Clinical & Experimental Allergy

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RESEARCH LETTER OPEN ACCESS

Respiratory Viral Infections and the Tonsillar Transcriptome: An Exploratory Study

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Received: 13 November 2025 | **Revised:** 2 April 2026 | **Accepted:** 7 April 2026

To the Editor,

Palatine tonsils are secondary lymphoid organs and part of the mucosa-associated lymphoid tissue of the oropharynx. They are rich in recirculating lymphocytes and serve as sites for initiating innate immune response, followed by adaptive immunity mediated by B and T lymphocytes [1]. The immune response to respiratory viral infections is characterised by elevated secretion of proinflammatory cytokines, as well as chemokines and may be accompanied by impaired production of interferons (IFNs) [2]. Our aim was to analyse the relationship between respiratory viral infections and tonsillar immune responses. We hypothesised that common nasopharyngeal respiratory viruses account substantially for tonsillar gene expression previously associated with asthma.

Human tonsil samples were obtained from two cohorts (2008–2015) at Satakunta Central Hospital (Pori), Turku University Hospital (Turku) and Salo Regional Hospital (Salo), Finland. The study was approved by the relevant ethics Committees. Written informed consent was obtained from the subject or his/her parent. Tonsillectomy was performed by routine clinical practise, and tonsil samples were stored in RNA*later*. Blood was drawn for allergy tests, and nasopharyngeal aspirates were obtained for comprehensive respiratory virus testing. RNA isolation and intra-tonsillar microbial profiling were performed using RNA-sequencing data.

Samples positive for any nasopharyngeal virus (adenovirus, bocavirus-1, coronaviruses, enteroviruses, influenza A and B viruses, metapneumovirus, parainfluenza virus types 1–3, rhinovirus, or RSV groups A and B) were considered virus-positive. Samples that did not have any positive virus detection were included as virus-negative. Statistical analysis was performed in R (R version 4.3.2).

A total of 63 subjects were analysed. The median age was 10 years (range 2–31), 52% were male, and 45% exhibited allergic sensitisation. Seasonal variation and age showed no significant effect on the study parameters. Differences between groups were found in the indication for adeno−/tonsillectomy and the presence of respiratory symptoms within the preceding 2 weeks (both $p < 0.05$). However, these did not influence the results.

RNA-sequencing yielded 17,110 transcripts for analysis following data processing, normalisation and quality control. Differential expression analysis identified 514 genes (116 up-regulated and 398 down-regulated), of which 11 were associated with the immune system, including *CYLD* and *SOCS5* positively, *PSMA7*,

Summary

- We identified tonsillar transcripts and pathways associated with nasopharyngeal respiratory viral infections.
- Tonsillar transcriptomics revealed cross talk with viruses through Th1 cytokines, especially IFN-γ.

AMFR, *IMPDH2*, *HMGB1* and *PPIA* negatively associated with virus detection (FDR <0.05). Cytokine receptor-related genes, including *CXCL5* (*ENA-78*) and *RORA*, were up-regulated, while *CD40* and *S1PR2* were down-regulated.

Weighted gene co-expression network analysis identified three modules. Module eigengenes (hypothetical central genes) were calculated for each module. Overall, 1681 genes were identified in the cytokine-mediated-signalling module, of which 27 overlapped with differentially expressed genes (DEGs). Within this module, 10 cytokine-related genes (*CTF1*, *GPI*, *NPPC*, *GMFB*, *CXCL13*, *TNFSF11*, *GDF7*, *TNFSF4*, *IL7* and *NRG2*) were enriched in cytokine-mediated signalling and cytokine-cytokine receptor interaction pathways. The module was further enriched for Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to T helper (Th) 1-type immune response. These included cytokine-cytokine receptor interaction, cytokine-mediated signalling pathway, cytokine receptor, cytokine signalling in the immune system, signalling by interleukins, IFN-γ production, IL7 mediated signalling pathway and JAK–STAT signalling pathway (Figure 1A,B). We identified 883 genes in the innate immune activation module, of which 62 overlapped with DEGs, enriched in infectious disease, oxidative phosphorylation, IL-1 signalling, respiratory electron transport and noncanonical NF-κB pathways. The immune activation and proteostasis module contained 1296 genes, including 31 DEGs, enriched in mitochondrial function, metabolism, translation and protease-related pathways.

