Bile Acids in the Modulation of Antiviral Immune Response

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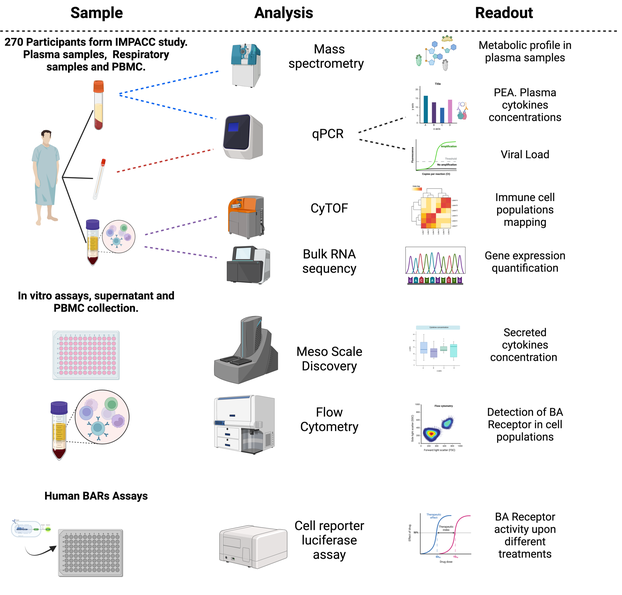
BACKGROUND

Bile acids (BAs) are cholesterol-derived metabolites been their primary role fatty solubilization, this metabolites are produce in the liver as primary BAs and they are secreted into the gut, where the microbiome subjects them to various chemical modifications, leading to the production of secondary BAs. One of these modifications is sulfation,that solubilize BAs for subsequent excretion via urine or feces.

BAs play a crucial role in host metabolism and immune regulation, they can trigger antiviral immune response by Type IFN.

In this study, we found that elevated levels of non-sulfated BAs were associated with mild COVID-19, whereas higher sulfated BA levels correlated with increased viral load, a pro-inflammatory environment, severe disease, and mortality. Functional assays revealed that non-sulfated BAs induced significant production of antiviral and immunoregulatory cytokines (e.g., IFN-α2a, TGF-β, IL-10, and TNF-α), while sulfated BAs failed to activate key BA receptors, including surface receptor TGR5 and nuclear receptors VDR and FXR,

These findings suggest that BA sulfation may play a critical role in shaping immune responses during SARS-CoV-2 infection and may influence COVID-19 disease severity.



RESULTS

Figure 1. Sulfated BAs in plasma during acute infection are associated with more severe COVID-19 disease.

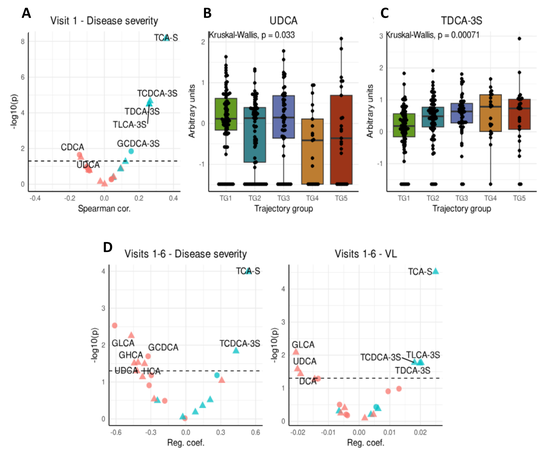


Figure 1. (A) Spearman correlations of BA species at the time of hospitalization reveal a dichotomy of BAs with sulfated being associated with severe disease (red) and non-sulfated with milder disease (cyan). **(B)** Correlation of non-sulfated (UDCA) and sulfated BA (TDCA-3S) with viral load a cross 5 trajectory groups. Comparison of means between groups in b and c was performed with Kruskal Wallis. **(C, D)** Spearman correlations of BA species across hospitalization and disease severity and viral load reveal a dichotomy of BAs with sulfated being associated with severe disease (red) and non-sulfated with milder disease (cyan).

Figure 2. Sulfated bile acids positively correlate with severe COVID-19 immune signatures as inflammation, higher neutrophil freq. and lower antigen presentation pathways.

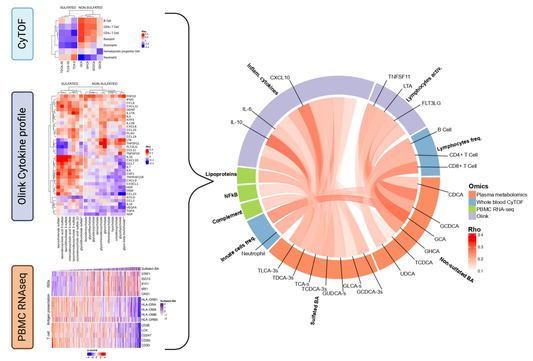


Figure 2. Integrated correlation network analysis of transcriptomics and metabolomics, where nodes denote features and edges the correlation coefficient between sulfated and non-sulfated BAs. Rho was used as an integration metric across OMICs.

