ADAPTIVE IMMUNITY DYSREGULATION IS ASSOCIATED TO THE DEVELOPMENT OF LONG-COVID

287

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BACKGROUND

The pathogenetic mechanisms behind the development of long-COVID (LC) are largely unknown. Because both plasma SARS-CoV-2 RNAemia and dysregulated immunity have been correlated with COVID-19 severity, we evaluated their associated with LC.

METHODS

- Population: unvaccinated hospitalized COVID-19 patients, studied during both acute-COVID-19 (T0) and 2-3 months from virological clereance (T1) (March-October 2020);
- LC definition: persistence ≥2mo after recovery of ≥1 symptom: anosmia, dysgeusia, fever, gastrointestinal symptoms, dyspnoea, fatigue, musculoskeletal pain, muscle weakness, brain fog;
- SARS-CoV-2 RNAemia: RT-qPCR of N-SARS-CoV-2;
- SARS-CoV-2-specific Abs: ELISA, pseudovirus neutralization, ID_{50}, Fc-mediated functions, %ADCC;
- T cells: immunophenotype and SARS-CoV-2 specificity upon 20h stimulation with S-N-M (AIM and ICS assays);
- B cells: immunophenotype and SARS-CoV-2-specific B cells detection (RBD-Biotin-Streptavidin-PE + RBD-Biotin-Streptavidin-PE-Vio770).

RESULTS

Table 1. Study population

	LC+ (*n*=38)	LC− (*n*=10)	*P* value LC+ *vs* LC− *
Age, years [median (IQR)]	60 (53–66)	51 (30–63)	0.1119
Sex [*n* (%)]			0.2917
Male	18 (47.4)	7 (70)	
Female	20 (52.6)	3 (30)	
Comorbidities [*n* (%)]			
Any comorbidity	22 (57.9)	4 (40)	0.4777
Hypertension	16 (42.1)	1 (10)	0.0744
Chronic heart disease	4 (10.5)	1 (10)	>0.9999
Chronic pulmonary disease	2 (5.3)	2 (20)	0.1871
Chronic kidney disease	1 (2.6)	0 (0)	>0.9999
Diabetes	4 (10.5)	0 (0)	0.5665
Neurologic disease	1 (2.6)	0 (0)	>0.9999
History of cancer	3 (7.9)	0 (0)	>0.9999
Obesity (BMI >30)	10 (26.3)	3 (30)	>0.9999
Smoke [*n* (%)]			0.4587
Yes	15 (39.5)	2 (20)	
No	23 (60.5)	8 (80)	
Symptoms at hospital admission [*n* (%)]			
Fever	35 (92.1)	10 (100)	>0.9999
Arthromyalgia	4 (10.5)	3 (30)	0.1469
Fatigue	30 (79)	8 (80)	>0.9999
Anosmia/dysgeusia	25 (65.8)	4 (40)	0.1639
Sore throat	1 (2.6)	2 (20)	0.1058
Cough	26 (68.4)	7 (70)	>0.9999
Chest pain	2 (5.3)	2 (20)	0.1871
Dyspnea	35 (92.1)	6 (60)	**0.0266**
Vomiting/nausea	1 (2.6)	2 (20)	0.1058
Diarrhea	6 (15.8)	2 (20)	0.6656
Duration of symptoms before hospital admission, days [median (IQR)]	7 (5–10)	11 (4.5–15.5)	0.1437
Radiologically documented pneumonia [*n* (%)]	36 (94.7)	9 (90)	0.5123
Maximum oxygen therapy [*n* (%)]			0.5774
None	2 (5.3)	1 (10)	
Low-flow systems	14 (36.8)	5 (50)	
CPAP/NIV/OTI	22 (57.9)	4 (40)	

Table legend: IQR: interquartile range; BMI: body mass index (kg/m^2); CPAP: continuous positive airway pressure; NIV: noninvasive ventilation; OTI: orotracheal intubation. **Statistical analyses*: Mann-Whitney U test, Fisher exact test or Chi-square, as appropriate.

Acutely ill hospitalized COVID-19 patients developing long-COVID show a dysregulated Ag-specific humoral, B- and T-cell response.

Table 2 LC symptoms

	LC+ (n=38)
Number of symptoms [*n* (%)]	
1	14 (36.8)
2	12 (31.6)
3	9 (23.7)
4	2 (5.3)
Fatigue [*n* (%)]	27 (71.1)
Dyspnea [*n* (%)]	16 (42.1)
Anosmia/dysgeusia [*n* (%)]	4 (10.5)
Diarrhea [*n* (%)]	3 (7.9)
Pain [*n* (%)]	11 (28.9)
Mnestic disorders [*n* (%)]	12 (31.6)
Pathologic 3-minute-walking test (SpO_2 drop ≥3%) [*n* (%)]	5 (13.2)

Figure 1. Similar SARS-CoV-2 RNAemia in LC+ and LC- patients

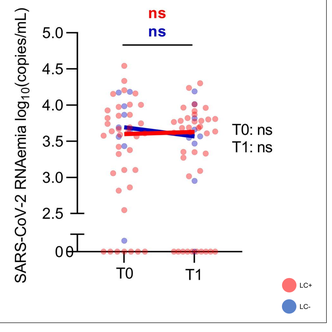


Figure 2. LC+ show a different magnitude and functionality of SARS-CoV-2-specific Abs (A-C) as well as magnitude of total SARS-CoV-2-specific B cells (D).

