CD2/LFA-3 AXIS COSTIMULATION OF CD57+CD28- CD8 T CELLS IN HIV AND ATHEROSCLEROSIS

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Nicole E. Winchester[1], Soumya Panigrahi[2], Anokhi Haria[3], Michael M. Lederman[2], Michael L. Freeman[2]

[1]Cleveland Clinic Lerner College of Medicine at Case Western Reserve University, Cleveland, OH, USA, [2]Rustbelt Center for AIDS Research, Division of Infectious Diseases and HIV Medicine, Department of Medicine, Case Western Reserve University/University Hospitals Cleveland Medical Center, Cleveland, OH, USA

BACKGROUND

- People with HIV (PWH) have an increased risk of cardiovascular disease, even after controlling for known CVD risk factors.
- Latent CMV infection is associated with increased CVD risk for people with and without HIV (PWoH).
- T cells from CMV+ individuals are enriched with an inflammescent CX3CR1+CD57+CD28- phenotype, suggesting they may require a pathway separate from the canonical CD28 pathway for optimal co-stimulation.

METHODS

1. PBMCs were extracted from 46 PWH, 15 PWoH, and atherosclerotic plaques of 8 PWoH
2. Expression of CD2, CD28, CD57, and CX3CR1 was analyzed by flow cytometry and compared between CMV seropositive and seronegative donors
3. Cells were stimulated with anti-CD3 + anti-CD28 or plate-bound (pb)LFA-3 for at least 72 hours before measurement of viability (by LiveDead Aqua), cell cycling/proliferation (by CellTrace Violet dilution and Ki-67 expression), cytokine and lytic granule expression (IFNγ, IL-2, MIP-1β, and TNF), glucose uptake, mitochondrial biogenesis, and BCL-2 expression.

RESULTS

CMV is associated with elevated CD2 expression on inflammescent CD8 T cells

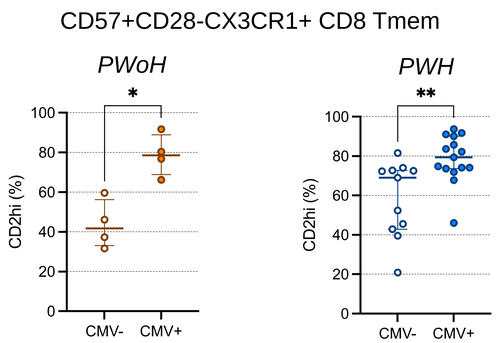


Figure 2. Percent of CD57+CD28-CX3CR1+ CD8+ Tmem with high CD2 expression was measured for PWoH (left) and PWH on long-term ART (right) and compared by CMV serostatus. *P<0.05, **P<0.01, Mann-Whitney U test.

CMV infection enhances **CD2** expression on inflammescent **CD57+CD28-CD8+ T** cells. These inflammescent cells are potently activated by LFA-3, which is highly expressed in inflamed **vasculature** of PWH or atherosclerosis

LFA-3 is a more potent co-stimulator of inflammatory cytokine and lytic granule synthesis than CD28 in PBMCs from CMV+PWH

