Targeting of HIV Infected Clones by Cognate Peptide Stimulation and Antiproliferative Drugs

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BACKGROUND

Clonal expansion of HIV infected CD4+ T cells is a barrier to HIV eradication. Previous studies suggested that interventions blocking cell proliferation can accelerate reservoir decay (1). We recently described (2) a marked reduction in clonally expanded infected CD4+ T cells in an elite controller (ES24) after chemoradiation for lung cancer with paclitaxel and carboplatin. We tested the hypothesis that this phenomenon was due to a higher susceptibility of frequently proliferating CD4+ T cell clones to the chemotherapeutic drugs.

METHODS

We studied a large CD4+ T cell clone with replication-competent provirus integrated into the ZNF721 gene (ZNF721i), which has been linked to the persistence of intact proviruses (Fig 1).
We stimulated CD8-depleted PBMCs from ES24 with the cognate peptide for the ZNF721i clone (peptide 61: Gag 241-255) for 20 hours on day 1. On day 5, the cells were exposed to paclitaxel and/or carboplatin, or the antiproliferative drug, mycophenolate mofetil (MMF). The cells were harvested on days 7 to 9. The frequency of ZNF721i cells was determined by integration site-specific digital PCR. Single-cell transcriptome analysis and T cell receptor sequencing was also performed on day 7 CD4+ T cells.

The ZNF721i provirus largely dominated the proviral landscape after chemoradiation

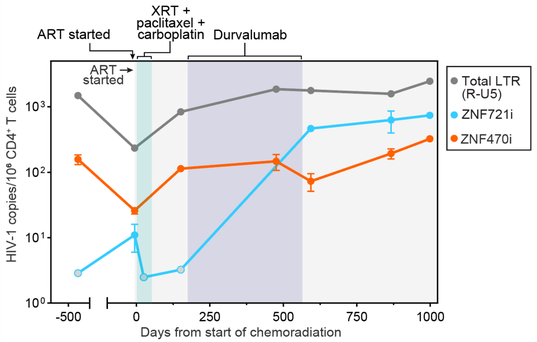


Figure 1. Longitudinal quantification of ZNF721i and ZNF470i proviruses before, during, and after treatment. In a previous study (1), we used provirus-specific dPCR assays to trace the dynamics of the two most abundant clones. Upon chemotherapy treatment, we noticed a sharp reduction in the frequency of clonally expanded and infected cells. After chemotherapy was completed, ZNF721i underwent a 70-fold expansion, while ZNF470i was persistent but stable during the whole follow-up. By stimulation with individual HIV-1 Gag peptides, we resolved the ZNF721i clone specificity at the epitope level. Cells harboring cells harboring ZNF721i responded to peptide 61 (Gag 241-255).

REFERENCES

1. Reeves et al., Nat Commun. 2018. PMID: 30446650
2. Dragoni, Kwaa et al., JCI. 2023. PMID: 37698927

SUMMARY

Combining antigen-driven stimulation with antiproliferative agents (Paclitaxel, MMF) could be a promising strategy to selectively target HIV-infected CD4+ T cell clones, independent of HIV expression

Treatment with Paclitaxel and MMF halts antigen-driven proliferation of the ZNF721i provirus

