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Research Article

Persistent SARS-Cov-2 Infection in an Immunocompromised Host. Role of the Cellular Immune Response

Duarte A[1]*, Cruces L[2,7], Nabaes Jodar MS[4,5], Goya S[5], Monzani C[2,7], Herrera M[2,7], Mazzitelli I[2,3], Turk G[2,3], Guevara D[6], Viegas M[4,5], Quiroga MF[2,3], Laufer N[2,3] and Cuesta MC[1]

[1]Servicio de Enfermedades Infecciosas Clínica Zabala, Buenos Aires, Argentina.

[2]CONICET-Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires, Argentina.

[3]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

[4]Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

[5]Laboratorio de Virología Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina.

[6]Laboratorio de Biología Molecular Swiss Medical Group, Buenos Aires, Argentina.

[7]Facultad de Medicina, Universidad de Buenos Aires, Argentina.

***Corresponding author: Duarte A**
Servicio de Enfermedades Infecciosas, Clínica Zabala. ZIP Code C1426AAB, Buenos Aires, Argentina.

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Abstract

We present a patient with B-cell depletion and persistent SARS-CoV-2 infection, for 19 weeks in whom the emergence of viral mutations was documented during its evolution, no humoral response was detected, however a specific cellular response was evidenced, highlighting the role of this in the control of infection.

Keywords: Persistent SARS-CoV-2; Immunocompromised; B-cell depletion; Cellular immune response; Mutations

Introduction

Since the onset of the pandemic, cases of persistent SARS-CoV-2 in immunocompromised patients with higher morbidity and mortality have been reported.

The dynamics of viral shedding, transmissibility and the ability to develop an effective humoral and cellular immune response, particularly given the continuous emergence of new variants and vaccination in these patients, continues to be a subject of research [1].

We present a patient with B-cell depletion and persistence of positive SARS-CoV-2 RT-PCR along 19 weeks despite having received treatment with corticosteroids, Convalescent Plasma (CP) and remdesivir. Direct genome sequences were obtained with a pattern of substitutions characteristic of the B.1.1.7 (alpha) lineage and the presence of mutations during its viral persistence. Although specific humoral response was not detected, there was evidence of a specific cellular response that was enhanced after the first dose of the vaccine.

Case report

70-year-old man with B-cell depletion due to mantle cell lymphoma receiving Rituximab maintenance. He was diagnosed with SARS-CoV-2 infection in April 2021 and was admitted to hospital twice because he developed three symptomatic courses of Covid-19, first mild, then severe, and finally severe-critical pneumonia with periods of improvement in between. Standard of care with oxygen therapy and dexamethasone was administered. In the last episode, he received empiric antibiotics, CP for 2 days and remdesivir for 10 days, showing a rapid clinical-radiological and gasometric response.

On day 66 of diagnosis, he was discharged. In outpatient controls for 19 weeks, persistence of detectable SARS-CoV-2 RT-PCR was observed with all Ct ≤ 26. The first negative RT-PCR was obtained on day 148 **(Figure 1A)**. Two months later, he received the first dose of vaccine for Covid-19.

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Additional and complementary assays were performed. Evaluation of SARS-CoV-2 RNA in plasma was performed on day 49 after symptom onset with a non-detectable result. In viral infectivity studies, cytopathic effect on Vero cells was not observed but SARS-CoV-2 RNA was detected in the cell culture supernatant. Direct viral sequences were obtained from 7 Nasopharyngeal Samples (NPS) for the region comprising amino-acids 340 to 574 of the S protein. All sequences presented the pattern of substitutions characteristic of the B.1.1.7 (alpha) lineage, with only 2 non-synonymous substitutions with respect to the reference sequence (GISAID Accession ID EPI_ISL_ 601443). In order to analyze the intra-host evolution throughout the persistent infection, NGS sequencing by Illumina platform was performed in four NPS with adequate viral RNA in quantity and quality at different time points of infection (40, 78, 98 and 125 days after the first positive PCR: T40, T78, T98 and T125, respectively). The analysis of the Variant Call Format (VCF) files showed a clear intrahost variation across the four analyzed samples at the amino acidic level (mainly in S and nsp3 proteins) (supplementary table A). At the sample level, mixed viral populations were found. The most interesting result was found in T78, which was taken after convalescent plasma therapy 56 days after diagnosis with a mixed population in four positions of the N Terminal Domain (NTD) of the S protein **(Supplementary Figure 1 A)**. The detailed results of the NGS analyses of the four samples are described in Supplementary material.