Taxonomic profiling of the tonsillar microbiota identified 45 phyla, with *Proteobacteria, Firmicutes, Fusobacteriota* and *Ascomycota* being the most predominant. Analysis of dominant families showed that *Streptococcaceae, Prevotellaceae* and *Fusobacteriaceae* were the most abundant. At the genus level, *Streptococcus* and *Fusobacterium* were the most prevalent taxa. Among the differentially abundant taxa, *Staphylococcus aureus* and the genus *Streptococcus* exhibited higher relative abundance in the virus-negative group (FDR <0.1). Comparison of microbial community composition revealed significant differences between virus-positive and virus-negative groups ($p = 0.04$;

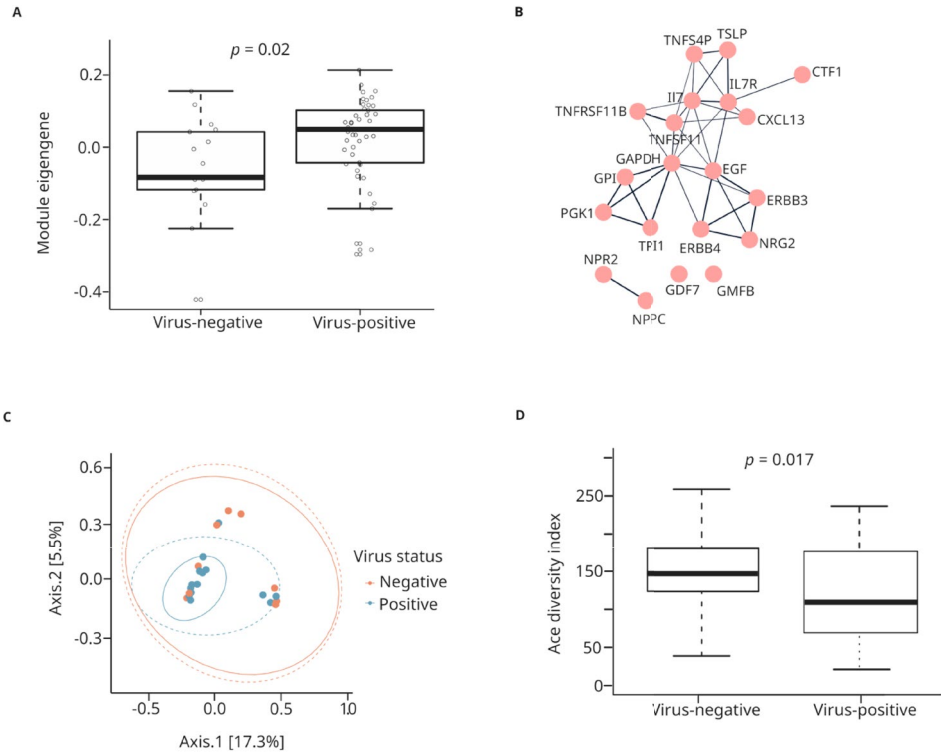

FIGURE 1 | (A) Module eigengene of the cytokine-mediated signalling module from weighted gene co-expression network analysis (WGCNA) in virus-positive and virus-negative samples. The cytokine-mediated-signalling module showed a positive correlation with the virus-positive group. (B) Cytokines and chemokines identified in this module were clustered according to the strength of their pairwise correlations (FDR <0.05), exhibiting strong co-expression within the module. (C) Principal coordinate analysis (PCoA) of tonsillar microbiota based on Canberra distance of transformed data demonstrates differences in beta diversity (between sample diversity) between virus-positive and virus-negative groups ($p = 0.04$). Each point represents an individual sample; colours indicate group status (virus-negative, red, $n = 11$; virus-positive, blue, $n = 21$). Partial overlap between groups is observed. (D) Alpha diversity (within-sample diversity) of the microbiota in virus-negative and virus-positive samples. Virus-negative samples showed higher alpha diversity compared to virus-positive group ($p = 0.02$).

