Antibody Dependent Cell Cytotoxicity of HIV-Infected CD4 T Cells

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Introduction

Human immunodeficiency virus (HIV) management requires daily administration of antiretroviral therapy (ART) drugs due to the presence of a latent reservoir. Recently, an increased resistance to apoptosis of latently infected cells has been linked to one of the mechanisms associated with HIV persistence[1,2]. While IL15 is being investigated to reduce latent reservoir[3,4], prior research by us and others identified the retinoids Isotretinoin and Acitretin as potential drugs that increase the sensitivity of HIV-infected cells to cell death[5,6]. Retinoids may be a potential approach to eliminate the latent reservoir as they can target receptors that mitigate proliferative effects and promote apoptotic activity of cells[7,8]. Moreover, previous data demonstrates that CD4 T cells express various retinoic acid receptors and retinoid X receptors (RAR and RXR)[9,10]. However, it is imperative first to determine which retinoids are effective at sensitizing infected cells to apoptosis and what receptors are required for this activity.

The aim of this study was to evaluate the ability of retinoids to enhance NK effector function against HIV infected CD4 T cells. We tested retinol (Vitamin A), 3 of its natural metabolites as well as 9 synthetic retinoid derivatives. 5 of these retinoids are FDA-approved.



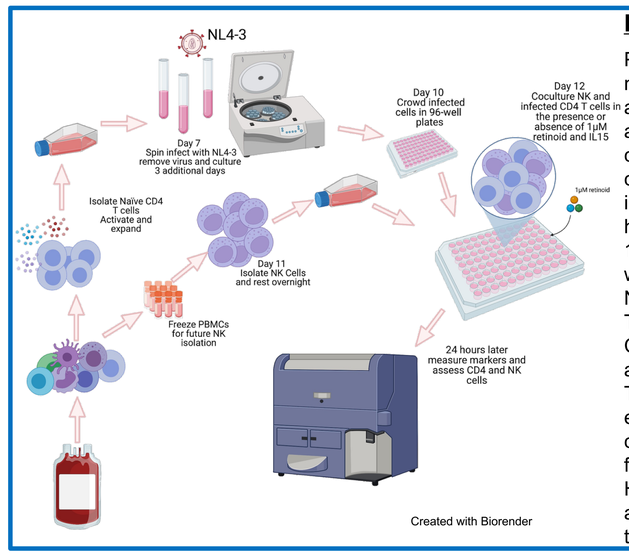


Figure 1. Workflow

PBMCs from male and female donors were used to isolate naïve CD4 T cells and NK cells. Naïve CD4 T cells were activated using aCD3/CD28 beads, expanded for 7 days, and then infected with the replication competent molecular clone NL43. On day 12, infected CD4 T cells were co-cultured with NK and treated with 1μM of a retinoid and ART in either the presence or absence of 100ng/mL of IL15 for 24 hours. Specific HIV-infected cell killing was calculated: 100*[1-(%infected targets with effectors / %infected targets without effectors)].

Next, we assessed the ability of retinoids Alitretinoin, Tazarotene Acid, and AM80 to enhance Antibody-Dependent Cell Cytotoxicity (ADCC) using the broadly neutralizing antibody N6 at different effector to target ratios.

To elucidate the mechanism/s by which these retinoids enhance NK killing of HIV-infected cells, we measured changes in several receptors involved in NK effector function. We measured changes in HLA-ABC, HLA-E, and HLA-F expression on target cells along with CD16 and the activating receptors NKG2C and KIR3DS1 on NK cells upon treatment with the indicated retinoids.

