Long-Term Immunodominant Profile of SARS-CoV-2 T-Cell Responses in Hybrid Immunity

390

Raúl Pérez-Caballero[1], Laia Bernad[1], Athina Kilpeläinen[1], Oscar Blanch-Lombarte[1], Luis Romero[1], Ruth Peña[1], G. Felipe Rodríguez-Lozano[1], Josep Maria Manresa-Dominguez[2], Bonaventura Clotet[1], Alex Olvera[1], Christian Brander[1], Eva María Martínez Caceres[2], Concepcion Violán[3], Pere Torán-Monserrat[3] and Julia G. Prado[1]

1. IrsiCaixa AIDS Research Institute, Badalona, Spain, Germans Trias i Pujol Research Institute (IGTP), Universitat Autònoma de Barcelona, Badalona, Spain. 2. Immunology Division, Laboratori Clinic Metropolitana Nord (LCMN), Hospital Universitari Germans Trias i Pujol, Badalona, Spain. 3. Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Unitat de Suport a la Recerca Metropolitana Nord, Mataró, Spain

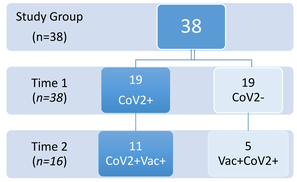
CROI 2024 March 3-6, 2024 Denver, Colorado

BACKGROUND

Characterization of long-term T-cell immunity against SARS-CoV-2 proteome is critical for understanding the implications of virus-specific hybrid immunity in protecting against re-infection and advancing vaccine design. Here, we conducted a high-resolution mapping of the breadth and magnitude of T-cell responses to the entire SARS-CoV-2 proteome over a 2-year follow-up period in individuals with hybrid immunity.

STUDY GROUPS

A



B

	CoV2+	CoV2+Vac+	CoV2-	Vac+CoV2+
Sampling	19	11	19	5
Sex, Female, n (%)	14 (74)	8 (73)	11 (58)	3 (60)
Days from Symptom Onset, median [IQR2]	124 [110-138]	833 [793-842]		302 [121.5-745.5]
Age, median [IQR]	52 [49-58]	55 [53-61]	47 [41-60]	49 [44.5-65.5]
Confirmed Positive PCR, n (%)	0 (0)	8 (73)	0 (0)	1 (20)
Positive serology S/N, n (%)	19 (100)	11 (100)	0 (0)	5 (100)
SARS-CoV-2 Vaccination, n (%)	0 (0)	11 (100)	0 (0)	5 (100)
Third dose, n (%)		11 (100)		5 (100)
mRNA-1273, n (%)		8 (73)		3 (60)
BNT162b2, n (%)		3 (27)		2 (40)

A. Flow chart of the longitudinal study design. We selected 38 ProHEpiC-19 participants (19 CoV2+ and 19 CoV2-) at time 1. Longitudinal samples were available after a 3-dose mRNA vaccination for 11 CoV2+Vac+ and 5 Vac+CoV2+, at 833 and 302 days from symptoms onset (DfSO, time 2).

