Open Forum Infectious Diseases

NOVEL ID CASES

Critical Presentation of a Severe Acute Respiratory Syndrome Coronavirus 2 Reinfection: A Case Report

Marta Massanella,[1] Anabel Martin-Urda,[2] Lourdes Mateu,[3,4] Toni Marín,[5] Irene Aldas,[5] Eva Riveira-Muñoz,[1] Athina Kipelainen,[1] Esther Jiménez-Moyano,[1] Maria Luisa Rodriguez de la Concepción,[1] Carlos Avila-Nieto,[1] Benjamin Trinité,[1] Edwards Pradenas,[1] Jordi Rodon,[6] Silvia Marfil,[1] Mariona Parera,[1] Jorge Carrillo,[1] Julià Blanco,[1,7] Julia G Prado,[1] Ester Ballana,[1] Júlia Vergara-Alert,[6] Joaquim Segalés,[6,8] Marc Noguera-Julian,[1,7] Àngels Masabeu,[2] Bonaventura Clotet,[1,3,7] Maria de la Roca Toda,[2] and Roger Paredes[1,3,7]

[1]IrsiCaixa-AIDS Research Institute and Germans Trias i Pujol Health Research Institute, Badalona, Catalonia, Spain, [2]Internal Medicine Department, Hospital de Palamós, Palamós, Catalonia, Spain, [3]Infectious Diseases Department, Hospital Germans Trias i Pujol, Catalonia, Spain, [4]Universitat Autònoma de Barcelona, Catalonia, Spain, [5]Pneumology Department, Hospital Germans Trias i Pujol, Catalonia, Spain, [6]Institute of Agrifood Research and Technology Centre de Recerca en Sanitat Animal, Campus de la Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain, and [7]Universitat de Vic Central de Catalunya, Vic, Catalonia, Spain, and [8]Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfections have been reported; however, most cases are milder than the primary infection. We report the first case of a life-threatening critical presentation of a SARS-CoV-2 reinfection.

Methods. A 62-year-old man from Palamós (Spain) suffered a first mild coronavirus disease 2019 (COVID-19) episode in March 2020, confirmed by 2 independent SARS-CoV-2 nasopharyngeal polymerase chain reaction (PCR) assays and a normal radiograph. He recovered completely and tested negative on 2 consecutive PCRs. In August 2020, the patient developed a second SARS-CoV-2 infection with life-threatening bilateral pneumonia and Acute respiratory distress syndrome criteria, requiring COVID-19–specific treatment (remdesivir + dexamethasone) plus high-flow oxygen therapy. Nasopharyngeal swabs from the second episode were obtained for virus quantification by real-time PCR, for virus outgrowth and sequencing. In addition, plasma and peripheral blood mononuclear cells during the hospitalization period were used to determine SARS-CoV-2–specific humoral and T-cell responses.

Results. Genomic analysis of SARS-CoV-2 showed that the virus had probably originated shortly before symptom onset. When the reinfection occurred, the subject showed a weak immune response, with marginal humoral and specific T-cell responses against SARS-CoV-2. All antibody isotypes tested as well as SARS-CoV-2 neutralizing antibodies increased sharply after day 8 postsymptoms. A slight increase of T-cell responses was observed at day 19 after symptom onset.

Conclusions. The reinfection was firmly documented and occurred in the absence of robust preexisting humoral and cellular immunity. SARS-CoV-2 immunity in some subjects is unprotective and/or short-lived; therefore, SARS-CoV-2 vaccine schedules inducing long-term immunity will be required to bring the pandemic under control.

Keywords. immune responses; life-threatening COVID-19; reinfection; SARS-CoV-2; secondary infection.

High levels of population immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will be needed to control the coronavirus disease 2019 (COVID-19) pandemic. A handful of cases of SARS-CoV-2 reinfection have been reported worldwide since September 2020 [1–7], casting doubts on the durability of immune responses to this virus. Whereas infections by the closely related betacoronaviruses severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus elicit long-lasting protective immunity [8, 9], immune responses to common-cold coronaviruses are short-lived [10]. Reinfections may occur after a few months, but usually with mild symptoms [11]. SARS-CoV-2 reinfection cases have generally been mild except in 1 case, and none have been life-threatening. Here we report the first critical presentation of a SARS-CoV-2 reinfection and investigate its virological and immune correlates.