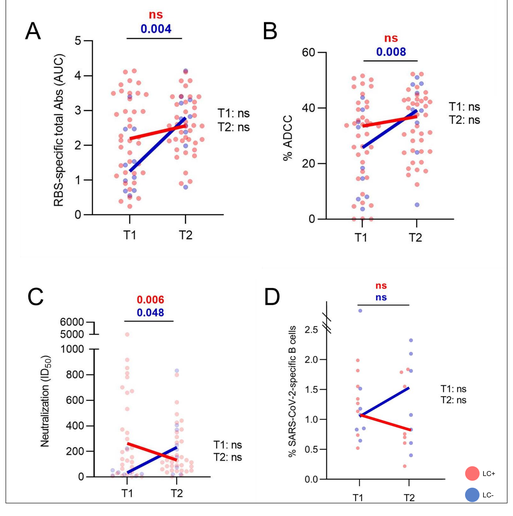


Figure 3. LC+ showed a similar maturation profile of B cells (A) but a different pool of class-switched IgA+ memory B cells (MBCs) (B) compared to LC-.

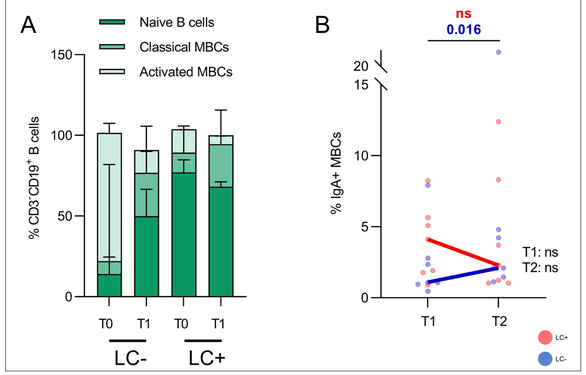
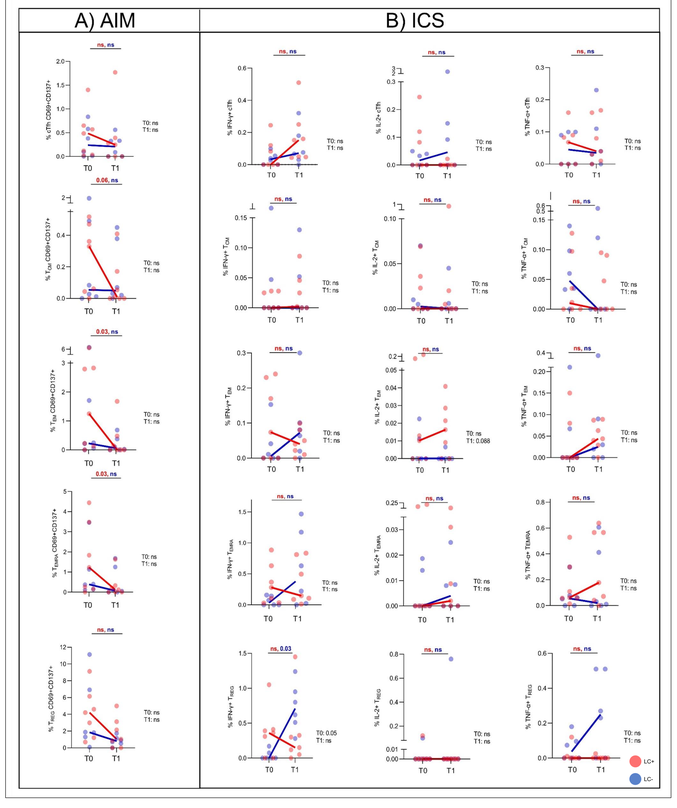


Figure 4. LC+ showed a decline over time of CD69+CD137+ CD4 T cells (A) and a different IFN-γ functionality (B).



CONCLUSIONS

Acutely ill hospitalized COVID-19 patients developing LC feature a dysregulated SARS-CoV-2-specific humoral, B- and T cell response which does not seem dependent on a persistent SARS-CoV-2 RNAemia nor to coarse maturation defects of total non-Ag-specific B cells. These data suggest a link between a dysregulated Ag-specific immunity and LC development. The fine understanding of the factors contributing to such dysregulation in LC patients is strongly needed.

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