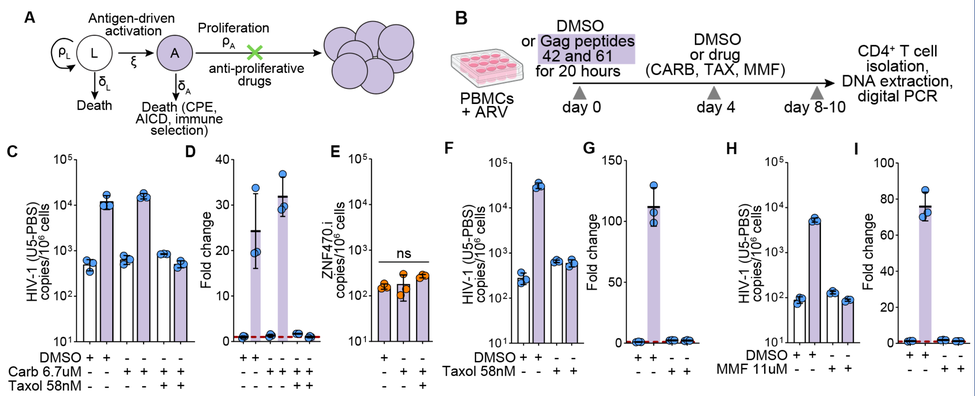


Figure 2. Reduction in the proliferation of gag-reactive infected cells upon treatment with chemotherapeutic drugs. Using dPCR, we assessed the effect of chemotherapeutic drugs following stimulation of infected cells with their cognate peptide. A) Schematic overview of the proposed effect of anti-proliferative drugs on cell expansion. B) Schematic overview of the ex-vivo experiment used to assess the effects of chemotherapeutic drugs. C), D), F) and G) Impact of carboplatin, carboplatin+paclitaxel, paclitaxel, and MMF on infected cell proliferation upon stimulation. Purple-shaded stacked bars represent the gag peptide stimulated condition. E) As a control, we quantified a non-reactive Gag clone (ZNF470i) to assess the specificity of the antiproliferative effect.

Paclitaxel specifically blocked cells proliferating in response to Gag, rather than T cell activation

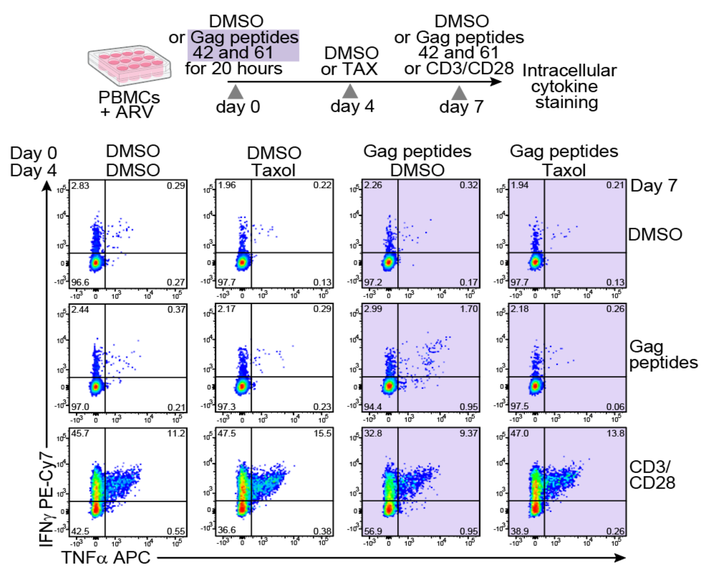


Figure 3. Intracellular cytokine staining showed that paclitaxel abrogated proliferation of Gag-reactive cells. We set up a 7-day expansion assay with the cognate peptide in the presence or absence of paclitaxel. Upon cognate peptides stimulation, we observed a marked expansion of the cells that expressed IFNγ and TNFα.
Paclitaxel reduced the number of cells that secreted these cytokines in response to the peptides. However, cells in the culture still made a robust response to stimulation with anti CD3 antibodies, suggesting that the drug specifically targeted antigen-specific cells.

Paclitaxel treatment selectively inhibited the proliferation of Gag-reactive cytolytic clonotypes

