Evaluation of T cell dysfunction in Post Acute Infection Syndromes

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Introduction

Post-acute infection syndromes (PAISs) like long COVID, post treatment Lyme disease syndrome (PTLDS) and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) are a wide range of complex conditions characterized by cognitive, emotional and neurological symptoms that vary in frequency and severity over time.[1-3] These conditions frequently lead to persistent symptoms such as fatigue, pain, difficulty concentrating and headaches that continue long after the initial infection has resolved. An estimated 100 million individuals are currently affected worldwide, with an annual incidence projected at 1–2% of the population due to ongoing exposure to infections and vaccines.[4]

PAISs are often associated with a dysfunction of the immune system, notably progressive T cell exhaustion marked by diminished effector activity, reduced antigen killing, upregulation of exhaustion markers, metabolic reprogramming, mitochondrial dysfunction, and increased oxidative stress.[5, 6] Without proper intervention, T cell dysfunction may progress to terminal exhaustion.

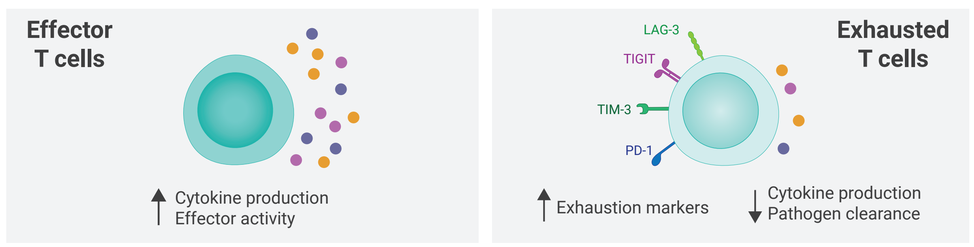

Figure 1. Chronic antigen stimulation following a chronic infection can cause T-cell exhaustion, resulting in low level of effector T cell activity, minimal antigen killing activity, upregulation of T-cell exhaustion markers.

Chronic antigenic stimulation plays a key role in T cell exhaustion. Persistent exposure to viral antigens can drive the upregulation of inhibitory receptors and metabolic dysfunction in T cells, contributing to immune dysregulation observed in PAIS.[7-9]

Despite their prevalence, no early diagnostic tools exist. To address this gap, Virax Biolabs has initiated clinical studies to explore T cell-based diagnostics, aiming to improve early detection and patient outcomes.

Results

We used flow cytometry to analyse the expression of T cell exhaustion markers following prolonged antigenic stimulation. PBMCs from healthy donors were stimulated for four days with peptide pools derived from SARS-CoV-2, EBV, and CMV. The expression of PD-1, LAG-3, TIM-3 and CD39 was assessed to evaluate the impact of stimulation on T cell functionality.

Flow cytometry analysis demonstrated a significant upregulation of exhaustion markers on CD4⁺ and CD8⁺ T cell subsets following non-specific stimulation of PBMCs induced by anti-CD3, anti-CD28 and anti-CD2 antibodies:

- PD-1 and LAG-3 showed robust increases at day 4 in both CD4⁺ and CD8⁺ T cells, indicating pronounced activation-induced exhaustion.
- CD39 was clearly elevated following stimulation, highlighting activation-induced differentiation toward exhaustion.
- TIGIT expression did not significant change post-stimulation, suggesting a selective regulation of exhaustion markers during short-term polyclonal activation.

