Gut–Lung Axis Disruption in Long COVID Uncovered by Integrated Immuno-Epithelial-Microbial Profiling 758

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Three months after SARS-CoV-2 infection, persistent symptoms are linked to a sustained disruption of the gut-lung axis, marked by epithelial barrier dysfunction, γδ T-cells activation and impairment, and microbial translocation.

BACKGROUND

- SARS-CoV-2 infects both the respiratory and gastrointestinal tracts, and is associated with dysbiosis in both compartments;
- During infection, microbial translocation (MT) ranges from secondary bacterial products to viable bacterial particles in more severe cases;
- γδ T cells, part of innate system with adaptive features, are enriched at mucosal site, and are altered during infection;
- These processes have been studied separately and limited to acute infection, despite the respiratory and gastrointestinal tracts are interconnected through a bidirectional communication known as gut-lung axis;
- We aim to evaluate the interplay between gut- and lung-epithelial damage, MT, and γδ T cells in a longitudinal cohort of hospitalized unvaccinated COVID-19 individuals.

METHODS

- Patients: unvaccinated, hospitalized COVID-19 subjects during acute phase (T0) and at 3-month from symptoms onset (T1), classified as having LC (LC+) or not (LC−) long COVID. Plasma, whole blood and PBMCs were collected at both time points;
- Circulating biomarkers: epithelial permeability and cell death (eCAD, CK-18/M65), gut and lung epithelial damage (I-FABP, SPD), and MT (LBP, β-glucan, EndoCab) were assessed with ELISA;
- Microbiome: circulating microbiome was assessed through 16S rRNA gene sequencing on whole blood (MiSeq Illumina);
- γδ T cells: isolated from PBMCs and characterized through multiparametric flow cytometry (phenotypes: Vδ1, Vδ2, HLA-DR, CD38, PD-1, CD103, CD3, CCR9, CD4, CD8, α4β7; funcitonality: TNF-α, IL-17, IFN-γ upon PMA/IONO stimulation).

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RESULTS

Table 1. LC+ subjects show comparable demographic characteristics as compared to LC- subjects.

	LC+ (*n*=21)	LC− (*n*=19)	*p-value LC+ *vs* LC−
Age, years [median (IQR)]	55 (45-69)	55 (56-67)	0.888
Sex [*n* (%)]			0.133
Male	14 (66.6)	17 (89.5)	
Female	7 (33.4)	2 (10.5)	
Comorbidities [*n* (%)]			
Hypertension	11 (52.1)	6 (31.6)	0.752
Chronic heart disease	7 (33.4)	5 (26.3)	0.216
Chronic pulmonary disease	1 (4.7)	1 (5.3)	0.736
Diabetes	2 (9.5)	1 (5.3)	>0.999
History of cancer	1 (4.7)	0 (0)	>0.999
Obesity (BMI > 30)	4 (19)	4 (21)	>0.999
Virologic clearance, days [median (IQR)]	35 (25-42)	28 (24-35)	0.515
SARS-CoV-2 RNAemia, log_{10}(cp/mL) [median (IQR)]			
T0	0 (0-3.5)	3 (0-3.5)	0.784
T1	0 (0-3.5)	3.1 (0-3.5)	0.176
T1-T0	0 (-0.6 - 0)	0 (-0.2 – 3.15)	0.711
Acute COVID-19 symptoms [*n* (%)]			
Fever	20 (95.2)	18 (94.7)	0.942
Cough	15 (71.4)	9 (47.4)	0.121
Chestpain	1 (4.8)	1 (5.3)	0.942
Asthenia	4 (19.0)	5 (26.3)	0.459
Dyspnea	14 (66.7)	7 (36.8)	0.060
Nausea	3 (14.3)	2 (10.5)	0.719
Diarrhoea	1 (4.8)	1 (5.3)	0.942
Anosmia/dysgeusia	1 (4.8)	0 (0.0)	0.963
Arthromyalgia	0 (0.0)	1 (5.3)	0.287
Acute COVID-19 severity [*n* (%)]			
ICU admission	0 (0)	2 (10.5)	0.219
Low flow oxygen therapy	12 (57.1)	12 (65.2)	0.755
NIV/CPAP	9 (42.9)	4 (21)	0.186
OTI	0 (0)	2 (10.5)	0.488
Acute COVID-19 treatments, [*n* (%)]			
Immunomodulators	1 (4.8)	0 (0)	0.335
Anti-inflammatory	2 (9.5)	0 (0)	0.167
Antivirals	19 (90.5)	16 (84.2)	0.550
Anticoagulants	9 (42.9)	5 (26.3)	0.273
Antibiotics	4 (19.0)	2 (10.5)	0.451
SARS-CoV-2 pneumonia [*n* (%)]	21 (100)	16 (84.2)	0.058
Follow-up, months from symptoms' onset to follow-up visit [median (IQR)]	3 (3-4)	3 (3-3)	0.257
Early persistent symptoms (EPS) [*n* (%)]			
Asthenia	16 (76.2)	-	-
Dyspnoea	15 (71.4)	-	-
Anosmia/dysgeusia	3 (14.3)	-	-
Diarrhea	0 (0)	-	-
Arthromyalgia	4 (19)	-	-
Mnestic disorders	6 (28.6)	-	-

Legend. IQR: interquartile range; BMI: Body Mass Index (kg/m2); ICU: Intensive Care Unit; NIV: noninvasive ventilation; CPAP: continuous positive airway pressure; OTI: orotracheal intubation; Immunomodulators: tocilizumab; Anti-inflammatory: Celecoxib; Antivirals: Remdesivir, Lopinavir/ritonavir, Darunavir/cobicistat; Anticoagulants: heparin; Antibiotics: Azithromycin, or Teicoplanin; virologic clearance: number of days required to obtain a negative SARS-CoV-2 RT-PCR result on a nasopharyngeal swab; SARS-CoV-2 RNAemia: levels of SARS-CoV-2 Nucleocapsid RNA in plasma samples; T0: acute COVID-19 time point; T1: follow-up time point. *Statistical analyses: Mann-Whitney U test or Fisher exact test, as appropriate.

