Pathology Advanced Translational Research Unit
Department of Pathology and Laboratory Medicine
Emory University School of Medicine
Emory Vaccine Center — Where Science Meets Hope.

The complexity of Bile Acids underlies their protective innate response and interferon signaling During COVID-19 Disease

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BACKGROUND

- How do changes in BA levels affect the antiviral immune response in SARS-CoV-2-infected individuals?

Bile acids (BAs), synthesized by hepatocytes and modified by commensal bacteria, are critical regulators of immune cell function, particularly within innate immunity. Among their roles, BAs mediate antiviral signaling via TGR5, a receptor expressed on immune cells that modulates transcriptional responses to viral infections. While BAs are known to influence immune responses to various viral infections, the specific impact of BA levels on immune cells remains underexplored, particularly in the context of SARS-CoV-2 infection.

METHODS

We utilized single-cell RNA/DNA analysis from the Immunophenotyping Assessment in a COVID-19 Cohort (IMPACC), a prospective longitudinal study with detailed clinical, virological, and immunological data, to investigate the impact of bile acids (BAs) on innate antiviral immunity. Statistical analyses were conducted using R Studio v4.4.0, with Mast test P-values applied for module score calculations and pathway analysis.

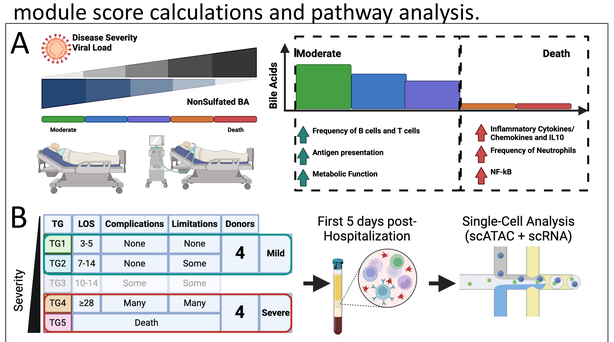


Figure 1. (A) Overall Working Model of Bile Acids in SARS-CoV-2 Infection. (B) Experimental design of Multiome analysis, categorizing four donors as mild (trajectories 1–2) and four as severe (trajectories 4–5).

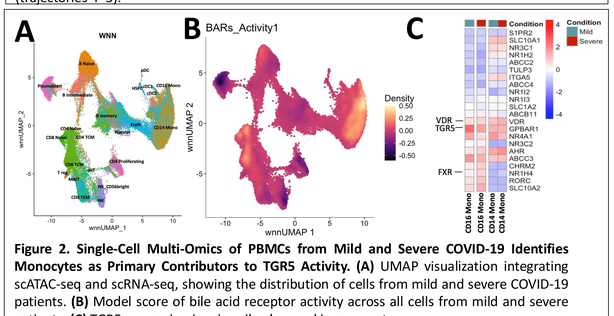
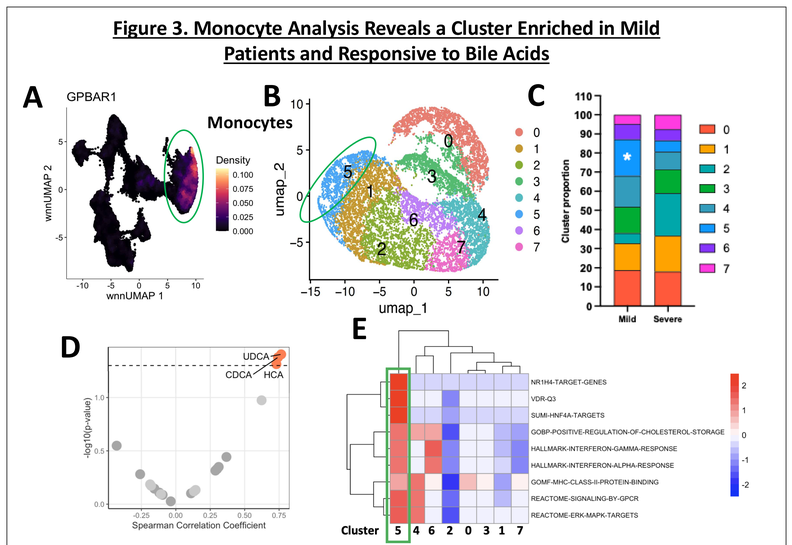


Figure 2. Single-Cell Multi-Omics of PBMCs from Mild and Severe COVID-19 Identifies Monocytes as Primary Contributors to TGR5 Activity. (A) UMAP visualization integrating scATAC-seq and scRNA-seq, showing the distribution of cells from mild and severe COVID-19 patients. **(B)** Model score of bile acid receptor activity across all cells from mild and severe patients. **(C)** TGR5 expression is primarily observed in monocytes.