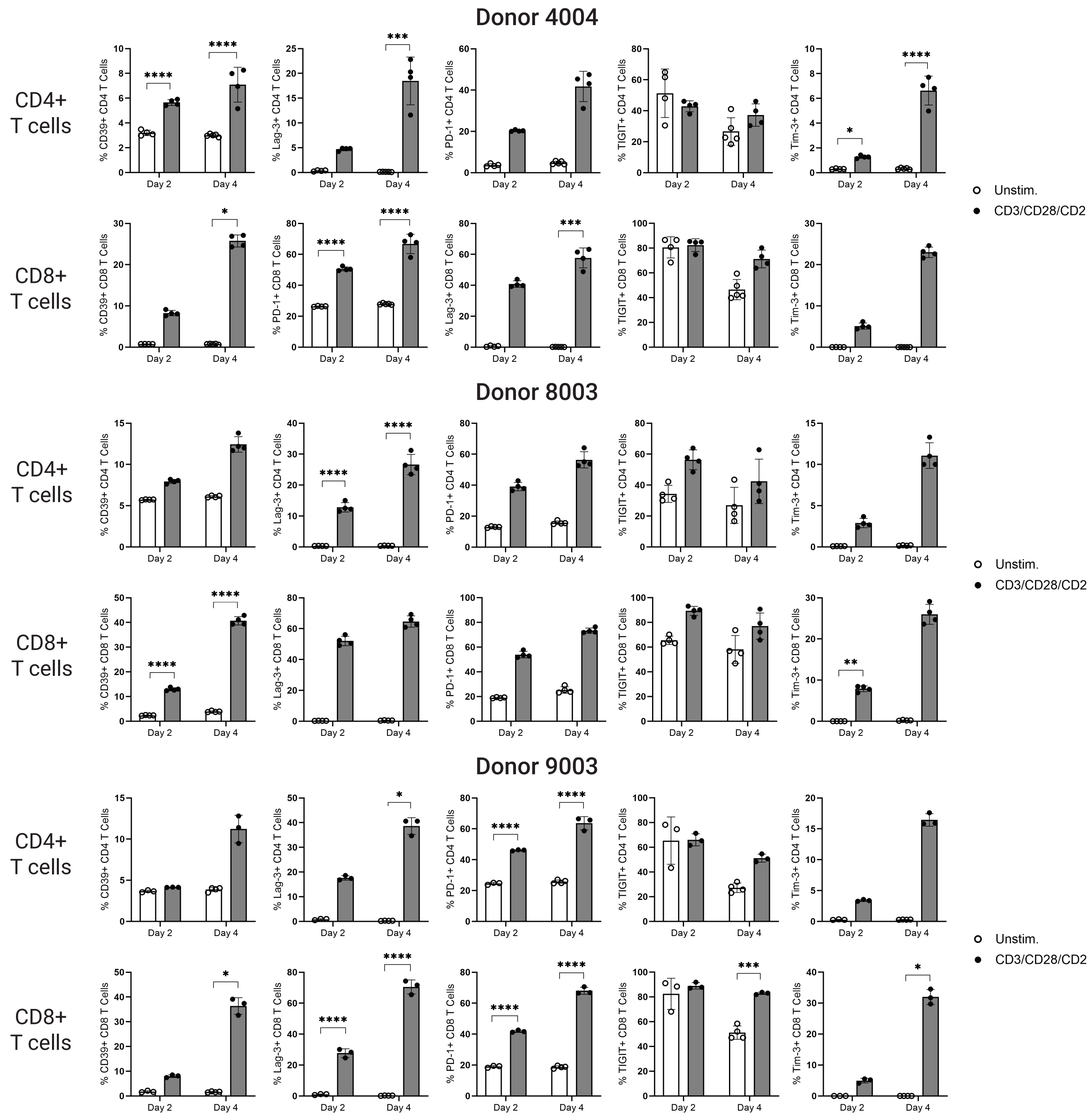

Figure 2. Flow cytometric analysis of the expression of exhaustion markers on CD4⁺ and CD8⁺ T cell subsets following non-specific stimulation. PBMCs were stimulated with anti-CD3, anti-CD28 and anti-CD2 antibodies for up to 4 days.

Additionally, stimulation with pathogen-specific peptide pools (SARS-CoV-2, EBV, and CMV) resulted in detectable, but significantly less pronounced increases in exhaustion marker expression, consistent with selective activation of antigen-specific memory T cells.

