High-resolution mapping of T-cell hybrid immunity to the entire SARS-CoV-2 proteome

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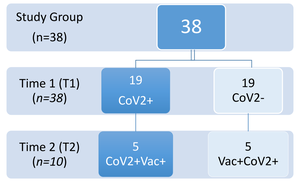
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BACKGROUND

To understand T-cell responses to SARS-CoV-2, it is essential to define the contribution of infection versus immunization to virus-specific hybrid immunity. Here, we characterized the breadth and magnitude of T-cell responses to the entire SARS-CoV2 proteome over a 2-year follow-up period in infected and vaccinated (CoV2+Vac+) and vaccinated and infected (Vac+CoV2+) individuals.

STUDY GROUPS

A



B

	CoV2+	CoV2+Vac+	CoV2-	Vac+CoV2+
Sampling	19	5	19	5
Sex, Female, n (%)	14 (74)	4 (80)	11 (58)	3 (60)
Days from Symptom Onset, median [IQR2]	124 [110-138]	816 [735.5-834]		302 [121.5-745.5]
Age, median [IQR]	52 [49-58]	60 [54-63]	47 [41-60]	49 [44.5-65.5]
Confirmed Positive PCR, n (%)	0 (0)	3 (60)	0 (0)	1 (20)
Positive serology S/N, n (%)	19 (100)	5 (100)	0 (0)	5 (100)
SARS-CoV-2 Vaccination, n (%)	0 (0)	5 (100)	0 (0)	5 (100)
Third dose, n (%)		5 (100)		5 (100)
mRNA-1273, n (%)		4 (80)		3 (60)
BNT162b2, n (%)		1 (20)		2 (40)

A. Flow chart of the longitudinal study design. We selected 38 ProHEpiC-19 participants (19 CoV2+ and 19 CoV2-) at time 1 (T1). Longitudinal samples were available after a 3-dose mRNA vaccination for 5 CoV2+Vac+ and 5 Vac+CoV2+, at 816 and 302 days from symptoms onset (DfSO, time 2, T2).

B. Table 1. Epidemiological data of study groups. The ProHEpiC-19 cohort is a prospective longitudinal study starting in March 2020 involving 7,776 healthcare workers in Spain.

METHODS

We measured the breadth and magnitude of IFN-γ T-cell responses by ELISpot assay in cryopreserved PBMCs, using a 15-mer overlapping peptide (OLP) library of 2,790 SARS-CoV-2 peptides.

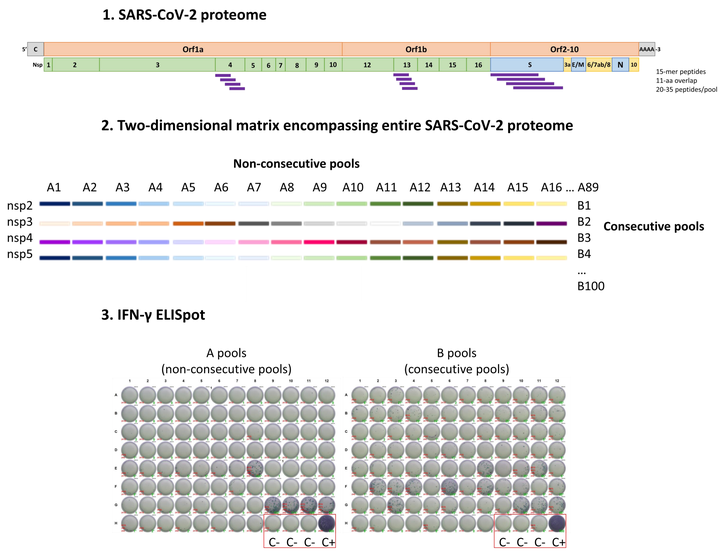


Fig 1. Graphic representation of the ELISpot mega matrix for the analysis of SARS-CoV-2 specific T-cell responses. 1. Overlapping peptide pools (15 aa, 11-aa overlap) spanning the whole SARS-CoV-2 proteome. **2.** A two-dimensional matrix containing non-consecutive A pools (n=89) and consecutive B pools (n=100) of 20-35 peptides encompassing all regions of the SARS-CoV-2 proteome. **3.** IFN-γ ELISpot was used for the analysis of T-cell responses to SARS-CoV-2 peptide pools.