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

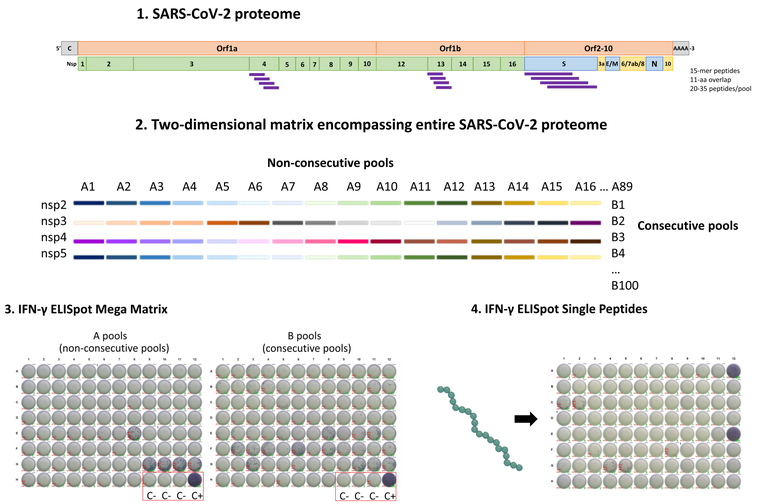


Fig 1. Graphic representation of the IFN-γ ELISpot mega matrix approach and single peptides analysis of SARS-CoV-2 T-cell responses. 1. Overlapping peptide pools (15 aa, 11-aa overlap) spanning the whole SARS-CoV-2 proteome. **2.** A two-dimensional matrix containing non-consecutive A pools (n=89) and consecutive B pools (n=100) of 20-35 peptides encompassing all regions of the SARS-CoV-2 proteome. **3.** IFN-γ ELISpot was used to analyze T-cell responses to SARS-CoV-2 peptide pools. **4.** Single-peptide matrix deconvolution.

Long-term T-cell hybrid immunity primed by SARS-CoV-2 infection holds broader and higher magnitude of responses

RESULTS

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

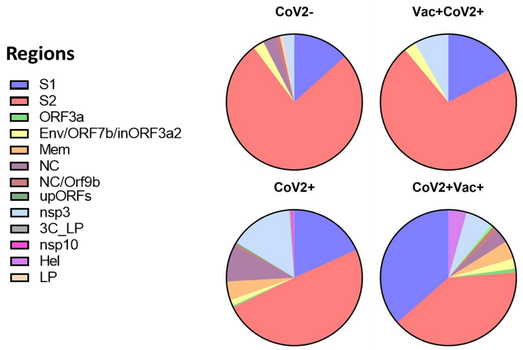


Fig 2. Pie charts represent the immunodominant T-cell responses across the entire SARS-CoV-2 proteome between study groups. We described T-cell responses to 13 regions (48.1%) in the SARS-CoV-2 proteome. Nsp3, Spike 1 (S1), and Spike 2 (S2) are the most immunodominant regions with the greatest magnitude of T-cell responses.

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

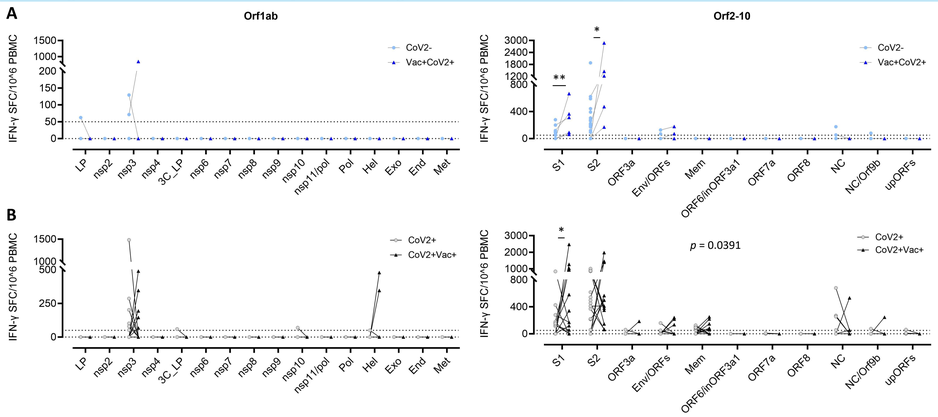


Fig 3. Scatter plots show the magnitude of T-cell responses in CoV2- and Vac+CoV2+ (A), and CoV2+ and CoV2+Vac+ (B) overtime. Stronger T-cell responses are observed in Orf2-10 regions (structural proteins) in all groups throughout the follow-up. Vaccinees showed significant T-cell responses against S1 (Vac+CoV2+ p = 0.0054; CoV2+Vac+ p = 0.0330) and S2 (Vac+CoV2+ p = 0.0288) proteins. Within the Orf1ab, Nsp3 is shown as the protein with the greatest T-cell response in CoV2+ and CoV2+Vac+. Dash line indicates the positivity threshold.