Assays to detect IgG against Spike (COVIDAR) and against nucleocapsid (N, CMIA) proteins were performed on several samples, including those obtained after vaccination **(Figure 1A)** but all showed negative results. However, specific cellular responses against N and S proteins were evidenced. Cellular immune response was evaluated at months 2 and 3 during infection and also 3 months after the last positive PCR result. For that, ELISpot was performed to detect Interferon-gamma producing cells after stimulation with SARS-CoV-2 Wuhan variant peptide pools and proteins. More specifically, PBMCs were stimulated in the presence of Spike, RBD and Nucleocapsid proteins or peptide pools from S and N viral proteins (co-stimulation with anti-human CD28 and CD49d was performed).

A gradual increase in the response against Spike and Nucleocapsid peptide pools was observed, reaching the highest values after the PCR negative result. In particular, Spike-specific responses were markedly higher compared to N-specific responses as time progressed, i.e., month 3 after infection and month 3 after the first negative SARS-CoV-2 PCR result **(Figure 1B)**. No changes were observed over the response profile to Spike, Nucleocapsid nor RBD protein antigens **(Supplementary materials Figure 1B)**.

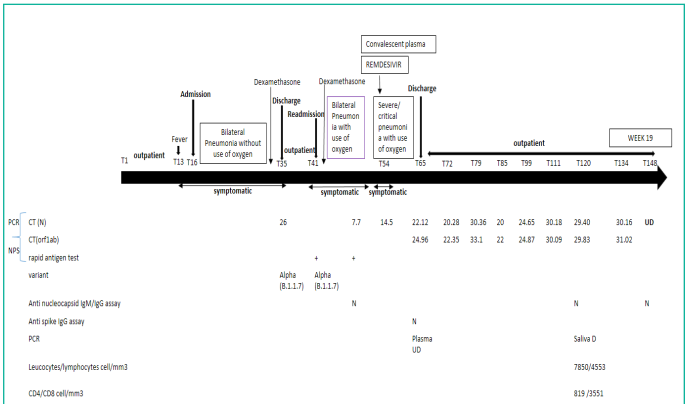
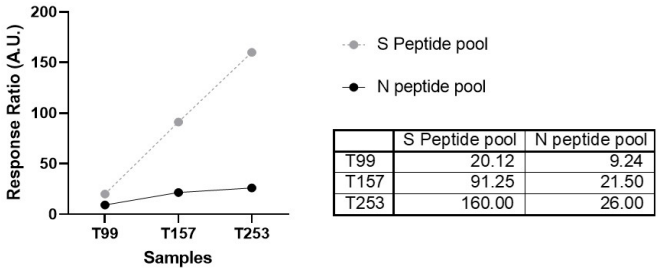


Figure 1A: Timeline of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-related investigations. Ct, cycle threshold; orf 1ab: gen ORF 1ab; N, nucleocapsid; T: time; IgM/IgG: immunoglobulin M/G; PCR: Real time polymerase chain reaction; NPS: nasopharyngeal.



	S Peptide pool	N peptide pool
T99	20.12	9.24
T157	91.25	21.50
T253	160.00	26.00

Figure 1B: Cellular immune response after in vitro stimulation of PBMCs with SARS-CoV-2 peptide pools**.** IFN-γ ELISpot assays were performed to determine the frequency of Ag-experienced T cells in peripheral blood at the indicated times (T99, T157 and T253). Stimulation of BPMCs with peptide pools encompassing S protein or N protein was performed. Afterwards, IFN-γ producing cells were determined as illustrated in the *Supplementary materials* section.

Discussion

Given that the dynamics of SARS CoV-2 is the result of a complex balance between its replication, the immune response and possible therapeutic options, it is expected that immunosuppressed patients may offer the virus better opportunities to persist.

The humoral immunity deficit of our patient due to his underlying disease and the anti-tumor treatment he received explains the absence of seroconversion detected even after vaccination, as well as the difficulty to eliminate the virus [1].

Coronaviruses accumulate mutations at a much slower rate than other RNA viruses, and most of these have little or no phenotypic impact. However, some can significantly influence viral transmissibility and the ability of the virus to escape the host's immune response [2-4].

Whole genome sequencing of SARS-CoV-2 in persistent infections is key to understanding and expanding knowledge about intra-host evolution and variant acquisition (o diversity acquisition). These modifications could impact the ability to confer resistance to neutralizing antibodies leading to partial immune escape or otherwise compensate for infectivity deficits associated with other non-synonymous substitutions.