Figure 1C). Alpha diversity, assessed using the ACE index, was higher in virus-negative participants compared with those with viral infection ($p = 0.02$; Figure 1D).

Of the identified genes associated with the antiviral immune response, *HMGB1* has been associated with antiviral immune activation and increased cytokine and chemokine production [3]. *CYLD*, a negative regulator of the RIG-I–mediated antiviral response, was up-regulated in our data, suggesting a mechanism to prevent excessive activation of antiviral signalling and inflammation [4]. Increased *CXCL5* expression is consistent with its known function in mediating neutrophil recruitment during early antiviral responses and additionally influencing B-cell localisation by regulating *CXCL13* expression [5]. In contrast, *PSMA7*, which suppresses innate immunity via the RIG-I–MAVS pathway, was down-regulated in virus-positive samples, potentially enhancing IFN responses and facilitating viral clearance [6].

Our finding that the virus-negative state is associated with increased microbiome diversity is supported by others and may reflect more robust immune functions in individuals with a more diverse microbiome [7]. The greater abundance of *streptococcus* and *S. aureus* could relate to actual disease entity in virus-negative tonsillectomy patients.

The present results suggest that respiratory virus detection was independently and positively associated with type 1 immune responses. Th1 cells play a critical role during acute respiratory infections by producing numerous cytokines such as IFN-γ. IFN-γ has antiviral effects through the induction of IFN-stimulated genes and inflammatory mediators that restrict viral replication [8]. Our study demonstrates that nasopharyngeal viral infection may be closely associated with alteration in cytokine responses and IFN-related gene expression within the tonsils. Moreover, these associations may provide prognostic value for both short- and long-term clinical outcomes [9].

Author Contributions

Conception and study design: Tuomas Jartti, Cezmi A. Akdis. Collection of data: Lotta E. Ivaska, Emilia Mikola, Antti Silvoniemi, Tuomo Puhakka. Data management and analysis: Tanzeela Hanif, Lotta E. Ivaska, Oscar Palomares, Sanna Toppila-Salmi, Mubeccel Akdis, Cezmi A. Akdis, Tuomas Jartti. Lead study coordinator: Tuomas Jartti. Drafting and writing of the manuscript: Tanzeela Hanif, Lotta E. Ivaska, Antti Silvoniemi, Tuomas Jartti. Critical revision of the manuscript: Tanzeela Hanif, Lotta E. Ivaska, Antti Silvoniemi, Emilia Mikola, Tuomo Puhakka, Oscar Palomares, Sanna Toppila-Salmi, Mubeccel Akdis, Cezmi A. Akdis, Tuomas Jartti.

Acknowledgements

Open access publishing facilitated by Turun yliopisto, as part of the Wiley - FinELib agreement.

Funding

This work was supported by Sigrid Juselius Foundation, Helsinki, Tampere Tuberculosis Foundation, Tampere, Instrumentarium Foundation, Helsinki, Ella and George Ehrnrooth Foundation, Helsinki, Finnish and Norwegian Medical Foundation, Helsinki, Maud Kuistila Foundation, Helsinki, Ida Montin Foundation, Kerava, TYKS Foundation, Turku. L.I. was supported by the Finnish ORL-HNS Foundation, Tampere and the Sakari Alhopuro Foundation, Turku. All in Finland.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Supporting text and figures of this study are available in an external repository (Open Science Framework: https://osf.io/fupkd/files/osfstorage).

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