III. Breadth of T-cell responses and percentage of responders

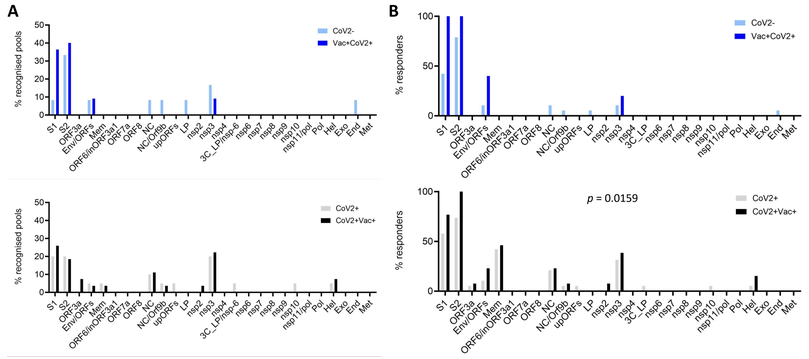


Fig 4. Bar graphs show the breadth of T-cell responses (A) defined by the number of responding pools per region across the SARS-CoV-2 proteome, and percentage of responders (B) to SARS-CoV-2 proteome regions.

IV. SARS-CoV-2 single peptides after deconvolution

A

Vac+CoV2+			
Region	**Pool**	**OLP ID**	**OLP Sequence**
inORF3a	B67	CoV-2_inORF3a2-2778	CSLCLQLAVVVCNSL
S1	B52	CoV-2_S1-2094	VGYLQPRTFLLKY
S1	B52	CoV-2_S1-2095	YLQPRTFLLKYNENGTI
S2	B81	CoV-2_S2-2229	CSNLLLQYGSFCTQL
S2	B82	CoV-2_S2-2239	AQVKQIYKTPPIKDF
S2	B82	CoV-2_S2-2240	KQIYKTPPIKDFGGF
S2	B82	CoV-2_S2-2242	PPIKDFGGFNFSQIL
S2	B83	CoV-2_S2-2266	ALLAGTITSGWTFGA
S2	B83	CoV-2_S2-2267	GTITSGWTFGAGAAL

B

CoV2+ Vac+			
Region	**Pool**	**OLP ID**	**OLP Sequence**
Nsp3	B14	CoV-2_nsp3-458	FEYVHTTDPSFLGRY
ORF1ab	B37	CoV-2_Hel-1666	NRFNVAITRAKVGIL
exORFM	B31	CoV-2_Mem-2509	ILRGHLRIAGHHLGR
NC	B63	CoV-2_NC-2703	SGTWLTYTGAIKL
ORF1a	B19	CoV-2_nsp3-611	TNIVTRCLNRVCTNY
ORF9b	B64	CoV-2_ORF9b-2742	RKTLNSLEDKAFQL
S1	B50	CoV-2_S1-2036	AIHVSGTNGTKRF
S1	B52	CoV-2_S1-2093	AAYYVGYLQPRTFLL
S1	B52	CoV-2_S1-2095	YLQPRTFLLKYNENGTI
S1	B53	CoV-2_S1-2119	RKRISNCVADYSVLY
S1	B53	CoV-2_S1-2120	SNCVADYSVLYNSA
S1	B54	CoV-2_S1-2148	ERDISTEIYQAGSTP
S1	B54	CoV-2_S1-2156	LQSYGFQPTNGVGY
S2	B82	CoV-2_S2-2240	KQIYKTPPIKDFGGF
S2	B82	CoV-2_S2-2258	CAQKFNGLTVLPPLL
S2	B83	CoV-2_S2-2266	ALLAGTITSGWTFGA
S2	B83	CoV-2_S2-2267	GTITSGWTFGAGAAL

Table 2A-B. SARS-CoV-2 single peptides from Vac+CoV2+ **(A)** and CoV2+Vac+ **(B)** groups identified after mega matrix deconvolution.

CONCLUSIONS

- These results define immunodominant long-term T-cell responses in S, Nsp3, NC, Env, and M proteins in the SARS-CoV2 proteome in the context of hybrid immunity.
- 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 than Vac+CoV2+ 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 novel vaccine design.
- We propose a vaccine design including Membrane and Nsp3 proteins in addition to S to elicit broader T-cell responses

Contac us: rperez@irsicaixa.es

jgarciaprado@irsicaixa.es

Generalitat de Catalunya
Departament d'Empresa i Coneixement
Secretaria d'Universitats i Recerca

#Yomecorono

Instituto de Salud Carlos III · Gobierno de España · Ministerio de Economía y Competitividad · FEDER Fondo Europeo de Desarrollo Regional · Unión Europea "Una manera de hacer Europa" · Horizon Europe · PERIS 2016 2020