# HLA-E disguises HIV-infected T cells

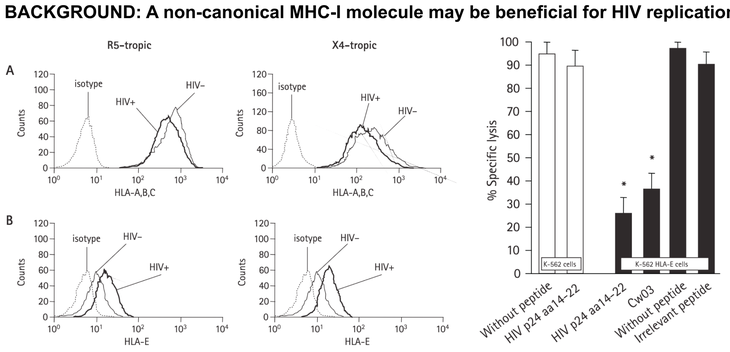
## Lessons in HIV control from an autologous T Cell:Natural Killer Cell differentiation and co-culture system

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### ABSTRACT:

It has often been difficult to understand the molecular interplay occurring in primary immune cells during HIV infection without requiring many rare clinical samples or using immortalized cell lines which can sometimes have questionable relevance. We have recently developed a model in true primary CD4+ T cells isolated from the blood of healthy donors, in which we are able to facilitate efficient knock-out (~99%) and knock-in (~80% of alleles) at a locus of interest, allowing specific genetic intervention to interrogate HIV replication in true immune cells. These CD4+ T cells then undergo a differentiation into Th1-like cells highly expressing one or both HIV-1 co-receptors, and which facilitates infection by replication competent CCR5-tropic and CXCR4-tropic virus. Co-culture experiments with autologous natural killer cells isolated from PBMCs of the same donor are able to inhibit replication in real time in this model, as shown by a 0.4 to 6-fold drop in co-culture supernatant relative to T cell alone supernatant. It is thought that NK-mediated cell killing of infected cells is inhibited through upregulation of HLA-E. Because clinical reports have demonstrated a drop in canonical type I HLA molecules, and an upregulation of the non-canonical HLA-E protein, and in vitro experiments in cell lines suggest HIV can hijack HLA-E to inhibit NK-cell mediated lysis by presenting a specific p24 peptide, we aimed to test the role of T cell HLA-E expression in HIV-1 infection in true T and NK cells. Using the editing, differentiation, and co-culture model described, we were able to efficiently knock out HLA-E in T cells, causing a virtual complete loss of surface HLA-E protein in T cells from 5 healthy donor individuals. These cells and matching control knock-out cells targeting the human beta globin locus were edited, differentiated, and infected, then challenged with replication competent NL4-3 and autologous NK cells were added at a 1:10 effector to target ratio. After 3 weeks of infection, the HLA-E knock-out cells from all 5 donors showed an increased level of control of virus replication in the presence of NK cells, suggesting that HIV does use HLA-E to inhibit NK-mediated killing of infected cells. While control knock-out cells demonstrated an average of 3-fold drop in infection in the presence of autologous NK cells, HLA-E knock-out cells had a 28-fold average drop in p24 concentration, with one donor showing as much as an 82-fold drop in infection. These results are consistent with both clinical and cell culture results, suggest that HIV-1 is able to evade NK-cell mediated lysis through upregulation of HLA-E, and highlight the utility of developing novel models of genetic intervention for study of HIV replication in primary cells.

### BACKGROUND: A non-canonical MHC-I molecule may be beneficial for HIV replication



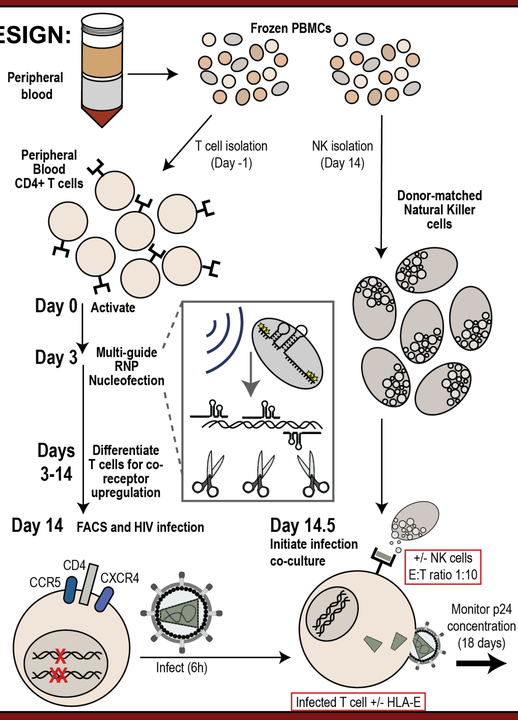

- HIV infected cells down-regulate canonical HLA proteins, and upregulate the non-canonical HLA-E

Nattermann et al. *Antiviral Therapy*, 2005

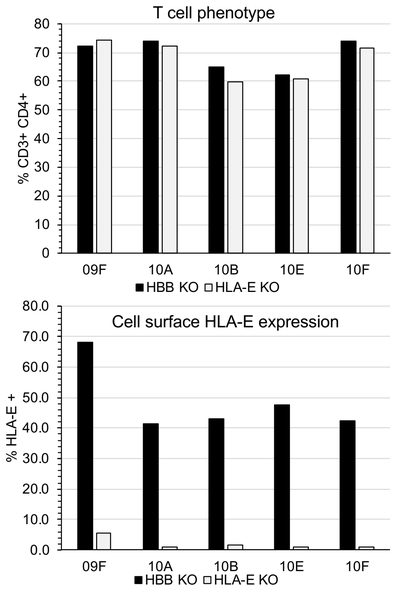
- A specific HIV capsid (p24) peptide on HLA-E can inhibit NK cell mediated lysis