CASE REPORT

The patient was a 62-year-old male hospitalist physician from Palamós (Girona) with previous history of mild asthma, hypertension, dyslipidemia, liver steatosis, hyperuricemia, and overweight (body mass index ≥30 kg/m^2). He was receiving treatment with olmesartan, hydrochlorothiazide, and allopurinol. Occasionally, he received oral corticosteroids for his asthma, and intra-articular slow-release triamcinolone plus hyaluronic acid to treat recurrent episodes of right knee pain and swelling.

First Episode of SARS-CoV-2 Infection

Being previously well, on 23 March 2020, he developed fever of 38°C, diarrhea, anosmia, dysgeusia, cough, intense asthenia, and arthromyalgias. Two consecutive nasopharyngeal PCR tests on 24 and 25 March were positive for SARS-CoV-2. With a normal radiograph (Figure 1A), the subject was confined at home and received treatment with hydroxychloroquine per oral (PO) for 7 days (400 mg twice daily on day 1, followed by

Received 10 March 2021; editorial decision 15 June 2021; accepted 21 June 2021.

Correspondence: Marta Massanella, PhD, Hospital Germans Trias i Pujol, Ctra de Canyet s/n, 08916 Badalona, Catalonia, Spain (mmassanella@irsicaixa.es).

Open Forum Infectious Diseases®2021

https://doi.org/10.1093/ofid/ofab329

200 mg twice daily on days 2–7) plus azithromycin 500 mg PO once daily for 3 days. Symptoms improved, but the anosmia, dysgeusia, asthenia, and shortness of breath did not completely resolve until mid-April. On 2 April, a COVID-19 immunoglobulin M (IgM)/immunoglobulin G (IgG) rapid test (Leccurate, Lepu Medical) was negative, and on 7 and 8 April, 2 nasopharyngeal swab PCRs tested negative for SARS-CoV-2. In preparation for a trekking excursion, on 30 May he received 1 dose of intra-articular triamcinolone 40 mg plus hyaluronic acid in the right knee.

Second Episode

On 28 August, 3 days after returning to work from vacation in northern Spain, he developed intense arthromyalgias, headache, fever, cough, and dyspnea. He prescribed himself treatment with oral azithromycin 500 mg once daily for 3 days, inhaled salmeterol, and oral prednisone (30 mg PO the first day, followed by 15 mg PO once daily thereafter). Again, he underwent 2 consecutive nasopharyngeal SARS-CoV-2 PCR tests on 31 August (hospital laboratory) and 1 September (private laboratory), both of which were positive. On 2 September, he was admitted to the emergency room of the Hospital de Palamós for worsening dyspnea and cough. He reported chills, fever 39ºC, myalgias, anosmia, and ageusia. His respiratory rate was 36 breaths/minute, his heart rate was 100 beats/minute, and he had bilateral inspiratory crackles. The electrocardiogram was normal and the chest radiograph showed bilateral alveolar-interstitial infiltrates (Figure 1B). Upon admission, he had lymphopenia and high C-reactive protein, with normal D-dimer and procalcitonin. Baseline arterial blood gas (ABG) (fraction of inspired oxygen [FiO_2], 0.21) was pH = 7.45, partial pressure of oxygen in arterial blood (PaO_2) = 80.5 mm Hg, and partial pressure of carbon dioxide ($PaCO_2$) = 31.0 mm Hg (PaO_2/FiO_2: 383 mm Hg).