T-cell responses are broader and of higher magnitude in CoV2+Vac+ individuals

RESULTS

I. Immunodominant T-cell responses across the SARS-CoV-2 proteome

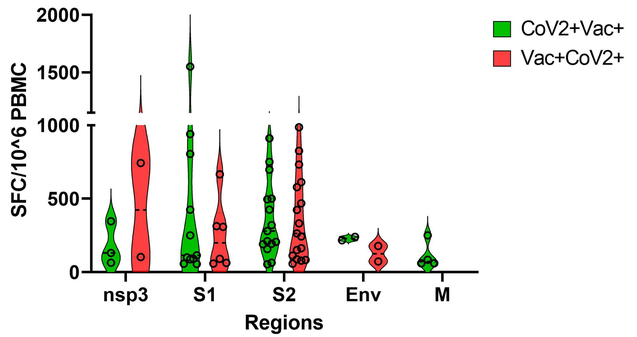


Fig 2. Violin plot represents the magnitude of immunodominant T-cell responses across the entire SARS-CoV-2 proteome between study groups at T2. T-cell responses against nsp3, Spike 1 (S1), Spike 2 (S2), envelope (Env) and membrane (M) are shown.

II. Breadth of T-cell responses against SARS-CoV-2 proteome

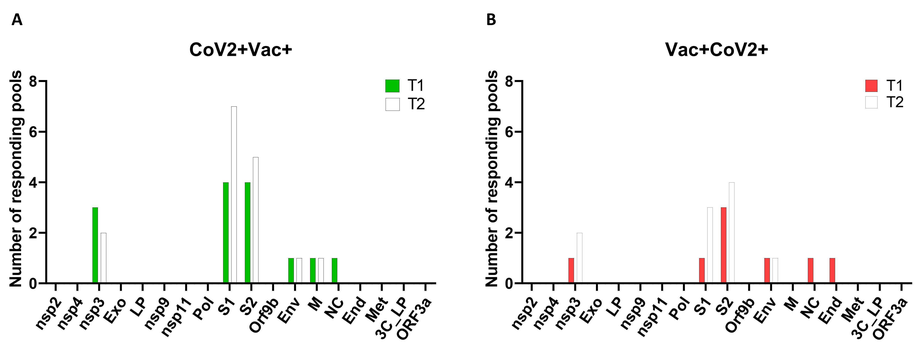


Fig 3. Bar graphs showing breadth of responses defined by the number of responding pools per region across the entire SARS-CoV-2 proteome. During the follow-up, CoV2+Vac+ and Vac+CoV2+ showed responses to 6 out of 18 regions over the entire SARS-CoV-2 proteome. At T2 CoV2+Vac+ and Vac+CoV2+ increased the number of responses in S1 (7-3) and S2 (5-4) pools. Furthermore, Vac+CoV2+ increased at T2 the T-cell response to nsp3 pools (2). In addition, CoV2+Vac+ had an exclusive and sustained response to M, whereas response to End region was only shown in Vac+CoV2+ at T2. T-cell responses to NC pools were found in T1.