RESULTS

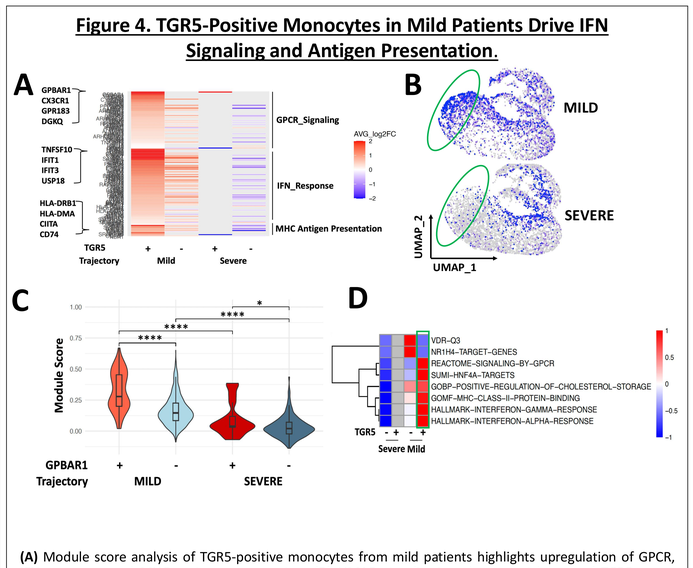
Bile acid (BA) signaling through TGR5-positive monocytes involves VDR and FXR, triggering antigen presentation while synergizing with IFN-γ. Moreover, BA can modulate antiviral T cell activation via VDR and FXR. These findings highlight the crucial role of bile acids in regulating both adaptive and innate antiviral immune responses, underscoring their potential as mechanistic biomarkers for SARS-CoV-2 clearance

Figure 3. Monocyte Analysis Reveals a Cluster Enriched in Mild Patients and Responsive to Bile Acids



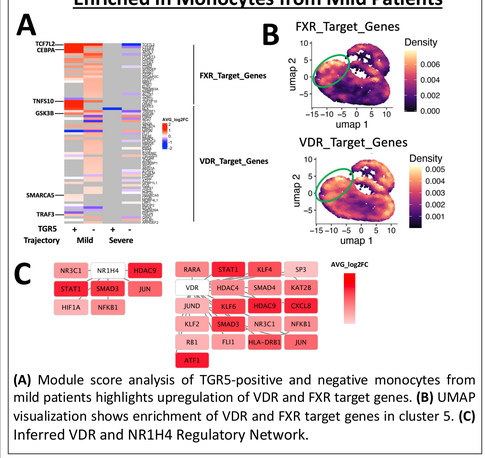
(A) TGR5 (GPBAR1) expression is primarily observed in monocytes. **(B)** UMAP visualization of scRNA-seq monocyte data identifies eight distinct clusters (0–7) annotated using *Seurat*. **(C)** Cluster proportion analysis reveals enrichment of cluster 5 in mild patients. **(D)** Positive correlation between cluster 5 and plasma bile acid levels, including UDCA, CDCA, and HCA. **(E)** Gene set enrichment analysis (GSEA) shows upregulation in bile acid receptor pathways, cholesterol storage, IFN-γ/β signaling, GPCR signaling, and ERK pathways.

Figure 4. TGR5-Positive Monocytes in Mild Patients Drive IFN Signaling and Antigen Presentation.



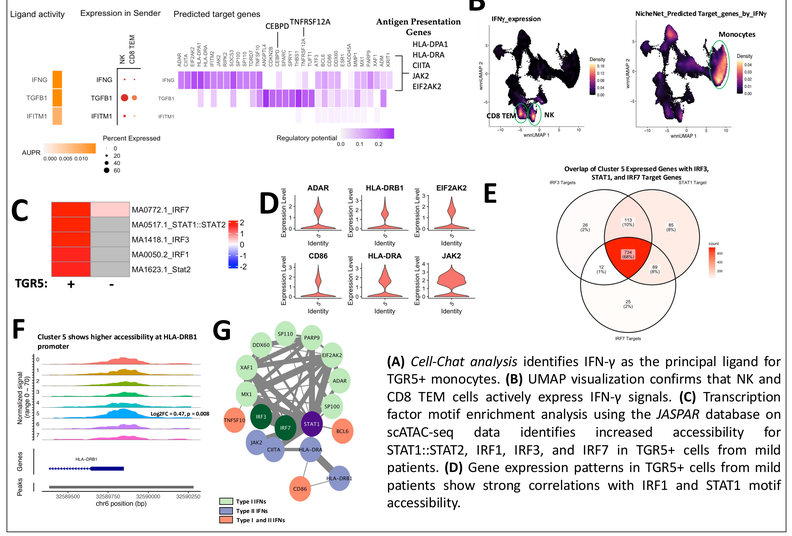
(A) Module score analysis of TGR5-positive monocytes from mild patients highlights upregulation of GPCR, IFN response, and MHC antigen presentation. **(B)** UMAP visualization shows enrichment of TGR5 target module scores in mild patients. **(C)** Statistically significant enrichment of TGR5 target molecules. **(D)** GSEA shows enrichment of cholesterol storage, IFN-γ/β, GPCR signaling, and ERK pathways in cluster 5 TGR5-positive cells, while VDR and FXR (NR1H4) target genes are enriched in TGR5-negative cells.

