# Cholesterol Synthesis Pathways in Monocyte-Derived Macrophages Link CMV and HIV to Atherosclerosis

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## Background

- Cytomegalovirus (CMV) is a widespread herpesvirus that establishes latent infection in hematopoietic progenitor cells
- Latent CMV infection is associated with increased inflammation and inflammation-related disorders, particularly cardiovascular disease (CVD)
- CMV co-infection is nearly universal among people with HIV (PWH), who exhibit a two-fold increase in risk for CVD compared to people without HIV (PWoH).
- CMV can reactivate as latently infected cells differentiate into macrophages. Reactivation is usually asymptomatic in immunocompetent hosts but may contribute to CMV-associated inflammation and CVD.

## Methods

- Monocyte-derived macrophages (MDMs) from PBMCs that were differentiated using autologous serum, a model that preserves the influence of systemic biomarkers found *in vivo,* and collected for bulk RNA sequencing[1].
- Pathway and gene set enrichment analyses were used to compare the MDM transcriptomes with previously published datasets from CVD or *in vitro* CMV infection.

## References

[1]Bowman, E. R, et al. (2020). PLoS Pathog., 16: e1008869.

[2]Schwartz, M., et al. (2023). Nat. Microbiol., 8: 455–468.

[3]Folkersen, L., et al. (2012). Mol. Med., 18: 669–675.

[4]Lee, K., et al. (2013). Atherosclerosis, 226: 74–81.

## Results

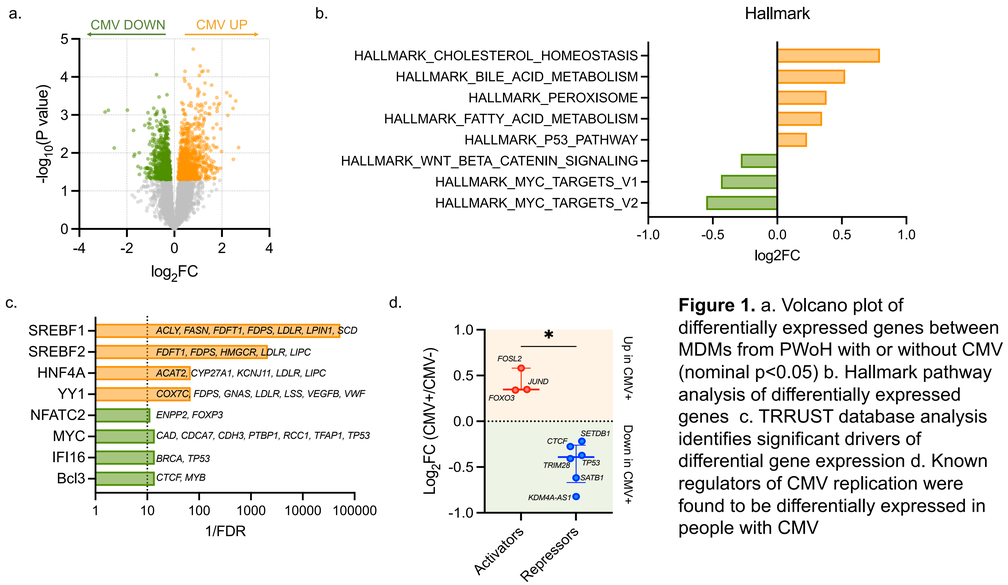



**Figure 1.** a. Volcano plot of differentially expressed genes between MDMs from PWoH with or without CMV (nominal p<0.05) b. Hallmark pathway analysis of differentially expressed genes c. TRRUST database analysis identifies significant drivers of differential gene expression d. Known regulators of CMV replication were found to be differentially expressed in people with CMV

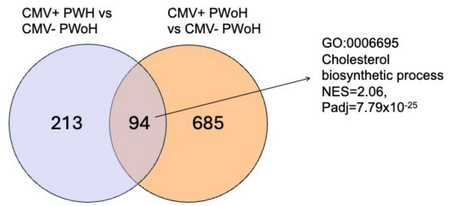



**Figure 2.** Venn diagram of upregulated genes in MDMs from CMV+ PWH and CMV+ PWoH shows an enrichment for the cholesterol biosynthesis pathway within the overlap

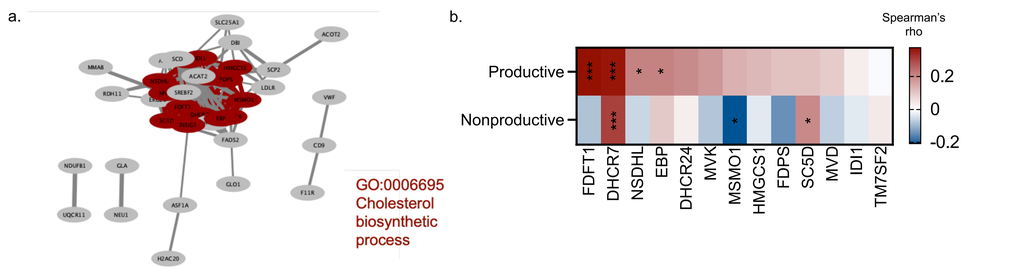



**Figure 3.** a. STRING network analysis demonstrates involvement of cholesterol biosynthetic process in upregulated genes in MDMS and *in vitro* CMV infection[2] (GSE165291) b. Correlation between SV40-GFP (proxy of infection efficiency) and cholesterol-linked genes upregulated during CMV infection

## Acknowledgements

This research is funded by NIH grants AI162150 and HL158592, with additional support from the Richard J. Fasenmyer Foundation.

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## Results cont.

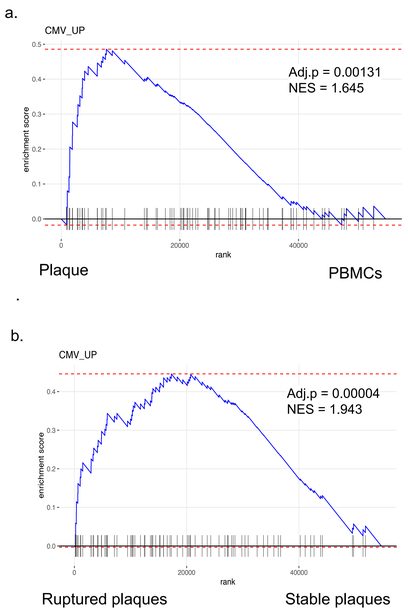



**Figure 4.** a. GSEA of differentially expressed genes between atherosclerotic plaques and PBMCs (GSE21545) shows significant enrichment of top 100 most significantly upregulated genes in MDMs from people with CMV[3] b. GSEA of differentially expressed genes between macrophages derived from ruptured atherosclerotic plaques and stable atherosclerotic plaques[4] (GSE41571)

## Conclusions

- MDMs from people with CMV are enriched in cholesterol synthesis, which is shared between PWH and PWoH with CMV.
- The genes for SREBP1 and SREBP2, known regulators of lipid metabolism, were identified as significant drivers of expression of upregulated genes, including *FDFT1*, *FDPS*, and the statin target *HMGCR*.
- *FDFT1* expression was associated with viral load in *in vitro* CMV infection, suggesting that MDMs from people with CMV are transcriptionally permissive for CMV replication.
- Gene set enrichment of the upregulated genes in atherosclerotic tissue is consistent with a proatherogenic role for MDMs in people with CMV.