On 2 September (day 5 postsymptoms), he initiated remdesivir (200 mg the first day, followed by 100 mg/day for 5 days), dexamethasone (6 mg/day PO), and enoxaparin (40 mg/day subcutaneously), as well as intravenous (IV) amoxicillin-clavulanate, which was eventually withdrawn when urine pneumococcal and *Legionella* species antigens and bacterial sputum and blood cultures were negative. Initially, he remained with oxygen saturation 96%–97% with nasal cannulas 2 L/minute (FiO_2 = 24%), but showed worsening of his radiologic infiltrates with persistence of elevated inflammation markers. A lung computed tomography angiogram on 3 September ruled out pulmonary thromboembolism and confirmed extensive bilateral lung infiltrates with areas of pneumonic consolidation (Figure 1C). He then received 2 doses of tocilizumab (600 mg IV) on 3 and 4 September, and dexamethasone dosing was increased to 20 mg/day. Despite progression of the lung infiltrates (Figure 1D), he remained clinically stable until 7 September (day 10 postsymptoms), when his respiratory status deteriorated abruptly, with ABG (FiO_2 = 35%) of pH = 7.45, PaO_2 = 55 mm Hg, and $PaCO_2$ = 38 mm Hg (PaO_2/FiO_2: 157 mm Hg). He was transferred to the respiratory intermediate care unit at Hospital Germans Trias i Pujol in Badalona for hypoxemic respiratory failure on 8 September. He was started on high-flow oxygen therapy (HFOT) (50 L/minute, 87%) with an ABG of pH = 7.48, PaO_2 = 105 mm Hg, and $PaCO_2$ = 37 mm Hg (PaO_2/FiO_2: 121 mm Hg); the ratio of oxygen saturation index was 5.95 at 6 hours. High-flow respiratory support was maintained during the following 4 days with progressive improvement, and

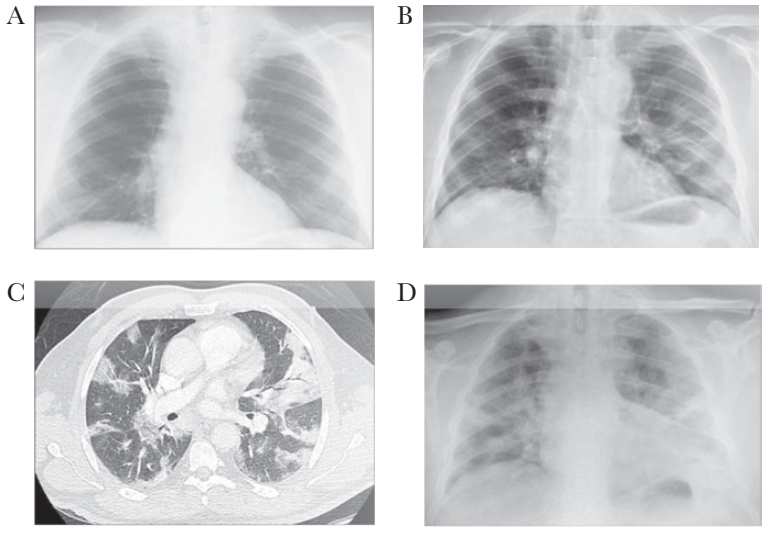

Figure 1. Clinical characterization of the reinfected patient. *A*, First coronavirus disease 2019 (COVID-19) episode, 30 May 2020, with no radiologic infiltrates. Panels *B–D* correspond to 3 consecutive days of the second COVID-19 episode in September 2020, that is, 2 September (day of hospital admission, showing bilateral lung infiltrates, *B*), 3 September (lung angiogram that ruled out a lung thromboembolism but showed extensive bilateral lung infiltrates with left pneumonic consolidation, *C*) and 4 September (with clear radiologic progression in 48 hours, *D*).

was withdrawn after an ABG (0.35% 30 L/minute HFOT) of pH = 7.43, PaO_2 = 102 mm Hg, and $PaCO_2$ = 38 mm Hg (PaO_2/FiO2: 291 mm Hg). Dexamethasone dosing was reduced to 6 mg/day and tapered until withdrawal after 14 days. Given his favorable evolution, the patient was transferred to the infectious diseases ward, where conventional oxygen support was gradually reduced and completely withdrawn on 17 September. The patient was finally discharged home on 18 September without further complications.