III. Magnitude of T-cell responses against SARS-CoV-2 proteome

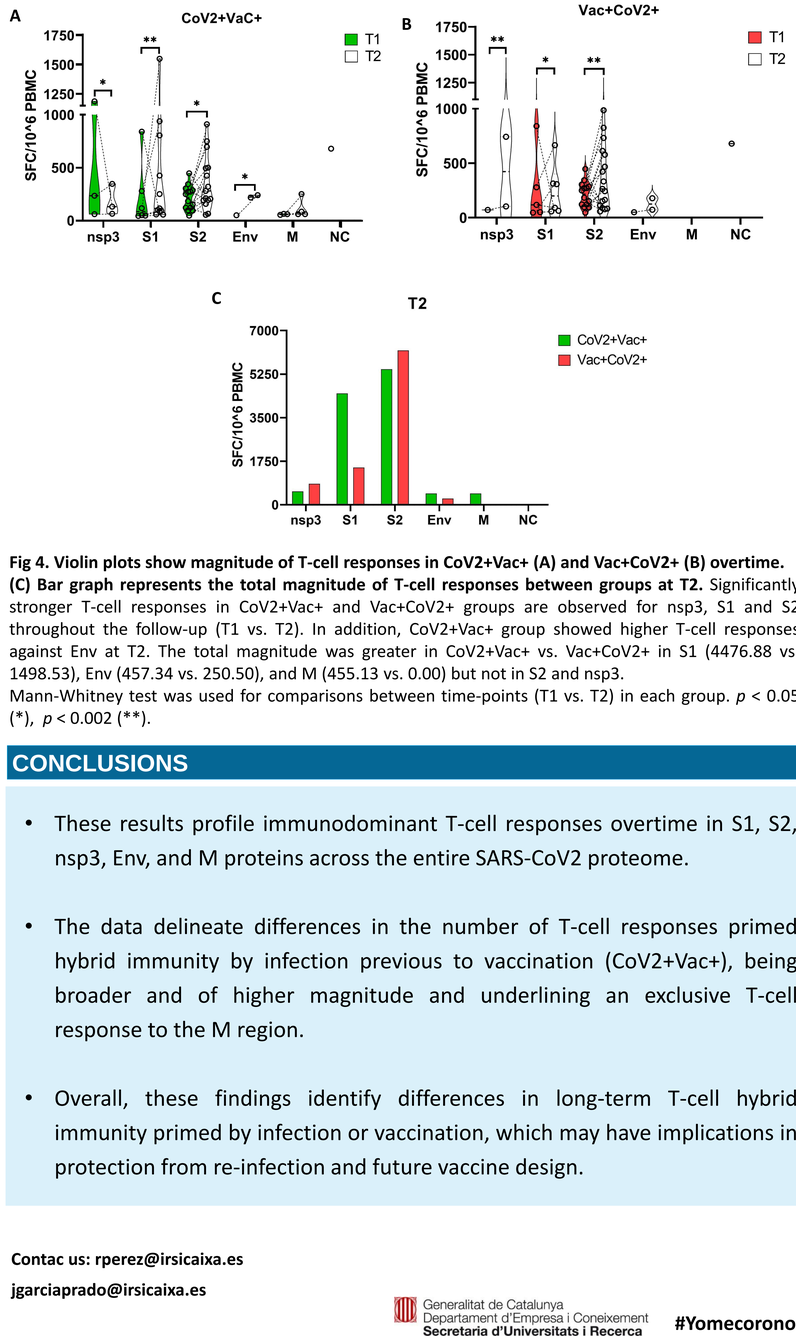


Fig 4. Violin plots show magnitude of T-cell responses in CoV2+Vac+ (A) and Vac+CoV2+ (B) overtime. (C) Bar graph represents the total magnitude of T-cell responses between groups at T2. Significantly stronger T-cell responses in CoV2+Vac+ and Vac+CoV2+ groups are observed for nsp3, S1 and S2 throughout the follow-up (T1 vs. T2). In addition, CoV2+Vac+ group showed higher T-cell responses against Env at T2. The total magnitude was greater in CoV2+Vac+ vs. Vac+CoV2+ in S1 (4476.88 vs. 1498.53), Env (457.34 vs. 250.50), and M (455.13 vs. 0.00) but not in S2 and nsp3.
Mann-Whitney test was used for comparisons between time-points (T1 vs. T2) in each group. $p < 0.05$ (*), $p < 0.002$ (**).

CONCLUSIONS

- These results profile immunodominant T-cell responses overtime in S1, S2, nsp3, Env, and M proteins across the entire SARS-CoV2 proteome.
- The data delineate differences in the number of T-cell responses primed hybrid immunity by infection previous to vaccination (CoV2+Vac+), being broader and of higher magnitude and underlining an exclusive T-cell response to the M region.
- Overall, these findings identify differences in long-term T-cell hybrid immunity primed by infection or vaccination, which may have implications in protection from re-infection and future vaccine design.

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