### EXPERIMENTAL DESIGN:

- Isolation of PBMCs from healthy donors
- CD4+ T cell purification by negative selection
- Knock-out of control gene (*HBB*) or *HLA-E* in CD4+ T cells
- Differentiation and infection of edited T cells
- Isolation of donor-matched Natural Killer (NK) cells
- Addition of autologous NK cells to HIV infected T cells to quantify long term HIV control in the presence or absence of HLA-E

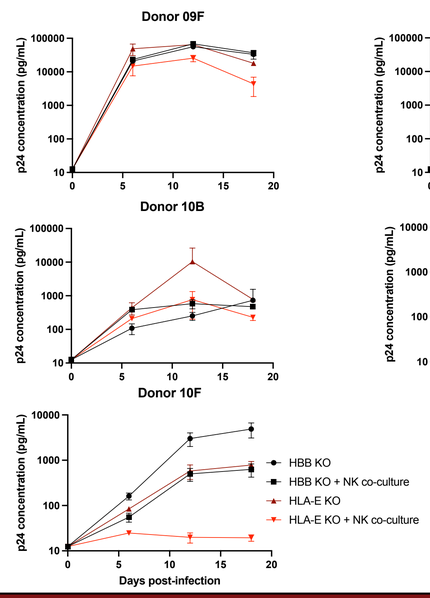



### RESULTS: Highly efficient *HLA-E* KO in primary CD4+ T Cells



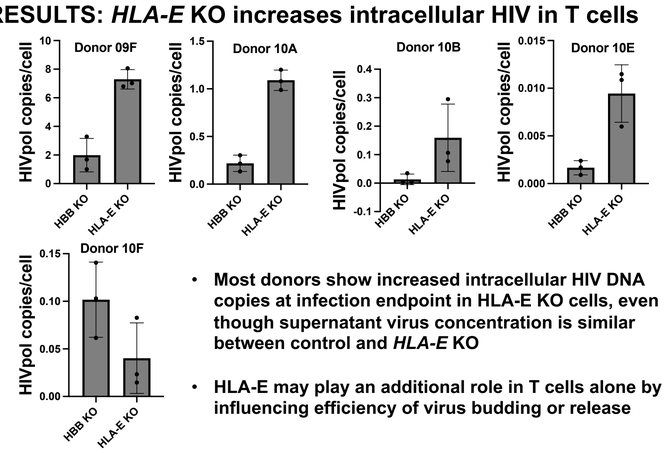

- *HLA-E* KO causes loss of HLA-E protein without disturbing T cell phenotype or HIV co-receptor expression levels

### RESULTS: *HLA-E* KO increases NK cell control of HIV replication



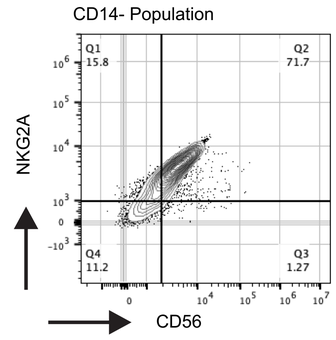

- NK cells from donors 10A, 10E, and 10F inhibit replication in the presence of HLA-E
- All donors show increased virus control in the presence of NK cells after *HLA-E* KO

### RESULTS: *HLA-E* KO increases intracellular HIV in T cells

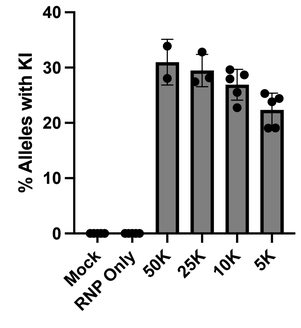



- Most donors show increased intracellular HIV DNA copies at infection endpoint in HLA-E KO cells, even though supernatant virus concentration is similar between control and *HLA-E* KO
- HLA-E may play an additional role in T cells alone by influencing efficiency of virus budding or release

### FUTURE DIRECTIONS: NK cell editing to study virus recognition




- Differentiation of HSPCs into NK-like cells



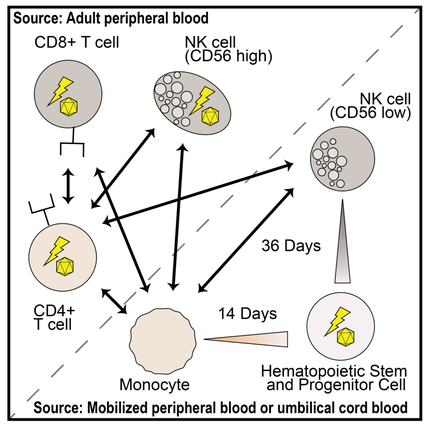

- Editing of terminally differentiated NK cells

### CONCLUSIONS:

- Development of a novel T cell-NK cell co-culture system for genetic intervention and study of virus control *ex-vivo*
- T cells maintain normal levels of phenotypic markers and co-receptor expression in the presence or absence of HLA-E
- HLA-E KO in CD4+ T cells may increase proviral load in susceptible cells
- HLA-E inhibits NK cell control of HIV infection
- This system now allows investigation of proposed mechanisms occurring in 'elite controllers', rare patients who are naturally able to control HIV infection without antiretroviral therapy

### NEW PRIMARY CELL MODEL SYSTEMS FOR HIV INFECTION:

- New gene editing and differentiation models allow interrogation of HIV replication mechanisms in primary cells
- Allow understanding of person-to-person variability in infection
- Bi-directional understanding of the infection interplay between different cell types




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