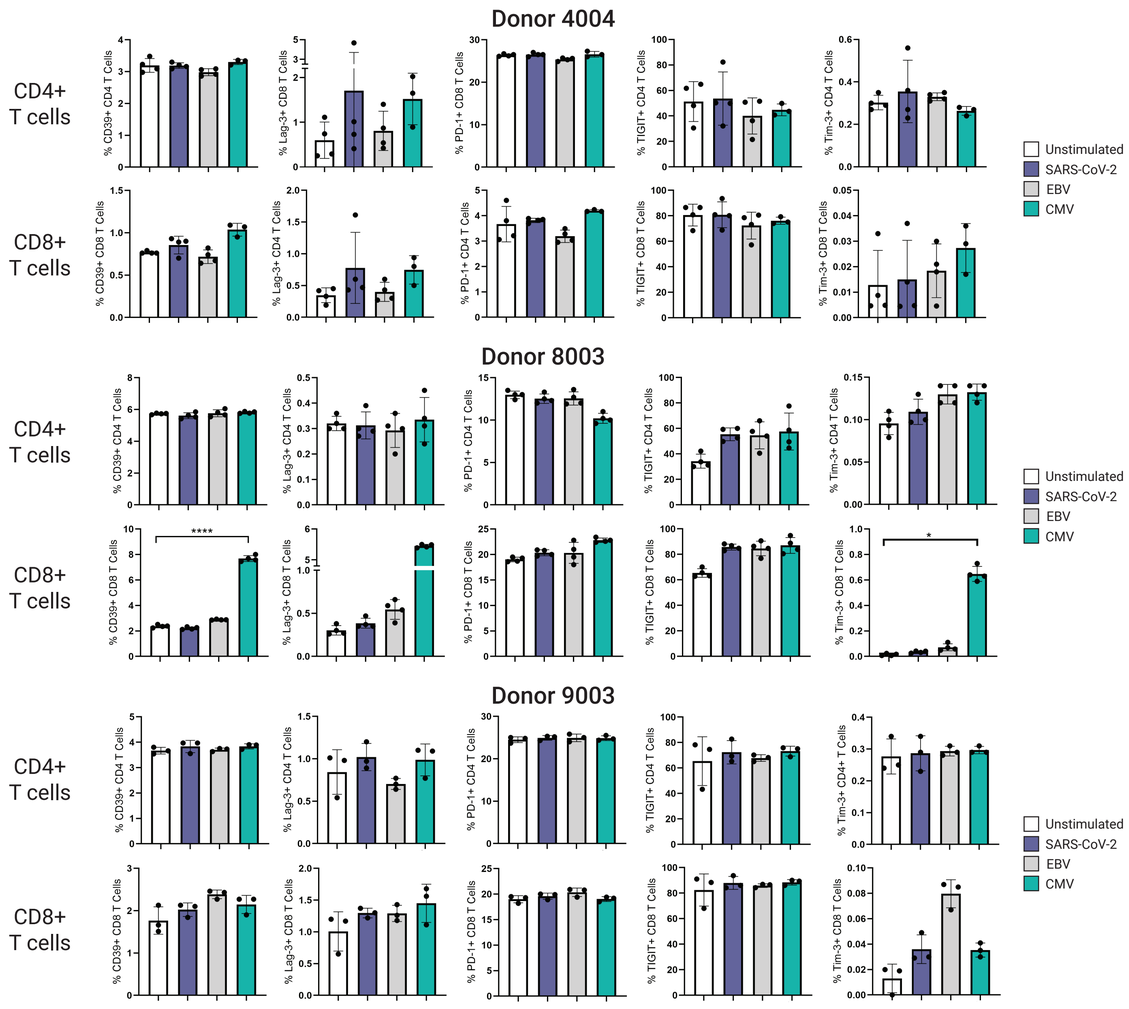

Figure 3. Flow cytometric analysis of the expression of exhaustion markers on CD4⁺ and CD8⁺ T cell subsets following stimulation with infection-derived peptide pools.

