DIVERGENT ADAPTIVE IMMUNE RESPONSES DEFINE TWO TYPES OF LONG COVID

D09

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Background:

More than 10% of patients infected with SARS-CoV-2 experience a Long COVID syndrome, characterized by the persistence of a diverse array of symptoms where fatigue predominates. The role of the adaptive immune response in Long COVID remains poorly understood, with contrasting hypotheses suggesting either an insufficient antiviral response or, alternatively, an excessive immune response that would trigger autoimmune damage.

Objective:

We set to characterize humoral and cellular responses in Long COVID patients prior to SARS-CoV-2 vaccination.

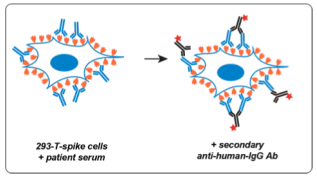
PERSICOT cohort:

Long COVID patients (LC, n=44) were included based on (1) an initial SARS-CoV-2 infection documented by PCR or the conjunction of two major signs of COVID-19 and (2) the persistence or resurgence of symptoms for over 3 months. They were compared to convalescent COVID patients with resolved symptoms (CO, n=25) and uninfected healthy blood donors (HD, n=14).

	Healthy donors (n=14)	Convalescent patients (n=25)	Long COVID patients (n=44)	
			Seronegative (n=17)	Seropositive (n=27)
Age (Mean, +/-SD)	30.6 (+/-9.4)	35.5 (+/-14.4)	43 (+/-7.5)	46.7 (+/-9)
Sex (female, %)	10 (71.4%)	16 (64%)	14 (82.4%)	22 (81.5%)
Time post-infection (Mean +/-SD)	/	7.4 (+/-4.5)	12.2 (+/-1.8)	13.8 (+/-3.4)
Positive PCR test (%)	/	24 (96%)	2 (11.8%)	21 (77.8%)
Number of symptoms (Median)	/	4	4	5
Anosmia and/or agueusia	/	10 (40%)	5 (29.4%)	17 (63%)

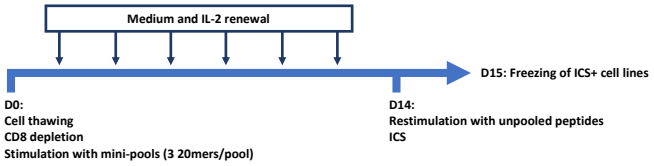
Methods:

- IgG and IgA antibodies specific for the SARS-CoV-2 spike were detected by a sensitive S-flow assay, which measures antibody binding to spike-expressing HEK 293T cells.

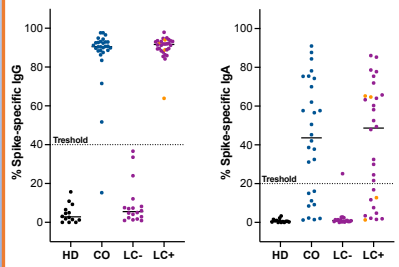


The S-flow test measures antibody binding to native spikes expressed at the surface of transduced HEK 293T cells (Grzelak L. *et al.*, Sci. Transl. Med., 2020, 12: eabc3103). IgG and IgA detection thresholds were set at 40% and 20% bound cells, respectively.

- For CD4⁺ T cell response analyses, cytokine production was measured by intracellular cytokine staining at D14 on primary T cell lines stimulated by immunodominant peptides (20mers) derived from the S, M, and N viral proteins (3 peptides per protein).



Anti-SARS-COV-2 antibody seroconversion distinguishes two populations of Long COVID patients:



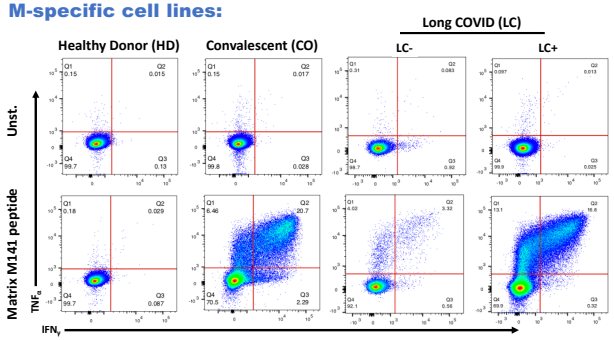
HD: healthy donors, CO: convalescent patients, LC-: seronegative Long COVID patients, LC+: seropositive Long COVID patients

- Analysis of Spike-specific IgG and IgA by S-flow highlighted heterogeneity among Long COVID patients. Bimodal IgG data distribution revealed two groups, with seronegative Long COVID (LC-) patients, who did not seroconvert, and seropositive Long COVID (LC+) patients, who had IgG titers as high as those of convalescents.

- All but one LC- patients were also seronegative for spike-specific IgA.

- Four Long COVID patients seronegative by standard spike-specific ELISA assay (orange dots) were reclassified as seropositive LC+ by S-flow assay, demonstrating the higher sensitivity of S-flow and the interest of this assay to document a previous SARS-CoV-2 infection.

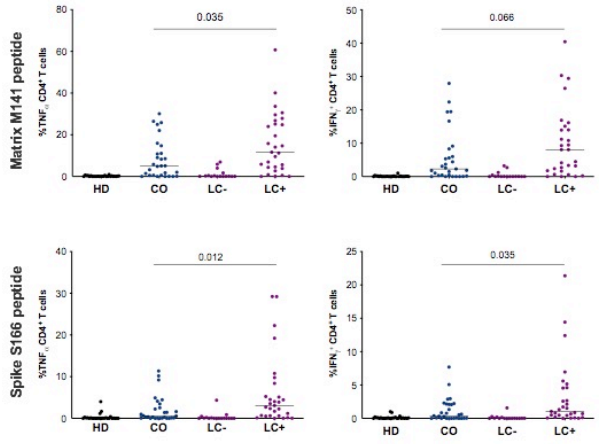
Examples of CD4+ T cell responses observed in M-specific cell lines:



In this example, IFN-γ and TNF-α expression was analyzed in CD4+ T cell lines specific for the SARS-CoV-2 Matrix M141 peptide by intracellular cytokine staining (ICS).

A strong response was detected for both a convalescent and a seropositive Long COVID patient (LC+), while the response in the seronegative Long COVID patient (LC-) was limited but detectable.

Heterogeneity of CD4+ T cell responses in Long COVID:



Medians are shown; Statistical test: Mann Whitney; HD: healthy donors; CO: convalescents; LC-: seronegative Long COVID patients; LC+: seropositive Long COVID patients

- Seropositive LC+ patients showed significantly stronger responses against both Matrix M141 and Spike S166 immunodominant peptides compared to convalescent patients.

- Seronegative LC- patients showed only weak CD4⁺ T cells responses to Matrix M141 and Spike S166 immunodominant peptides. However, these responses appeared higher than those of heathy donors for 5 out of 17 LC- patients.

Conclusions:

- ❑ These findings highlight divergent adaptive immune responses among Long COVID patients, with a group characterized by seroconversion and particularly strong CD4⁺ T cell responses, and a second group characterized by low or undetectable antibody and cellular responses.
- ❑ Weak but detectable CD4⁺ T cell responses may help document previous SARS-CoV-2 infection in a subset of seronegative Long COVID patients.
- ❑ Further studies are ongoing to determine whether the etiology and the duration of symptoms differ in the two groups of Long COVID patients.

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