The alpha variant (B.1.1.7), one of the first emerging lineages carrying S: N501Y, spread in southeastern England in early 2020 and quickly became dominant in the world. Despite being significantly more transmissible than wild-type genotypes, Alpha was not associated with significant immune escape from the neutralizing activity of convalescent or vaccinated sera [3,4].

In our patient, the study of the genome on 7 samples showed the presence of the successful variant B.1.1.7, with the presence of intravariant mutations. Viral infectivity was demonstrated by the presence of SARS CoV-2 RNA in the viral culture supernatant, despite the absence of cytopathic effect.

This case highlights a still unresolved problem of immunocompromised hosts, which can spread infectious virus for longer periods of time and, since it is not possible to carry out routine viral cultures to demonstrate their infectivity, alternative criteria are required to define isolation times. The problem becomes even more complex when considering that they are also a permanent source of emergence of variants and therefore

perpetuation of the ongoing pandemic with implications for the effectiveness of currently available therapeutics and vaccines.

Several studies provide data on the virus and its evolutionary dynamics, on humoral immunity but the role of the cellular response in these patients is less well understood. Trials that analyze the responses of these patients to vaccines maintain that when they are evaluated through the combination of serology and cell-based assays, a lower response is observed compared to healthy individuals [1-4].

Patients with hematologic malignancies show complex and escalating immunologic consequences of SARS-CoV-2, including T-cell depletion, malignant B-cell vulnerability and impaired seroconversion with subsequent chronic or recurrent COVID disease. Experience with the SARS-CoV-2 treatment in this group is still scarce [5].

Remdesivir aims to reduce viral load before the virus triggers a powerfully devastating hyper inflammatory response. In immunocompetent patients it is administered within the first 7-10 days after the onset of symptoms, since viral replication spontaneously decreases thereafter, and subsequent therapy does not prevent the possible cytokine storm [6,7]. Remdesivir monotherapy is associated with treatment failure, but combination with CP appears to be a promising therapy [8-10].

This case indicates that the simultaneous use of remdesivir and CP for protracted COVID-19 in B-cell deficient patients could lead to viral clearance when antigen-specific T cells are elicited. These results also can argue against the concept that the beneficial effect of remdesivir and CP is achieved exclusively when they are administered at the beginning of the infection [11,12].

We believe that this case report reinforces the importance of antigen-specific T-cell response for the virus control and impact on clinical outcome. A more thoughtful knowledge about the SARS CoV-2 specific cell response and mechanism to enhance it may contribute to new strategies for monitoring and clinical decision-making in high-risk patients with COVID-19. In conclusion, our results highlight the importance of the immunocompromised patient as a model of viral persistence and source of variants and therefore epidemiological concern about the implications regarding the perpetuation of the pandemic and loss of therapeutic strategies and efficacy of available vaccines.

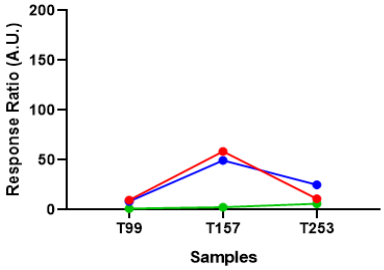
We also highlight the importance of the different pillars of the immune response in these patients, which are decisive for controlling and limiting the dynamics of the viral infection.

Supplementary materials

SARS-CoV-2 specific cellular responses. PBMC were plated on sterile 96-well plates (MultiScreen IP plates; Millipore), coated with mouse anti-human IFN-γ monoclonal antibody (BD Biosciences) at $2x10^5$ cells/well. SARS-CoV-2 Spike, RBD, Nucleocapside proteins (kindly provided by Dr. A. Gamarnik, Leloir Institute, Buenos Aires, Argentina), Spike or Nucelocapside peptide pools (BEI Resources, NIAID, NIH. NR-52402, and NR-52404) were titrated beforehand and added in duplicate wells (final concentration 10µg/ml for proteins and 2µg/ml for peptide pools). Negative (medium plus 0.05% DMSO) and positive (PHA 10µg/ml Sigma-Aldrich) controls were included for each donor. Plates were developed using biotinylated anti-human IFN-γ monoclonal antibody, streptavidin-peroxidase complex, and AEC (3-amino-9-ethylcarbazole) substrate reagent set (BD

Table A. Overview of the SARS-CoV-2 variant allele frequencies across four samples.
The table summarizes the positions in the viral genome and the variant frequencies in the different samples using a cut off value of 5%. The reference allele corresponds to the hCoV-19/Wuhan/WIV04/2019 genomic sequence (EPI_ISL_402124) and variant frequencies are indicated in parentheses. The symbol ? represents low coverage sites. In addition, the locations of deletions, insertions and synonymous and nonsynonymous mutations with their respective amino acid changes are shown.