METHODS

Patient Consent Statement

The subject provided written informed consent for using diagnostic images and clinical history, as well as for the prospective collection of nasopharyngeal swabs, saliva, and blood samples to characterize the viral and immunological correlates of the second SARS-CoV-2 episode (Supplementary Methods). No remaining sample materials were available from the first episode. The study was approved by the Hospital Universitari Germans Trias i Pujol Ethics Committee Board (reference PI-20-217).

RESULTS

Virology

High levels of SARS-CoV-2 particles (12.0 $\log_{10}$ copies of RNA/mL) were detected by quantitative real-time PCR in nasopharyngeal swab and saliva samples at day 3 postsymptoms (31 August), decreasing progressively thereafter in both compartments (Figure 2A). An infective SARS-CoV-2 isolate was recovered in Vero E6 cells from the 31 August nasopharyngeal swab sample. The full SARS-CoV-2 genome sequence was classified as genotype B.1.79 (G). According to Nextclade analysis [12], it contains 7 known amino acid substitutions (S:L18F, S:A222V, S:D614G, nsp12:P323L, N:A220V, ORF14:L67F, ORF10:V30L) and 14 nucleotide mutations, 11 of which were private. To infer the origin of the virus, we aligned the viral genome against the full GISAID database (16 October 2020) and selected the 100 best Spanish and the 100 best global hits for phylogenetic reconstruction (Figure 2B and Supplementary Data). All best BLAST hits corresponded to sequences annotated on 11 August or later, which were clearly divergent from March and April sequences, strongly suggesting a recent origin of the reinfection virus.

Humoral Immunology

Plasma and serum IgG, immunoglobulin A, and IgM antibodies against SARS-CoV-2 receptor-binding domain (RBD), nucleoprotein (NP), and spike antigens were determined using an in-house sandwich enzyme-linked immunosorbent assay. Antigen-free conditions were used for background subtraction. IgG antibodies against SARS-CoV-2 NP (but not against RBD or spike) were detectable at very low levels 6 days after symptom onset (Figure 2C). Thereafter, all antibody isotypes as well as SARS-CoV-2 neutralizing antibodies increased sharply after day 8 postsymptoms (Figure 2D). Neutralizing antibody levels peaked at days 11–13 from symptoms and plateaued afterward.

Cellular Immunology

An interferon gamma (IFN-γ) enzyme-linked immunosorbent spot assay detected a weak response against SARS-CoV-2 NP and spike recombinant proteins 7 days after symptom onset (Figure 2E). T-cell response levels remained below the limit of detection up to day 19 after symptom onset, when a slight increase of T-cell responses was observed, particularly against spike. Direct intracellular quantification of IFN-γ, interleukin 2, and tumor necrosis factor–α production against S and NP recombinant proteins in $CD4^+$ and $CD8^+$ T cells (Figure 2F) revealed a consistent peak for all cytokines at day 11 postsymptoms, particularly against NP. However, absolute levels of intracellular cytokine production remained low. Finally, we identified spike-specific $CD4^+$ T-cell responses marked by $CD25^+OX40^+$ expression 18 days after symptom onset.

DISCUSSION

This first report of a SARS-CoV-2 reinfection with a life-threatening presentation has important clinical and public health implications. Five months after a mild SARS-CoV-2 infection confirmed by 2 independent SARS-CoV-2 PCRs, and following clinical and PCR resolution, this physician hospitalist suffered from a second SARS-CoV-2 infection with life-threatening, severe respiratory failure, which required intensive respiratory support and COVID-19–specific treatment and, fortunately, evolved well. The SARS-CoV-2 reinfection was firmly documented through (1) quantitative real-time PCR showing repeatedly high viral loads and expected kinetics in independent compartments; (2) the ability to obtain a productively infective SARS-CoV-2 in Vero E6 cells; and (3) the genomic analysis of SARS-CoV-2 showed that the virus had probably originated shortly before symptom onset.

