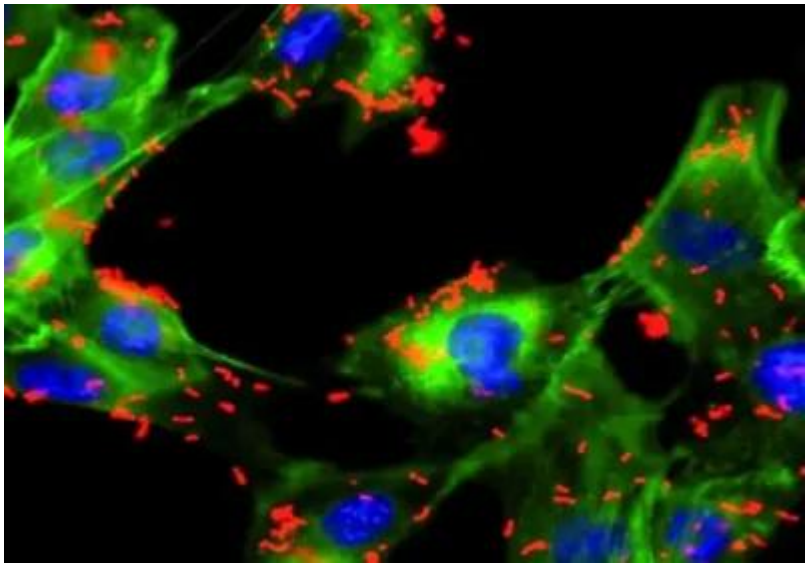
Understanding the functional characteristics of intracellular pathogens at the cellular level is important to address the obstacles associated with their effective treatment. In this article, the authors from India and Rwanda used single-cell RNA sequencing to investigate the diversity of intracellular microbes in peripheral blood mononuclear cells (PBMCs) of healthy individuals, in individuals positive for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and individuals who recovered from SARS-CoV-2 infection. They also examined possible relationships between microbial presence and host cellular responses under different pathophysiological conditions.

Possible interactions between intracellular pathogens, such as viruses, bacteria, fungi, and protozoan parasites when they concurrently infect the same host cells are significant for their interaction with the host's immune system and their ability to evade immune defenses. When T-cells express multiple inhibitory receptors, and cells infected with the first pathogen express multiple inhibitory ligands for those inhibitory receptors, a novel infection with the second pathogen, especially with a virulent pathogen that creates large antigen titer, can lead to an accelerated exhaustion of T-cells.
https://discovermednews.com/the-presence-of-concurrent-intracellular-pathogens-can-lead-to-t-cell-exhaustion-and-potentially-severe-consequences/



Many intracellular pathogens find shelter from humoral defenses commonly residing within highly efficient antimicrobial defense cells, such as macrophages and dendritic cells. Bacterial species *Mycobacterium Tuberculosis, Staphylococcus aureus,* and *Salmonella* can

reside within macrophages and perforate the phagosome of macrophages, hijacking the phagosomal processes. Neutrophils, fibroblasts, or epithelial cells can also serve as habitats for intracellular pathogens. The intracellular microbes may also inhabit specific host cell compartments, such as the endosome or cytosol, where they evade direct antibody attacks (Hiyoshi H, et al. Cell Host & Microbe 2022; 30, 163–170)
https://doi.org/10.1016/j.chom.2021.12.001