Figure 5. FXR and VDR Target Genes Are Enriched in Monocytes from Mild Patients



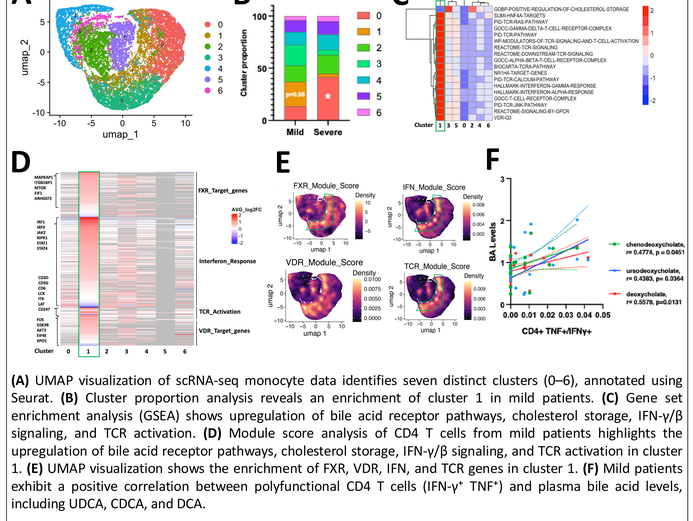
(A) Module score analysis of TGR5-positive and negative monocytes from mild patients highlights upregulation of VDR and FXR target genes. **(B)** UMAP visualization shows enrichment of VDR and FXR target genes in cluster 5. **(C)** Inferred VDR and NR1H4 Regulatory Network.

Figure 6. IFN-γ Synergizes with Bile Acids to Drive Antigen Presentation in TGR5+ Monocytes.



(A) *Cell-Chat analysis* identifies IFN-γ as the principal ligand for TGR5+ monocytes. **(B)** UMAP visualization confirms that NK and CD8 TEM cells actively express IFN-γ signals. **(C)** Transcription factor motif enrichment analysis using the *JASPAR* database on scATAC-seq data identifies increased accessibility for STAT1::STAT2, IRF1, IRF3, and IRF7 in TGR5+ cells from mild patients. **(D)** Gene expression patterns in TGR5+ cells from mild patients show strong correlations with IRF1 and STAT1 motif accessibility.

Figure 7. CD4 T cells in Mild Patients Drive IFN Signaling, TCR signaling, VDR/FXR target genes



(A) UMAP visualization of scRNA-seq monocyte data identifies seven distinct clusters (0–6), annotated using Seurat. **(B)** Cluster proportion analysis reveals an enrichment of cluster 1 in mild patients. **(C)** Gene set enrichment analysis (GSEA) shows upregulation of bile acid receptor pathways, cholesterol storage, IFN-γ/β signaling, and TCR activation. **(D)** Module score analysis of CD4 T cells from mild patients highlights the upregulation of bile acid receptor pathways, cholesterol storage, IFN-γ/β signaling, and TCR activation in cluster 1. **(E)** UMAP visualization shows the enrichment of FXR, VDR, IFN, and TCR genes in cluster 1. **(F)** Mild patients exhibit a positive correlation between polyfunctional CD4 T cells (IFN-γ⁺ TNF⁺) and plasma bile acid levels, including UDCA, CDCA, and DCA.

Working Model

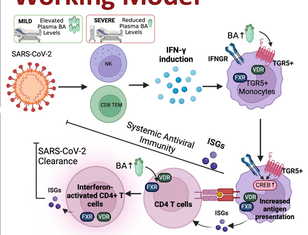


Figure 8. Single-cell analysis reveals heightened TGR5 expression in monocytes from mild COVID-19 patients, which is associated with enhanced IFN signaling, ERK1/2 activation, VDR/FXR target gene expression, and antigen presentation signaling compared to severe cases.

Mechanistically, BA signaling primarily occurs through TGR5-positive monocytes, involving VDR and FXR, and triggers antigen presentation while synergizing with IFN-γ, which also trigger ERK/CREB. This contributes to mild disease by limiting viral replication and suppressing inflammatory responses.

CONCLUSIONS

Our findings highlight the crucial role of bile acids in modulating both adaptive and innate antiviral immune responses, underscoring their potential as mechanistic biomarkers for SARS-CoV-2 clearance. This study provides novel insights into the complex interplay of BA signaling in immune regulation.

FUNDING SOURCES

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