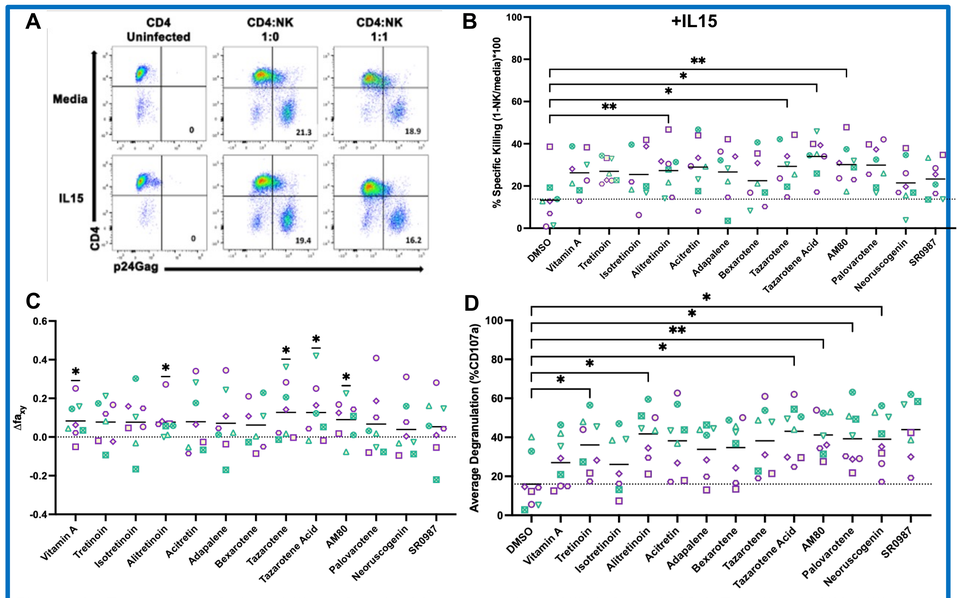


Figure 2. Evaluation of the ability of retinoids to sensitize HIV-infected CD4 T cells to NK killing. A. Representative flow cytometry plot. **B.** Specific killing relative to media control of HIV-infected CD4 T cells in the presence of IL15 and the indicated retinoids. **C.** Synergy analysis of specific killing between IL-15 and the indicated retinoids using the Bliss independence model **D**. Analysis of NK degranulation measured as %CD107a after coculture of HIV-infected CD4T cells cells in the presence of IL15 and the indicated retinoids. Purple symbols represent female (n=4) and green representing male (n=4) donors. One-way ANOVA with multiple comparisons was used to determine significance (*p<0.05, **p<0.01).