About the study

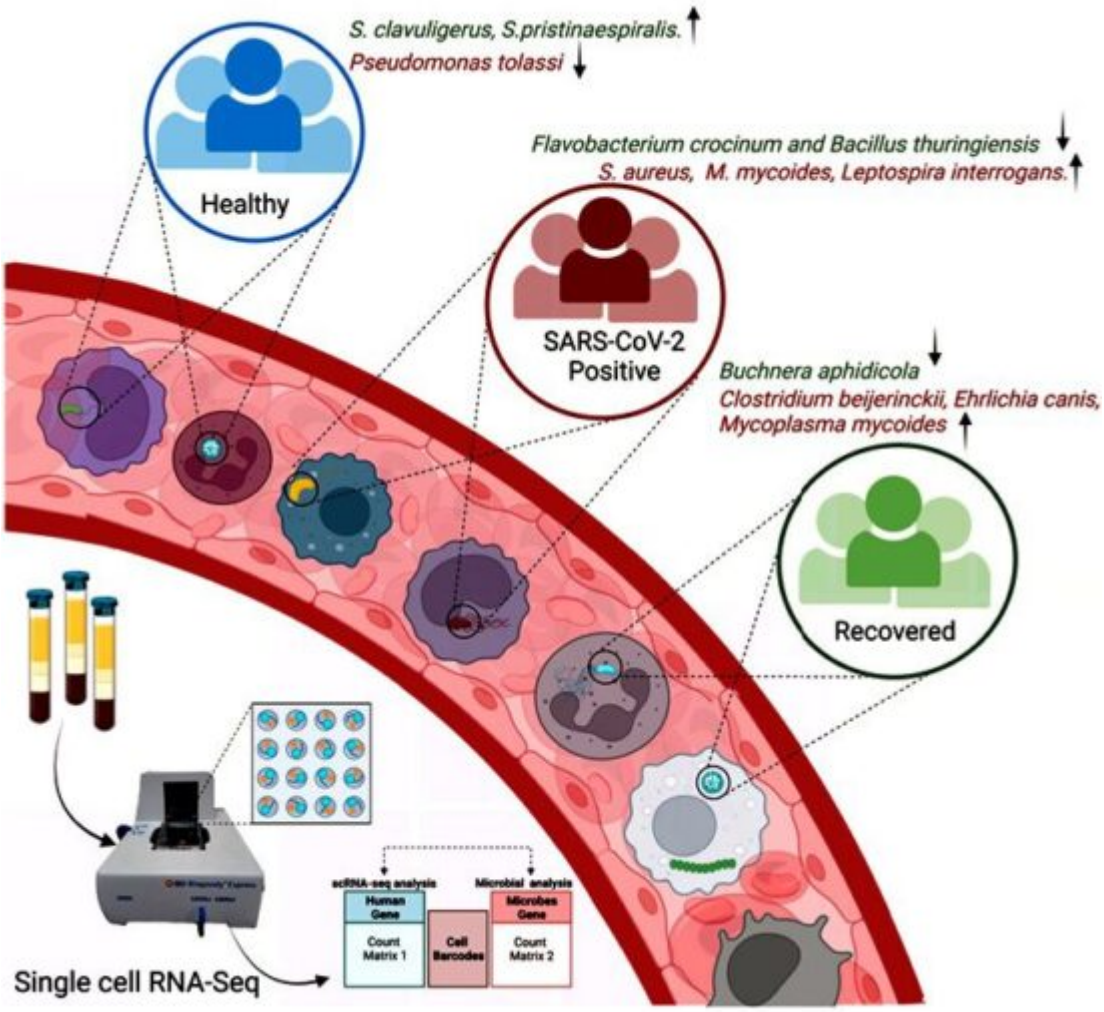
The study included 27 subjects, 14 positive for SARS-CoV-2, 10 recovered from COVID-19, and three healthy people. The authors used single-cell RNA sequencing, emphasizing that this technique offers a powerful window into the complex world of individual cells, especially microbial presence in the blood PBMCs.

A total of 97103 cells were captured post-filtration, 19026 from healthy individuals, 59792 from SARS-CoV-2 positive individuals, and 18285 from COVID-19 recovered individuals. Approximately 11 billion reads were generated from 97103 cells, about 9 billion were mapped to the human genome, while nearly 2 billion reads were unmapped.

Each group had differential proportions of human and non-human reads. In healthy people, the proportions were 82.98% human *vs.* 17.02% non-human. In the SARS-CoV-2-positive individuals, the proportion was 82.21% human *vs.* 17.79% non-human, and in individuals recovered from COVID-19, the proportion was 77.96% human *vs.* 22.09% non-human.