Fig. 1. At T0, LC+ subjects show increased MT along with epithelial lung injury which do not fully resolve at follow-up.

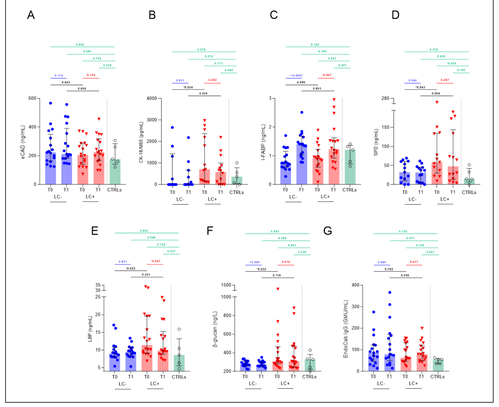


Fig. 2. LC+ subjects show no differences in taxonomic richness/evenness, community structure, or dispersion; however, they display enrichment of *Stenotrophomonas* at T0 and *Oxalobacteraceae* at T1.

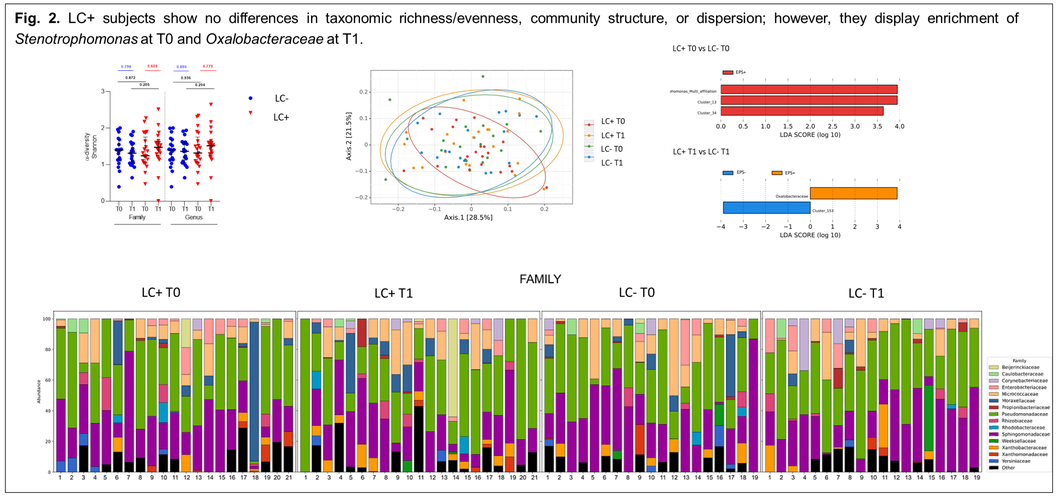
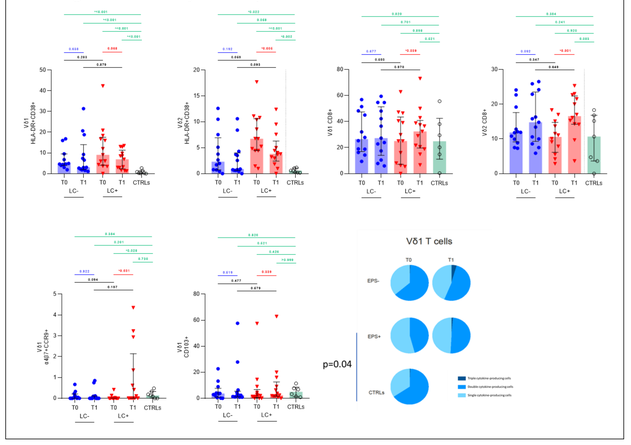


Fig. 3. At T0, LC+ subjects display increased activated γδ T cells and single-cytokine producing Vδ1, along with an increase over time of gut-homing Vδ1 and of cytotoxic γδ T cells.



CONCLUSIONS

Our findings support a model in which LC+ individuals display a mucosal-vulnerable phenotype characterized by early lung epithelial injury, selective microbial translocation, targeted γδ T cell activation with potentially reduced functionality, and progressive gut involvement. Together, these features indicate an alteration of the gut-lung axis. This framework highlights the gut-lung axis as a potential target for biomarker development and therapeutic strategies aimed at preventing or mitigating long COVID.

PLAIN LANGUAGE SUMMARY

Long-lasting symptoms after SARS-CoV-2 infection may stem from a breakdown in the normal communication between gut and lungs. Damage to these protective barriers may allow microbial substances to leak into the bloodstream, altering the immune system that normally monitors and protects them.