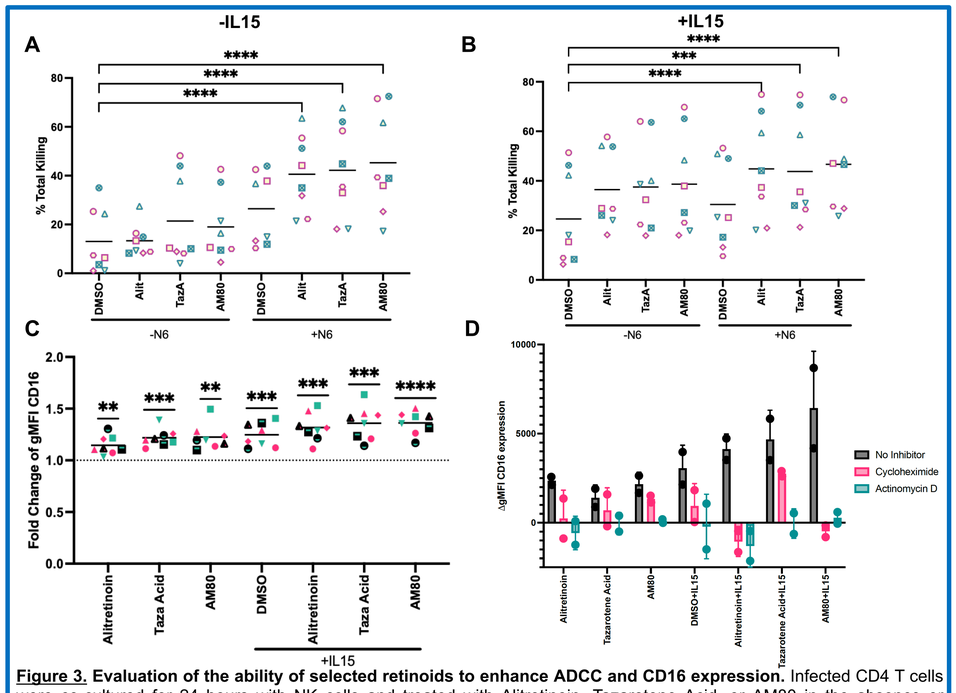


Figure 3. Evaluation of the ability of selected retinoids to enhance ADCC and CD16 expression. Infected CD4 T cells were co-cultured for 24 hours with NK cells and treated with Alitretinoin, Tazarotene Acid, or AM80 in the absence or presence of the N6 antibody and in the absence of IL15 **(A)** or the presence of IL15 **(B)**. Percent total killing was calculated as related to media control. Pink symbols represent female (n=4) and teal representing male (n=4) donors. One-way ANOVA with multiple comparisons was used to determine significance (***p<0.001, ****p<0.0001). **C.** Fold change relative to DMSO control of CD16 expression in NK cells treated with the indicated retinoid in the absence or presence of IL-15. Pink symbols represent female (n=4) and teal representing male (n=4) donors. One sample T test was used to determine significance (**p<0.01, ***p<0.001, ****p<0.0001). **D.** NK cells from two donors (1 male and 1 female) were treated with each retinoid (1μM) in the absence or presence of IL15 and in the presence of translation inhibitor, Cycloheximide, or the transcription inhibitor, Actinomycin D. CD16 expression relative to DMSO control was evaluated by flow cytometry.

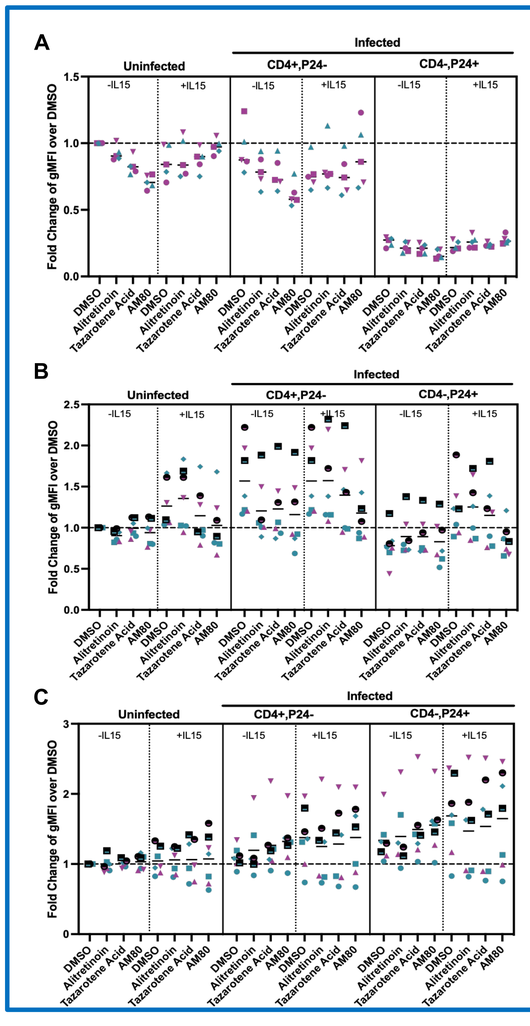


Figure 4. Evaluation of MHC-I expression on uninfected and infected CD4 T cells. A. Fold change expression of HLA-ABC relative to DMSO of uninfected controls after culture of uninfected and infected CD4 T cells with 1μM of Alitretinoin, Tazarotene Acid, or AM80 in the presence or absence of IL15 for 24 hours to measure HLA expression. Experiments were done in 5 donors. Purple symbols represent female and teal representing male donors. **B.** Fold change expression of HLA-E relative to DMSO of uninfected controls after culture of uninfected and infected CD4 T cells with 1μM of Alitretinoin, Tazarotene Acid, or AM80 in the presence or absence of IL15 for 24 hours to measure HLA expression. Experiments were done in 7 donors. Purple symbols represent female and teal representing male donors. **C.** Fold change expression of HLA-F relative to DMSO of uninfected controls after culture of uninfected and infected CD4 T cells with 1μM of Alitretinoin, Tazarotene Acid, or AM80 in the presence or absence of IL15 for 24 hours to measure HLA expression. Experiments were done in 7 donors. Purple symbols represent female and teal representing male donors.

