A Functional Assay To Measure The T cell Response To SARS-COV-2 In Primary Immunodeficiency Patients

Great Ormond Street NHS Hospital for Children

Arnold Awuah[1], Ava Zamani[2], Fariba Tahami[1], Mark Davis[1], Louis Grandjean[3], Matthew Buckland[1] & Kimberly C. Gilmour[1]

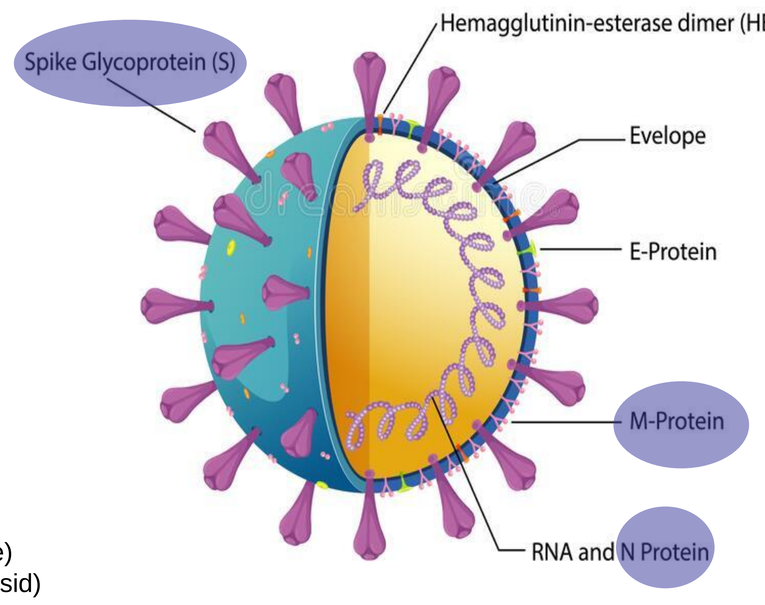
[1]Immunology, Great Ormond Street Hospital NHS Foundation Trust, [2]Affiliate to Immunology, Great Ormond Street Hospital NHS Foundation Trust, [3]Infectious Disease, Great Ormond Street Hospital NHS Foundation Trust

Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel respiratory virus with a wide range of clinical presentations known collectively as COVID-19. The severe respiratory illness accounts for increased hospital admissions and high mortality. Understanding the immune response to COVID-19 is a pre-requisite to identifying clinical correlates of exposure and immunity. This is of particular importance in vulnerable patients such as those with immunodeficiency. Detecting the antibody response to COVID-19 is essential to diagnostic testing, however the antibody response may wane over time, or may not be detectable in patients with antibody deficiency necessitating an examination of the role of the cell-mediated immunity. There is already evidence to suggest an important role of cellular immunity. T cells may provide long-lasting immunity against the virus, and a T cell response has been detected in seronegative individuals post-COVID-19.

A simple and practical method is essential to assess the T cell response in the clinical setting . A functional [3H]-thymidine incorporation assay to assess the T cell response to SARS-CoV-2 was developed with the aim of analysing a cohort of primary immunodeficiency (PID) patients at Great Ormond Street Hospital. Proliferation of T cells in response to three SARS-CoV-2 antigens was investigated in healthy controls as well as in patients with PID post-vaccination/infection.

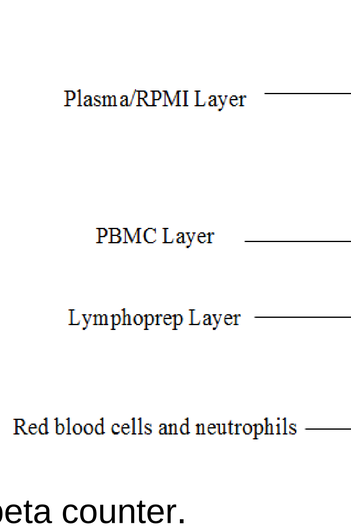
Structure of SARS-COV-2



M (Membrane)
N (Nucleocapsid)
S (Spike)