Genome position	PAIS-A9987 25/5/21 (T40)		PAIS-A9985 2/7/21 (T78)		PAIS-A9984 22/7/21 (T98)		PAIS-A9983 18/8/21 (T125)		Protein	Biological effect	Amino acid change
	Reference allele (Frec%)	Alternative allele (Frec%))	Reference allele (Frec%)	Alternative allele (Frec%)	Reference allele (Frec%)	Alternative allele (Frec%)	Reference allele (Frec%)	Alternative allele (Frec%)			
3158	A (100%)		A (60%)	G (40%)	G (100%)		G (100%)		nsp3	non synonymous variant	T147A
3684	A (100%)		A (100%)		A (88%)	T (12%)	A (100%)		nsp3	non synonymous variant	Q322L
3685	G (100%)		G (100%)		G (88%)	A (12%)	G (100%)		nsp3	non synonymous variant	Q322L
3686	C (100%)		C (100%)		C (88%)	A (12%)	C (100%)		nsp3	non synonymous variant	H323N
4230	C (87%)	T (13%)	C (100%)		C (100%)		C (100%)		nsp3	non synonymous variant	T504I
4233	A (78%)	G (22%)	A (10%)	G (90%)	G (100%)		A (22%)	G (78%)	nsp3	non synonymous variant	D505G
4237	T (34%)	G (66%)	T (100%)		T (100%)		T (100%)		nsp3	non synonymous variant	N506K
4752	T (100%)		T (100%)		T (100%)		C (15%)	T (85%)	nsp3	non synonymous variant	T678I
9971	A (100%)		A (100%)		A (100%)		A (86%)	T (14%)	nsp4	non synonymous variant	K473stop
10920	A (100%)		A (100%)		A (100%)		A (86%)	G (14%)	nsp5	non synonymous variant	D289G
12614	G (100%)		G (100%)		G (100%)		G (87%)	T (13%)	nsp8	non synonymous variant	D175Y
12624	C (100%)		C (100%)		C (100%)		C (80%)	A (20%)	nsp8	non synonymous variant	P178H
12854	C (78%)	T (22%)	C (11%)	T (89%)	T (100%)		T (100%)		nsp9	non synonymous variant	P57S
14050	G (100%)		G (100%)		G (100%)		G (74%)	A (26%)	nsp12	non synonymous variant	V204I
15026	CAT (100%)		CAT (100%)		CAT (100%)		CAT (83%)	C** (17%)	nsp12	deletion	I530Tfs*146
15444	G (100%)		G (100%)		G (100%)		G (75%)	GT (25%)	nsp12	insertion	C668Lfs*8
21618	C (100%)		C (100%)		C (88%)	T (13%)	C (100%)		S	non synonymous variant	S:T19I
22122	A (100%)		A (70%)	G (30%)	A (100%)		?		S	non synonymous variant	K187R
22208	C (100%)		C (79%)	T (21%)	C (100%)		?		S	non synonymous variant	L216F
22211	C (100%)		C (60%)	T (40%)	T (100%)		?		S	non synonymous variant	P217S
22214	C (100%)		C (77%)	A (23%)	C (100%)		?		S	non synonymous variant	Q218K
23423	C (100%)		C (70%)	T (29%)	C (15%)	T (84%)	T (100%)		S	non synonymous variant	P621S
23986	T (100%)		T (100%)		T (100%)		T (56%)	G (44%)	S	non synonymous variant	D808E
24506	G (100%)		G (100%)		T (10%)	G (90%)	G (100%)		S	non synonymous variant	S982A
25223	A (100%)		A (100%)		A (100%)		A (38%)	G (62%)	S	non synonymous variant	I1221V
26333	C (31%)	T (69%)	C (100%)		C (100%)		C (100%)		E	non synonymous variant	T30I
28309	A (38%)	T (62%)	A (100%)		A (100%)		A (100%)		N	synonymous variant	A12A
28975	GTCT 32%	G*** (68%)	TCT (100%)		GTCT (100%)		?		N	deletion	S235del
29247	C (100%)		C (100%)		C (100%)		C (55%)	T (45%)	N	non synonymous variant	T325I
29540	G (100%)		G (100%)		G (100%)		G (100%)		*		
29761	A (100%)		A (100%)		A (100%)		A (80%)	T (20%)	*		