Further analysis demonstrated that non-human reads belonged to microbial entities. Bacteria were predominantly identified in all groups, accounting for 9.44%, 12.06%, and 15.49% of the reads in the healthy, SARS-CoV-2-positive, and COVID-19 recovered groups, respectively. Archaea were identified in proportions of 6.46%, 4.54%, and 5.39% of the reads in the respective groups. Viruses were detected in 1.12%, 1.19%, and 1.16% of the reads in the respective groups.

76 bacterial species were further analyzed for their abundance across the three groups: healthy individuals *vs.* SARS-CoV-2 positive, SARS-CoV-2 positive *vs.* COVID-19-recovered, and healthy individuals *vs.* COVID-19-recovered. Out of 76 bacterial species tested, the abundance of 16 bacterial species was significantly different in at least one group.



The original illustration from the article of Yadav S et al. ISCIENCE 2023

Healthy individuals had a higher abundance of *Streptomyces pristinaespiralis* compared to SARS-CoV-2-positive and COVID-19-recovered individuals, a higher abundance of *S. clavuligerus* compared to COVID-19-recovered individuals, and a higher abundance of *Pseudomonas tolaasii* compared to SARS-CoV-2-positive individuals.

Individuals who recovered from COVID-19 had a higher abundance of *Buchnera aphidicola*, *Ehrlichia canis,* and *Clostridium beijerinckii* than SARS-CoV-2-positive or healthy individuals. It is important to note that all three bacterial species that were highly abundant in individuals who recovered from COVID-19 are opportunistic (*Buchnera aphidicola*,

Clostridium beijerinckii, and *Ehrlichia canis*). The authors noted that the increased abundance of opportunistic bacteria in individuals who have recovered from COVID-19 can be a result of a dysfunctional T-cell response.

SARS-CoV-2-positive individuals had a higher abundance of three species: *Clostridium botulinum, Bacillus thuringiensis,* and *Staphylococcus aureus* than healthy individuals and individuals who recovered from COVID-19. According to the authors, these three species are important in the context of disease progression.

The researchers further analyzed the abundance of 16 bacterial species that differed significantly in at least one group in 12 different cell types, including naïve T cells, naïve B cells, classical monocytes, neutrophils, natural killer (NK) cells, memory B cells, macrophages, memory T cells, regulatory T cells (Tregs), dendritic cells (DC), platelets, and plasma cells.

Intracellular microbes were found in 8 cell types in healthy individuals, in 12 cell types in SARS-CoV-2-positive individuals, and in 6 cell types in individuals who recovered from COVID-19. In all groups, microbial reads were found in six cell types involved in the antigen presentation process: Tregs, neutrophils, naïve T cells, naïve B cells, macrophages, and DC, suggesting their consistent presence in various health conditions.

Further analysis revealed that eight bacterial species, namely *Ehrlichia canis, Buchnera aphidicola, Pseudomonas tolaasii, S. clavuligerus, Streptomyces pristinaespiralis,* and *Escherichia albertii,* were expressed in six different cell types in all groups. *Ehrlichia canis* was more abundant in the naïve T cells and Tregs of SARS-CoV-2-positive individuals than in those of healthy individuals. The authors noted that *Ehrlichia canis* has a plausible role in the immune response mediated by T cells.

Bacterial species *Buchnera aphidicola, Streptomyces clavuligerus, S. pristinaespiralis*, and *Pseudomonas tolassi* were more abundant in memory B cells, naïve B cells, neutrophils, and platelets of healthy individuals than in those of SARS-CoV-2-positive individuals.

Conclusion

This study has revealed the diversity of intracellular microbes in the immune cells of healthy individuals, SARS-CoV-2-positive individuals, and COVID-19-recovered individuals. Of the 76 bacterial species tested, the abundance of 16 bacterial species was significantly different in at least one group.

Identification of bacterial species associated with different cell types sheds light on the intricate and complex nature of the interplay between the immune system and microbes during infection and recovery.

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Journal Reference

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