When the reinfection occurred, the subject had no evidence of preexisting humoral or cellular immune protection: either the first SARS-CoV-2 infection did not generate a protective immune response, or such response was lost between the 2 episodes. A rapid serology test performed between the 2 COVID-19 episodes was negative for both IgM and IgG. However, 6 days after the reinitiation of symptoms, a more sensitive in-house serological assay detected traces of anti-NP SARS-CoV-2 IgG. At that time, there was no neutralizing activity at all, and T-cell responses against spike and NP were very weak. The subsequent increase in humoral and cellular responses on days 11 and 18 postsymptoms, respectively, suggests that findings at day 6 were residual adaptative responses from the first COVID-19 episode, which did not protect him from a second infection.

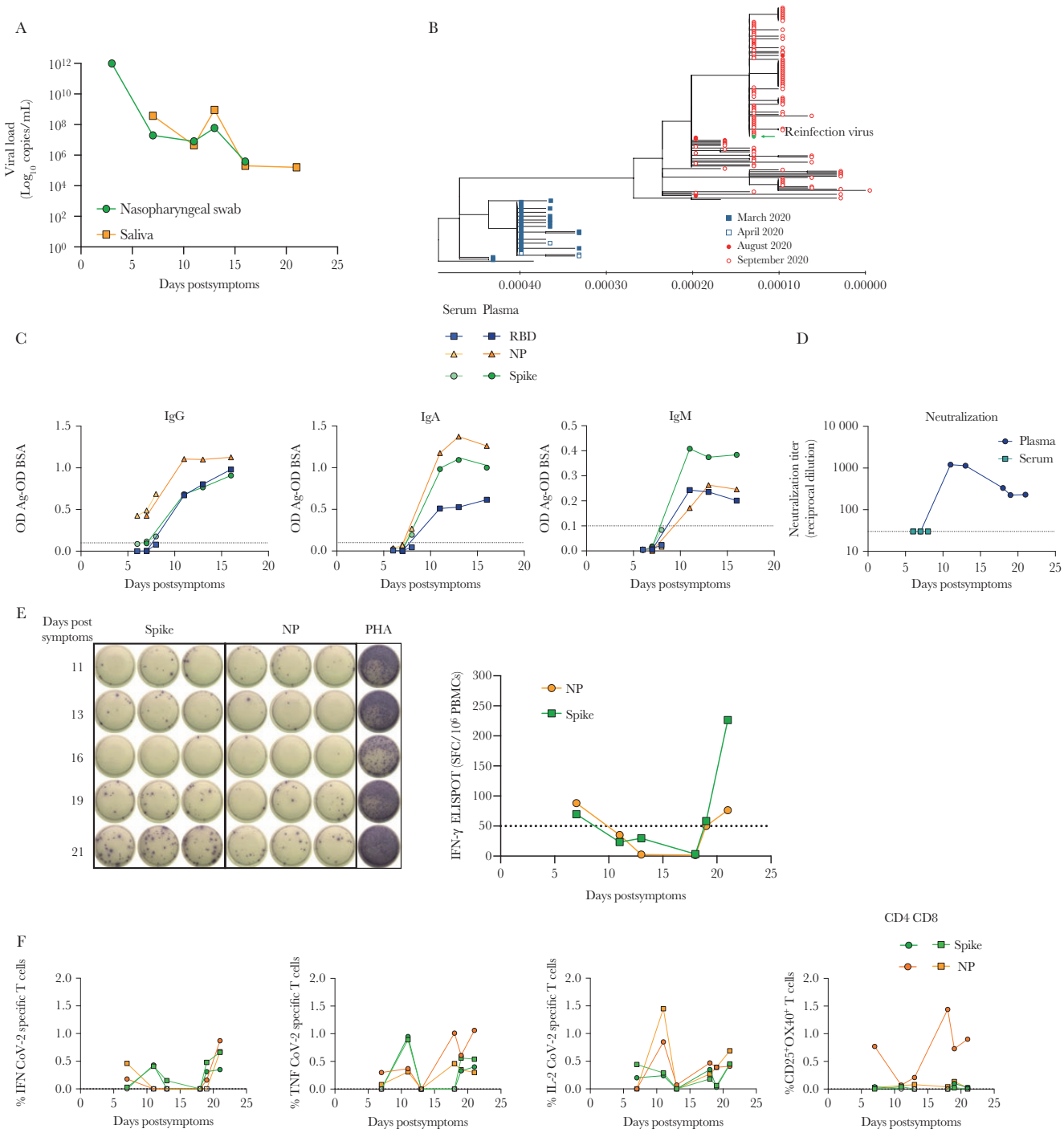

Figure 2. Virological and immunological characterization of the second episode of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfection. *A*, Viral load of SARS-CoV-2 was determined in nasopharyngeal swabs (green circles) and saliva (orange squares) by Real-Time Quantitative Reverse Transcription polymerase chain reaction up to 21 days post–symptom onset. Viral load values were $\log_{10}$ transformed. *B*, Phylogenetic tree reconstruction of SARS-CoV-2 including European samples from April–May (blue squares) and August–September 2020 (red circles). The sample obtained from our patient from 31 August 2020 is indicated in green. *C*, The presence of anti–SARS-CoV-2 antibodies, including immunoglobulin G (IgG, left panel), immunoglobulin A (IgA, middle panel), and immunoglobulin M (IgM, right panel), against receptor-binding domain (RBD, blue squares), nucleocapsid (NP, orange triangles), and spike (green circles) antigens (Ag) was quantified by enzyme-linked immunosorbent assay in serum or plasma samples up to 16 days postsymptoms. The signal was analyzed as the optical density (OD) with noise correction. The specific signal for each Ag was calculated after subtracting the background signal obtained for each sample in Ag-free wells. Dotted lines indicate the limit of positivity. *D*, The presence of neutralizing capacity was determined incubating a serial dilution of serum (light blue squares) or plasma (dark blue circles) with HIV reporter pseudoviruses expressing SARS-CoV-2 S