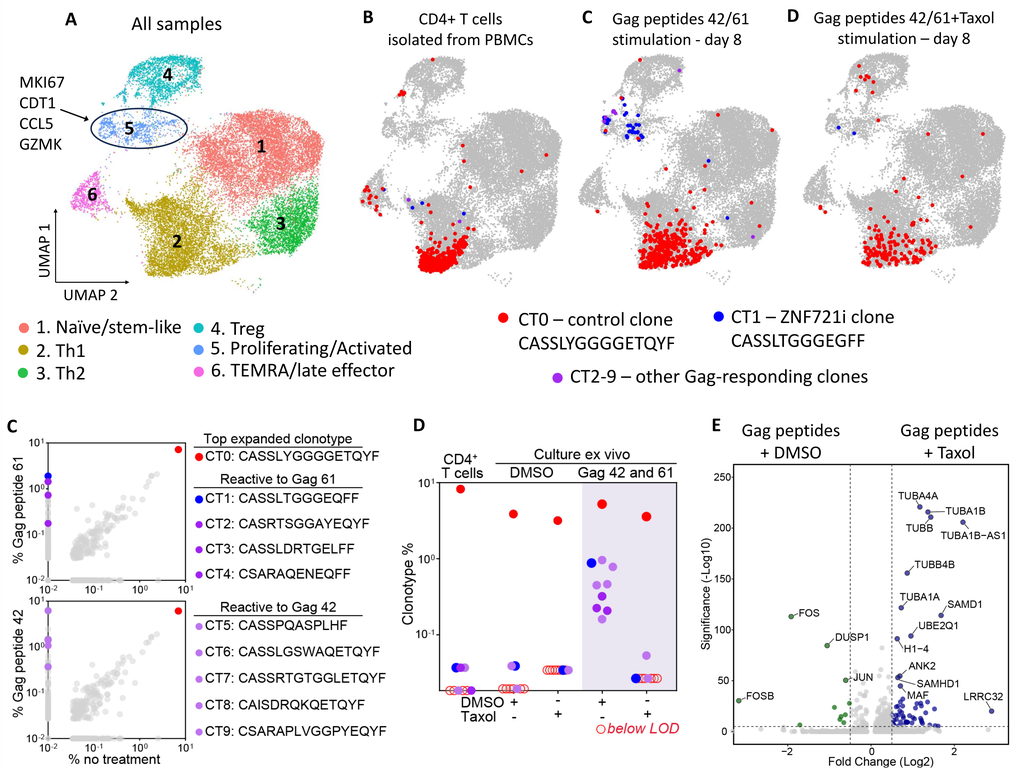


Figure 5. scRNA and TCR-seq shows peptide-induced expansion blocked by paclitaxel, arresting cells in G2 phase. To understand the mechanisms of paclitaxel in our culture system, we performed single-cell transcriptome and TCR sequencing of CD4+ T cells that were treated for 8 days with DMSO or stimulated with Gag peptides 42+61. At day 4, both conditions received either paclitaxel or no treatment. As a control, we analyzed CD4+ T cells directly isolated from PBMCs. **A)** UMAP of 6 clusters of CD4+ T cells defined by transcriptome. **B), C) and D)** UMAP showing a control clone (CT0, in red), the most abundant clonotype but not-affected by Gag peptides stimulation, the ZNF721i clone (CT1, in blue), and other gag peptides-reactive clones (in dark and light purple, corresponding to 42 and 61 peptide specificity, respectively), in untreated CD4+ T cell and stimulated cells either treated with paclitaxel or DMSO. **C)** Scatter plot bulk TCR DNA seq indicating the relative abundance of the clones of interest with no treatment or upon Gag peptides stimulation. **D)** Comparison of the relative abundance of the clones of interest, showing how paclitaxel treatment impacted the frequency of Gag peptide reactive clones. **E)** Volcano plot showing the differential gene expression of cells stimulated with Gag peptides, with or without paclitaxel. Cells treated with paclitaxel showed increase tubulin-associated genes expression, typical of cells in G2-phase arrest, and decreased expression of proliferation-associated genes.

CONCLUSIONS

Clonal expansion contributes significantly to the maintenance of HIV infected cells, and disrupting this process can affect the size of the reservoir. In this study, we showed that treatment with two antiproliferative drugs, paclitaxel and MMF, can specifically abrogate proliferation of activated cells. Indeed, the effect was observed only in cells specific to the cognate peptide, with no depletion of other expanded CD4+ T cell clones. The strategy of antigen-specific stimulation followed by treatment with an antiproliferative agent may lead to the selective elimination of clonally expanded HIV infected cells.

ACKNOWLEDGMENTS

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