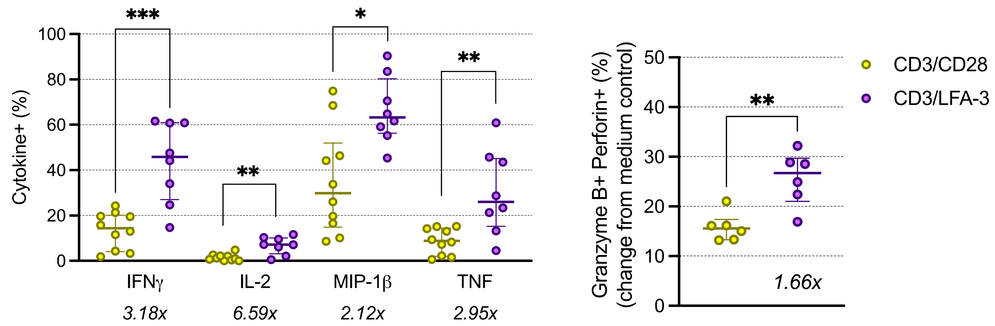


Figure 3. (Left) Percent of CD57+ CD8 Tmem expressing IFNγ, IL-2, MIP-1β, or TNF following indicated stimulations. *P<0.05 **P<0.01 ***P<0.001, Mann-Whitney. (Right) Percent of CD57+ CD8 Tmem expressing granzyme B and perforin above medium control background after 2d of stimulation. Significance determined by Mann-Whitney U test.

LFA-3 protein expression is elevated in human atherosclerotic tissues and in aortas of SIV/SHIV-infected rhesus macaques

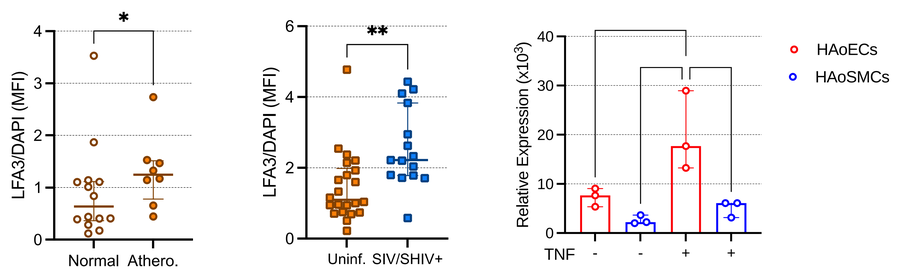
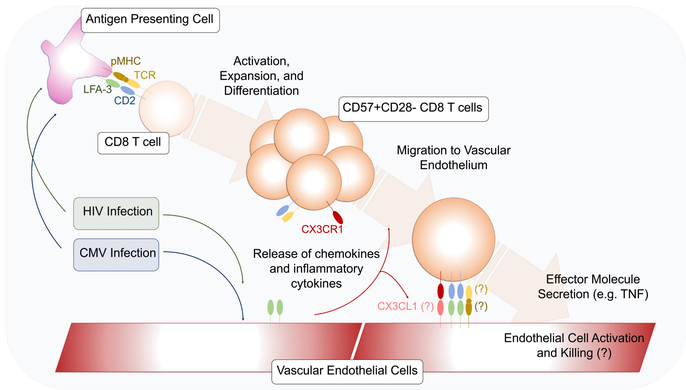


Figure 4. (Left) Quantity of LFA-3 expressed in the vasculature of PWoH with and without atherosclerosis, as measured by ratio of LFA-3 to DAPI MFI. (Middle) Quantity of LFA-3 expressed in the aortas of rhesus macaques by SIV/SHIV infection status, as measured by ratio of LFA-3 to DAPI MFI. *P<0.05, **P<0.01, Mann-Whitney. (Right) *LFA3* gene expression by human aortic endothelial (HAoECs) and smooth muscle (HAoSMCs) cells after stimulation with or without TNF for 7d. **P<0.01, significance determined by Kruskal-Wallis test..

CONCLUSIONS

- CMV infection is associated with high CD2 expression on CD57+CD28- CD8 memory T cells, regardless of HIV status.
- LFA-3 co-stimulation of inflammescent CD8 T memory cells potently enhanced cytokine and lytic granule production, more than CD28 co-stimulation.
- Proliferation, glucose uptake, mitochondrial biomass, and BCL-2 expression were similarly upregulated by LFA-3 and CD28 co-stimulation (not shown).
- LFA-3 protein is highly expressed in aortas of SIV/SHIV+ macaques and atherosclerotic plaques of PWoH. LFA3 gene expression is upregulated in aortic endothelial cells by TNF.

Proposed model of CMV driven endothelial damage by inflammescent CD8 T cells



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Cleveland Clinic Lerner College of Medicine

Case Western Reserve University EST. 1826