protein and then infecting ACE2-overexpressing HEK293T cells up to 21 days postsymptoms. Neutralization titer was calculated. *E*, T-cell responses were evaluated using the interferon gamma (IFN-γ) enzyme-linked immunosorbent spot (ELISPOT) assay against NP and spike recombinant proteins in peripheral blood mononuclear cells (PBMCs) up to 21 days postsymptoms. Triplicate wells at each time point are depicted as well as the phytohemagglutinin (PHA)–stimulated positive control (left panel). Longitudinal evolution of spot-forming cells (SFCs) per million PBMCs are shown (right panel). *F*, Intracellular cytokine production (IFN-γ, interleukin 2 [IL-2], and tumor necrosis factor alpha [TNF-α]) was evaluated in CD4 and CD8 T cells by flow cytometry after stimulation of PBMCs with NP and spike recombinant proteins. Activation-induced markers (CD25 and OX40) after stimulation were also evaluated. Samples below the limit of the detection of the assay are indicated as open symbols.

Subjects with asymptomatic or mild COVID-19 often develop weaker humoral and cellular immune responses than those with more severe forms of the disease [13–16]. Up to 20% of individuals do not seroconvert after first infection [17]. Neutralizing SARS-CoV-2 antibody levels may also decrease rapidly during the first 2 months after infection [13, 18]. Defective Bcl-6^{+} follicular helper T-cell generation and dysregulated humoral immune induction early in COVID-19 disease, including loss of lymph node germinal centers, have been postulated as a mechanistic explanation for the limited durability of antibody responses in coronavirus infections [19]. SARS-CoV-2–specific T cells often develop in the absence of specific antibodies [20], but the adaptive immune correlates following mild infections are still poorly understood.

In this subject, additional factors such as chronic intermittent exposure to corticosteroids and exposure to immune-modulating effects of hydroxychloroquine might have interfered with his ability to mount protective B- and T-cell adaptive immune responses following the first SARS-CoV-2 infection. In addition to being ineffective to treat SARS-CoV-2 infection, in randomized clinical trials hydroxychloroquine reduced CD4^{+} T-cell counts and increased HIV-1 replication [21], and led to clinical deterioration of chikungunya viral infections, due to delays on adaptive immunity [22].