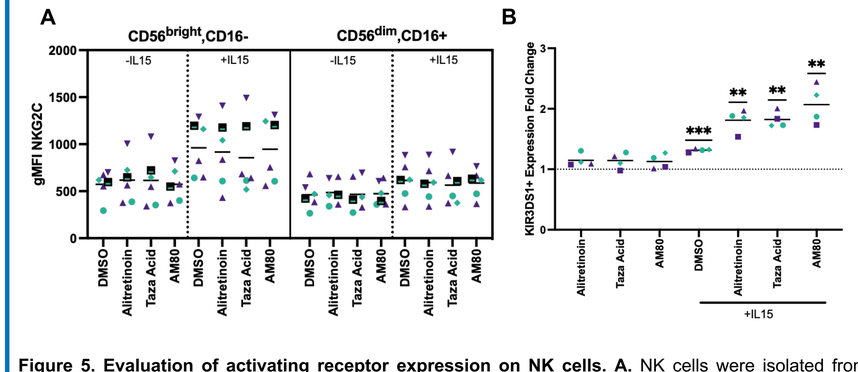


Figure 5. Evaluation of activating receptor expression on NK cells. A. NK cells were isolated from PBMCs and cultured with 1μM of Alitretinoin, Tazarotene Acid, or AM80 in the presence or absence of IL15 for 24 hours. We measured the activating receptor NKG2C on the two subpopulations of NK cells: $CD56^{bright}$, CD16- and $CD56^{dim}$, CD16+. Experiments were done in 6 donors. Purple symbols represent female (n=3) and green representing male (n=3) donors. **B.** NK cells were co-cultured with infected CD4 T cells in the presence or absence of 1μM of the indicated retinoid with or without IL15. KIR3DS1 expression was measured in the $CD56^{dim}$, CD16+ subpopulation of NK cells. Fold change relative to DMSO control. Purple symbols represent female (n=2) and green representing male (n=2) donors. One sample T test was used to determine significance (**p<0.01, ***p<0.001).

Conclusions

- We show that the combination of IL15 and several retinoids significantly enhanced NK killing and NK degranulation (Fig 2A,B,D). This interaction was found to be synergistic with regard to specific killing of infected CD4 T cells (Fig 2C).
- We show that Alitretinoin, Tazarotene Acid, and AM80 enhanced the ability of N6 to promote ADCC compared to DMSO control both in the absence and in the presence of IL15 (Fig 3A,B). Mechanistically, retinoids significantly enhanced CD16 expression at the transcriptional levels (Fig 3C,D).
- We show that retinoids did not change MHC-I expression on infected or uninfected CD4 T cells (Fig 4). Infected cells downregulated HLA-ABC and HLA-E (Fig 4A and B), but the addition of the retinoids did not influence this downregulation. On the other hand, HLA-F expression was upregulated in infected cells, but addition of the retinoids with and without IL15 did not consistently modulate this upregulation (Fig 4C).
- We show that IL-15 enhanced the HLA-E ligand NKG2C expression in the $CD56^{bright}$,CD16- population but not the $CD56^{dim}$, CD16+ population. Retinoids did influence this expression (Fig 5A).
- In co-culture experiments, we found that IL15 significantly increased the expression of the HLA-F ligand KIR3DS1 when compared to the DMSO control and that this increased expression is higher in the presence of all three tested retinoids (Fig 5B).
- In conclusion, we have identified three retinoids, Alitretinoin, Tazarotene Acid, or AM80, capable of sensitizing HIV-infected cells to NK natural cytotoxicity and ADCC. We are conducting experiments to confirm the mechanisms associated with this sensitization. Our studies may provide further evidence of small molecules that could be used clinically to reduce persistent reservoirs.

References

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