	Prot S	RBD	Prot N
T99	9.71	8.35	1.18
T157	58.50	49.50	2.50
T253	11.00	25.00	6.00

Supplementary Figure 1A: Cellular immune response after in vitro stimulation of PBMCs with SARS-CoV-2 protein antigens. IFN-γ ELISpot assays were performed to determine the frequency of Ag-experienced T cells in peripheral blood at the indicated times (T99, T157 and T253). Stimulation of BPMCs with Spike (S) protein, RBD protein or Nucleocapside (N) protein was performed. Afterwards, IFN-γ producing cells were determined as illustrated in the *Supplementary materials* section.

Supplementary Figure 1B: Weblogo analysis of the amino acid intrahost variability at the S protein. The y-axis indicates the residue frequency for each sampled time after the first COVID-19 positive test. The position at the S protein sequence is indicated at the top of each column. Symbol "?" indicates lack of sequencing coverage. Sequence logos were created using Weblogo 3 (http://weblogo.threeplusone.com/) according their intrahost residue frequency.

Biosciences). Plates were scanned on an ImmunoSpot reader and specific spots were counted using the ImmunoSpot software (Cellular Technology Ltd.).

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References

1. Haidar G, Mellors JW. Improving the Outcomes of Immunocompromised Patients With Coronavirus Disease 2019. Clin Infect Dis. 2021; 73: e1397-e1401.

2. Boehm E, Kronig I, Neher RA, Eckerle I, Vetter P, et al. Novel SARS-CoV-2 variants: the pandemics within the pandemic. Clin Microbiol Infect. 2021; 27: 1109-1117.

3. Gerdol M, Dishnica K, Giorgetti A. Emergence of a recurrent insertion in the N-terminal domain of the SARS-CoV-2 spike glycoprotein. Virus Res. 2022; 310: 198674.

4. García-Vidal C, Iglesias-Caballero M, Puerta-Alcalde P, Mas V, Cuesta-Chasco G, et al. Emergence of Progressive Mutations in SARS-CoV-2 From a Hematologic Patient With Prolonged Viral Replication. Front Microbiol. 2022; 13: 826883.

5. Cesaro S, Ljungman P, Mikulska M, Hirsch H, Lilienfeld-Toal M, et al. Recommendations for the management of COVID-19 in patients with haematological malignancies or haematopoietic cell transplantation, from the 2021 European Conference on Infections in Leukaemia (ECIL 9). Leukemia. 2022 Jun;36(6):1467-1480.

6. Abdul-Jawad S, Baù L, Alaguthurai T, Del Barrio I, Laing A, et al. Acute Immune Signatures and Their Legacies in Severe Acute Respiratory Syndrome Coronavirus-2 Infected Cancer Patients. Cancer Cell. 2021; 39: 257-275.e6.

7. Brown LK, Moran E, Goodman A, Baxendale H, Bermingham W, et al. Treatment of chronic or relapsing COVID-19 in immunodeficiency. J Allergy Clin Immunol. 2022; 149: 557-561.e1.

8. Beigel JH, Tomashek KM, Dodd LE, Mehta A, Zingman B, et al. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med. 2020; 383: 1813-1826.

9. Prendecki M, Clarke C, Edwards H, McIntyre S, Mortimer P, et al. Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression. Ann Rheum Dis. 2021; 80:1322-1329.

10. Mrak D, Tobudic S, Koblischke M, Graninger M, Radner H, et al. SARS-CoV-2 vaccination in rituximab-treated patients: B cells promote humoral immune responses in the presence of T-cell-mediated immunity. Ann Rheum Dis. 2021; 80: 1345-1350.

11. Jassem J, Marek-Trzonkowska NM, Smiatacz T, Arcimowicz L, Papak I, et al. Successful Treatment of Persistent SARS-CoV-2 Infection in a B-Cell Depleted Patient with Activated Cytotoxic T and NK Cells: A Case Report. Int J Mol Sci. 2021; 22: 10934.

12. D'Abramo A, Vita S, Maffongelli G, Beccacece A, Agrati Ch, et al. Clinical Management of Patients With B-Cell Depletion Agents to Treat or Prevent Prolonged and Severe SARS-COV-2 Infection: Defining a Treatment Pathway. Front Immunol. 2022; 13: 911339.