Figure 3. Cell-based Luciferase Reporter Assay. **(A, B)** Activation of the surface receptor TGR5 after 24 hours of incubation with various bile acids (BAs) and their sulfated counterparts. LCA was used as a positive control agonist. The data are presented separately as they originate from two independent experiments. **(C)** Competition assay between sulfated and non-sulfated BAs. In this assay, cells were pre-incubated for 3 hours with sulfated BAs, followed by 24-hour co-incubation with their non-sulfated counterparts. **(D)** Activation of the nuclear receptor FXR after 24 hours of treatment with different bile acids. On the left, GW4064 (purple) was used as a positive control agonist. **(E)** Activation of the nuclear receptor VDR after 24 hours of treatment with different bile acids. On the left, calcitriol (purple) was used as a positive control agonist. EC50 values were calculated using Non-linear Regression [Agonist] vs Response – Variable Slope (4 parameters).

Figure 4. Non-sulfated-BA trigger type I IFN production in TGR5 positive Monocytes.

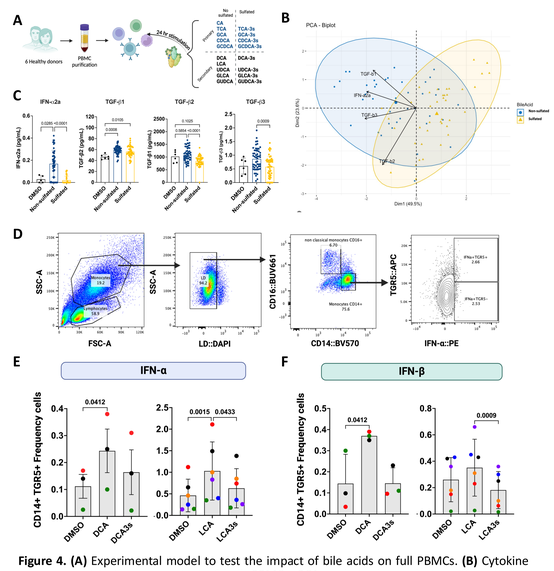
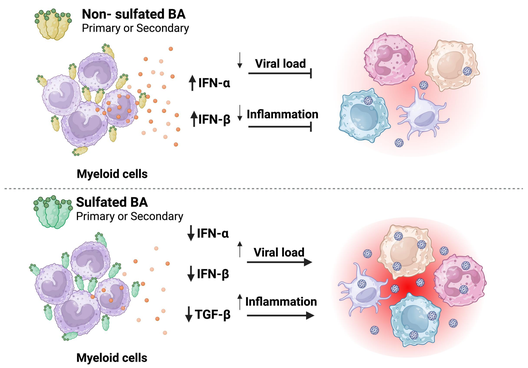


Figure 4. (A) Experimental model to test the impact of bile acids on full PBMCs. **(B)** Cytokine measurement in the supernatant of bulk PBMCs. PCA analysis shows cluster differentiation between sulfated and non-sulfated BAs in IFN-α2a and TGF-β production. **(C)** Cytokine quantification in the culture supernatant reveals that non-sulfated BAs induce IFN-α and TGF-β production in PBMCs. **(D)** Gating strategy for intracellular cytokine detection of Type I IFN by flow cytometry. **(E, F)** Scatter dot plots showing the frequency of TGR5-positive monocytes producing Type I IFN-α (E) and IFN-β (F) after treatment with the TGR5 agonists DCA and LCA, along with their respective sulfated counterparts, for 24 hours. For statistical analysis, the Kruskal-Wallis test without correction for multiple comparisons was performed.

CONCLUSIONS



1. Sulfated BAs positively correlate with several severe signatures of COVID-19.
2. BAs receptor activation assays showed that sulfated-BAs do not activate surface and nuclear receptors, making them unable to trigger the antiviral and anti-inflammatory activity, a feature of their non-sulfated form.
3. The detection of BA receptors by cytometry allows the identification of specific cell subsets that respond to BA stimulation. Type 1 interferon production occurs only when TGR5 positive populations are treated with non-sulfated BAs.
4. Our data shows that the levels of BAs and their conjugates shape host environment and the immune response that leads to protection from severe disease.

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NIH National Heart, Lung, and Blood Institute
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