Several factors might explain why this patient developed a life-threatening reinfection in contrast with previous cases [1–3, 6]. First, he was exposed to a high viral inoculum, as evidenced by the high SARS-CoV-2 titers shortly after reinfection (10^{12} log copies/mL), which is associated with increased disease severity [23–25]. Second, exposure to oral corticosteroids shortly after the initiation of symptoms might have contributed to worsening the clinical course of the second episode. The Randomised Evaluation of COVID-19 Therapy trial [26] demonstrated a trend toward increased mortality when dexamethasone was prescribed in subjects not requiring oxygen supplementation. Of note, the second infection happened in the absence of evident humoral or cellular protection and with no neutralizing activity. Thus, antibody-mediated enhancement was an unlikely mechanism for increased severity of the second infection in this case [27]. Finally, a recent study showed that factors associated with severe symptomatic SARS-CoV-2 reinfections did not differ from those determining severe primary disease [7]. The subject reported here had several of them, including increased age, obesity (body mass index $\geq$30 kg/m^2), and asthma.

CONCLUSIONS

Clinicians, public health officials, and the general public must be aware that life-threatening SARS-CoV-2 reinfections can occur. This case report and others suggest that, at least in some patients, immune responses to SARS-CoV-2 might be short-lived or be too weak to be protective. Symptomatic SARS-CoV-2 reinfections have been reported as early as 3 months after the first infection [1–6]. Systematic prospective cohort studies addressing the durability of SARS-CoV-2 immune responses are urgently needed. They will be key to understand the true frequency and the clinical, virological, and immunological determinants of SARS-CoV-2 reinfections, including life-threatening presentations. Long-term follow-up of SARS-CoV-2 vaccine-induced immune responses [28] will be required to assess their efficacy and durability, and thus the potential need for revaccinations.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We are deeply grateful to all participants and to the technical staff of IrsiCaixa for sample processing (L. Ruiz, E. Grau, R. Ayen, L. Gomez, C. Ramirez, M. Martinez, T. Puig) and staff from the Fight AIDS Foundation (A. Chamorro, J. Puig). We thank CERCA Programme/Generalitat de Catalunya for institutional support and the Foundation Dormeur. We would like to thank everyone who submitted sequences and metadata to GISAID.

Author contributions. E. B. and E. R.-M. performed and analyzed real-time quantitative polymerase chain reaction tests from all samples. M. N.-J. and M. P. performed and analyzed virus sequencing. J. R., J. V.-A., and J. S. grew the virus in vitro. J. C., M. R. C., and C. A.-N. determined and analyzed anti–SARS-CoV-2 antibodies. J. B., B. T., E. P., and S. M. determined and analyzed neutralization activity. J. G.-P., E. J.-M., and A. K. performed and analyzed specific T-cell responses (ELISPOT and intracellular staining). R. P., L. M., A. M.-U., A. M., I. A., M. T., and B. C. contributed to clinical management. M. M. and R. P. coordinated the study, collected all clinical and laboratory data, and wrote the manuscript. All authors discussed the results and approved the manuscript.

Patient consent statement. The subject provided written informed consent for using retrospective testing results, as well as for the prospective collection of samples. The subject provided consent for personal and clinical details along with any identifying images published in this study. The study was approved by the Hospital Universitari Germans Trias i Pujol Ethics Committee Board (King cohort extension, reference number PI-20-217).

Data availability statement. All data generated during this study are included in this published article and the Supplementary Data. All of the information on GISAID sequences used in this study can be found in the Supplementary Data.

Financial support. This work was partially funded by Grifols, the Departament de Salut of the Generalitat de Catalunya (grant numbers DSL0016 to J. B. and DSL015 to J. C.); the Spanish Health Institute Carlos III (grant numbers PI17/01518 and PI18/01332 to J. C. and COV20/00660 to J. G.-P.); and the crowdfunding initiatives "YoMeCorono" (https://www.yomecorono.com), BonPreu/Esclat, and Correos. E. P. was supported by a doctoral grant from Agencia Nacional de Investigatión y Desarrollo, Chile (grant number 72180406).