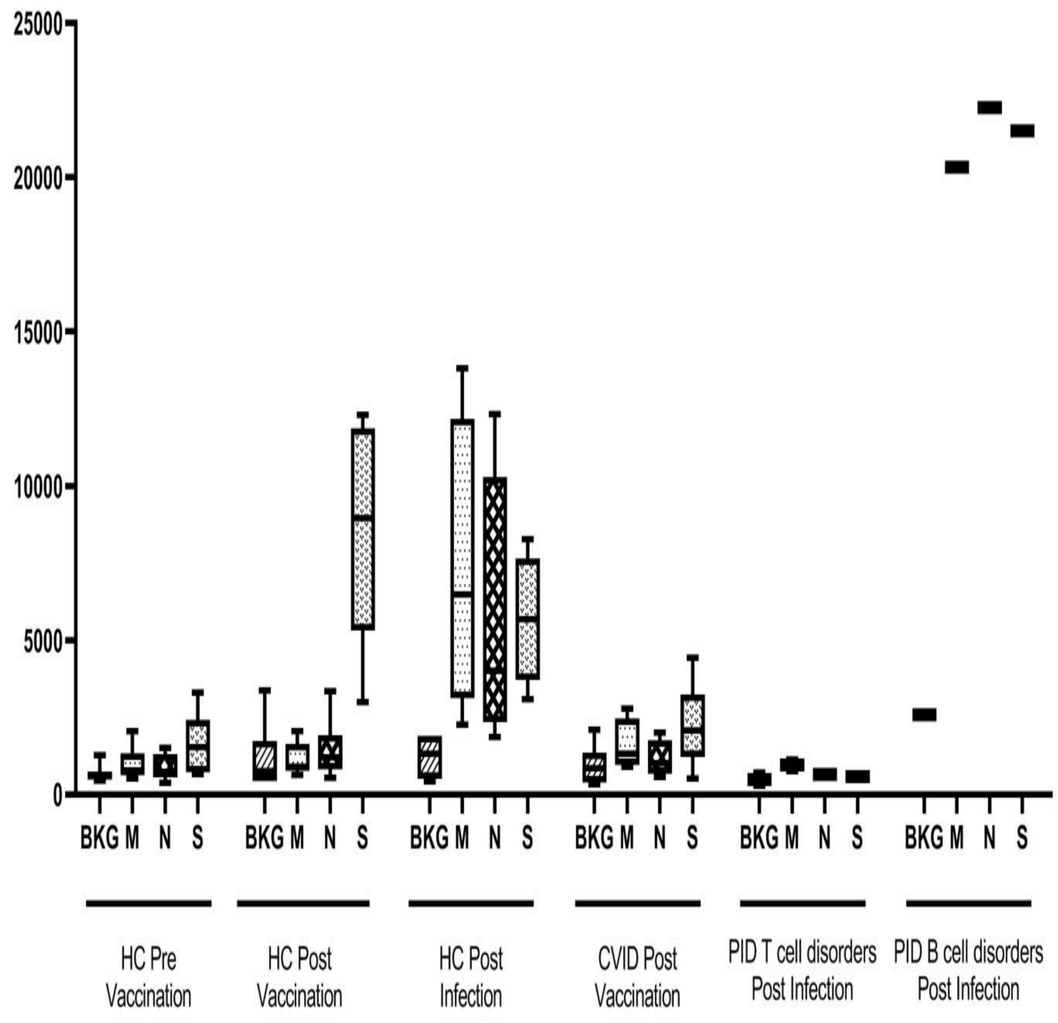
Testing methods for T cell response

- Heparinised blood is diluted with RPMI media and layered onto a Lymphoprep.
- PBMC separated from blood by density centrifugation.
- PBMC removed and wash with RPMI.
- PBMC resuspended in required RPMI.
- AB serum added.
- 96 well plates coated with Sars-Cov-2 antigens and PHA.
- Incubation of plates for 3-5 days.
- Pulsing with radioactive thymidine.
- Radioactivity measured on scintillation beta counter.
- Results analysed.



Subset of Results

			CPM				
					SARS-CoV-2 antigens		
Patient	Diagnosis	SARS-CoV-2 status	BKG	PHA	M	N	S
Healthy controls							
1	HC	Pre-vaccination	510	44742	892	556	1695
2	HC	Pre-vaccination	663	16328	1428	1432	2182
3	HC	Pre-vaccination	659	38723	653	817	739
4	HC	Pre-vaccination	1273	42686	2050	1508	2487
5	HC	Pre-vaccination	613	15825	618	579	706
6	HC	Pre-vaccination	456	16947	658	631	3307
7	HC	Pre-vaccination	610	42685	512	370	666
8	HC	Pre-vaccination	695	51356	1047	911	1372
9	HC	Post-infection	1901	35695	7273	1866	3085
10	HC	Post-infection	770	10037	2255	3788	5544
11	HC	Post-infection	425	18577	13811	12324	8265
12	HC	Post-infection	1880	15620	5702	4208	5813
13	HC	Post-vaccination	640	38550	939	1350	11712
14	HC	Post-vaccination	1177	29170	850	1063	10950
15	HC	Post-vaccination	849	25545	1506	1433	12301
16	HC	Post-vaccination	3359	18332	2040	3337	6962
17	HC	Post-vaccination	433	21918	825	907	6101
18	HC	Post-vaccination	429	13001	629	538	2999
Patients with PID							
19	CVID	Post-vaccination	2099	32708	2769	2007	2508
20	CVID	Post-vaccination	320	9852	ND	ND	512
21	CVID	Post-vaccination	1104	21164	ND	ND	1437
22	CVID	Post-vaccination	963	25869	1082	1030	1635
23	CVID	Post-vaccination	397	44820	906	557	2840
24	CVID	Post-vaccination	750	26451	1569	1008	4447
Patients with PID – T cell disorders							
25	T cell activation disorder	Post-infection	706	43961	760	594	690
26	Down syndrome	Post-infection	282	28839	1137	705	477
Patients with PID – B cell disorders							
27	XLA	Post-infection	2570	16137	20328	22269	21510



HC = 18 (8 pre vaccinated, 4 post infection, 6 post vaccination)
Patients = more than 180 with different conditions.

Graphical representation of results obtained from the project.

HC, Healthy controls; *CVID*, common variable immunodeficiency; *PID*, primary immunodeficiency; *XLA*, X-linked agammaglobulinaemia; *CPM*, counts per minute per suspension; *BKG*, background – unstimulated samples; PHA, Phytohaemagglutinin.

Conclusions

PHA proliferation (positive control) was normal in all patients tested. As expected healthy controls post infection proliferated to all three COVID antigens while post vaccination health controls showed a strong proliferative response to spike antigen alone.
Patients with CVID and T cell disorders failed to proliferate to COVID antigens and had responses near-equivalent to background. The XLA patient had the highest T cell proliferation to antigen exposure of the entire cohort, and proliferation to antigen post-infection mimicked the pattern seen in healthy controls post-infection. Further analysis of XLA/B cells disorder patients is required to confirm this finding.

References

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