Preliminary data from PAIS Assay

Clinical samples from patients with confirmed chronic illness were stimulated with a SARS-CoV-2 peptide pool and then analysed for a panel of cytokines known to be involved in immune responses and inflammation.

Significant changes were seen in the levels of all cytokines investigated in the patients with chronic illness compared to healthy controls, with marked differences detected in four cytokines, as detailed in the table below.

	IFN-g				IL-2				IL-10				Granzyme B			
Donor ID	Control -	Control +	SARS-Cov-2 activation	Stimulation ratio	Control -	Control +	SARS-Cov-2 activation	Stimulation ratio	Control -	Control +	SARS-Cov-2 activation	Stimulation ratio	Control -	Control +	SARS-Cov-2 activation	Stimulation ratio
Patient 1	35	2101	120	3.43	52	2905	93	1.79	823	1054	700	0.85	148	845	245	1.66
Patient 2	30	2064	205	6.83	45	1850	76	1.69	120	215	85	0.71	142	1284	211	1.49
Patient 3	33	2300	205	6.21	36	1989	101	2.81	650	945	780	1.2	96	743	188	1.96
Patient 4	35	780	20	0.57	58	2760	81	1.4	2215	2725	2640	1.19	82	655	154	1.88
Patient 5	45	298	33.3	0.74	25	2117	37	1.48	181	183	179	0.99	115	1017	186	1.62
Healthy Control 1	34	3904	181	5.32	120	3770	213	1.78	1218	2543	1773	1.46	69	2118	90	1.3
Healthy Control 2	50	3693	330	6.6	99	3028	194	1.96	1200	2643	1383	1.15	211	1068	311	1.47
Healthy Control 3	37	5194	151	4.08	79	4062	182	2.3	313	2594	431	1.38	190	4160	174	0.92
Healthy Control 4	37	4885	128	3.46	56	3637	312	5.57	707	2679	1190	1.68	123	3665	187	1.52
Healthy Control 5	21	5447	71	3.38	88	4047	108	1.23	728	2715	1873	2.57	154	2926	183	1.19

Table 1. Investigation of cytokine production by patients with chronic illness in reponse to stimulation with SARS-CoV-2 peptide pool.
Patient blood was collected and PBMCs isolated. The PBMCs were seeded into FluoroSpot plates and stimulated with a SARS-CoV-2 peptide pool to induce cytokine secretion by T cells. FluoroSpot analysis was then undertaken.

Av + 1.5x std dev	Cytokine production above average
Av + 1x std dev	
Av - 1x std dev	Cytokine production below average
Av - 1.5x std dev	

Clinical Study NCT06731179

Virax Biolabs initiated a clinical study in the United Kingdom aimed at assessing ViraxImmune™ performance in detecting T-cell dysfunction in confirmed PAIS patients. 160 participants will be enrolled, with the first samples collected in March 2025.

- Validated symptom questionnaires for chronic fatigue (FACIT-F) and cognitive impairment (MoCA) will also be assessed
- A Composite Score will be calculated for each patient based on the individual cytokine analyses undertaken, providing a single, easy-to-interpret value.
- A link to be established between the participant's T cell dysfunction and their symptoms of chronic fatigue and cognitive impairment.

Conclusion

- PAISs are often associated with a dysfunction of the immune system, notably progressive T cell exhaustion
- T cell exhaustion is well documented to correlate with reduced cytokine production, particularly of pro-inflammatory cytokines, resulting in impaired immune functionality
- Virax Biolabs is currently running a number of clinical studies to evaluate the role of T cell dysfunction associated with persistent symptoms associated with long COVid, ME/CFS and post treatment Lyme disease using its FluoroSpot-based assay capable of sensitive multiplexed quantification of cytokine secretion at the single-cell level
- This approach is expected to offer significant diagnostic potential for evaluating and monitoring immune status in chronic conditions

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