Potential conflicts of interest. J. B. and J. C. report personal fees from Albajuna Therapeutics, outside the submitted work. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. To KK-W, Hung IF-N, Ip JD, et al. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing [manuscript published online ahead of print 25 August 2020]. Clin Infect Dis **2020**. doi:10.1093/cid/ciaa1275.

2. Van Elslande J, Vermeersch P, Vandervoort K, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain [manuscript published online ahead of print 5 September 2020]. Clin Infect Dis **2020**. doi:10.1093/cid/ciaa1330.
3. Gupta V, Bhoyar RC, Jain A, et al. Asymptomatic reinfection in two healthcare workers from India with genetically distinct SARS-CoV-2 [manuscript published online ahead of print 23 September 2020]. Clin Infect Dis **2020**. doi:10.1093/cid/ciaa1451.
4. Duggan NM, Ludy SM, Shannon BC, et al. Is novel coronavirus 2019 reinfection possible? Interpreting dynamic SARS-CoV-2 test results through a case report. Am J Emerg Med **2021**; 39:256.e1–3.
5. Prado-Vivar B, Becerra-Wong M, Guadalupe JJ, et al. COVID-19 re-infection by a phylogenetically distinct SARS-CoV-2 variant, first confirmed event in South America. *SSRN* [Preprint]. Posted online 8 September **2020**. doi:10.2139/ssrn/3686174.
6. Tillett RL, Sevinsky JR, Hartley PD, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. Lancet Infect Dis **2021**; 21:52–8.
7. Murillo-Zamora E, Mendoza-Cano O, Delgado-Enciso I, Hernandez-Suarez CM. Predictors of severe symptomatic laboratory-confirmed SARS-CoV-2 reinfection. *Public Health* **2021**; 193:113–5.
8. Alshukairi AN, Khalid I, Ahmed WA, et al. Antibody response and disease severity in healthcare worker MERS survivors. Emerg Infect Dis **2016**; 22:1113–5.
9. Liu W, Fontanet A, Zhang PH, et al. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. J Infect Dis **2006**; 193:792–5.
10. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. Nat Med **2020**; 26:1691–3.
11. Galanti M, Shaman J. Direct observation of repeated infections with endemic coronaviruses. J Infect Dis **2020**; 223:409–15.
12. Hadfield J, Megill C, Bell SM, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics **2018**; 34:4121–3.
13. Long Q, Tang X, Shi Q, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med **2020**; 26:1200–4.
14. Peng Y, Mentzer AJ, Liu G, et al. Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent COVID-19 patients. *bioRxiv* [Preprint]. Posted online 8 June **2020**. doi:10.1101/2020.06.05.134551.
15. Trinité B, Tarrés-Freixas F, Rodon J, et al. SARS-CoV-2 infection elicits a rapid neutralizing antibody response that correlates with disease severity. *Sci Rep* **2021**; 11:2608.
16. Pradenas E, Trinité B, Urrea V, et al. Stable neutralizing antibody levels 6 months after mild and severe COVID-19 episodes. Med (N Y) **2021**; 2:313–20.e4.
17. Fill Malfertheiner S, Brandstetter S, Roth S, et al. Immune response to SARS-CoV-2 in health care workers following a COVID-19 outbreak: a prospective longitudinal study. J Clin Virol **2020**; 130:104575.
18. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. N Engl J Med **2020**; 383:1085–7.
19. Kaneko N, Kuo H-H, Boucau J, et al. The loss of Bcl-6 expressing T follicular helper cells and the absence of germinal centers in COVID-19. *SSRN* [Preprint]. Posted online 16 July **2020**. doi:10.2139/ssrn.3652322.
20. Gallais F, Velay A, Wendling M-J, et al. Intrafamilial exposure to SARS-CoV-2 associated with cellular immune response without seroconversion, France. *Emerg Infect Dis* **2021**; 27:113–21.
21. Paton NI, Goodall RL, Dunn DT, et al; Hydroxychloroquine Trial Team. Effects of hydroxychloroquine on immune activation and disease progression among HIV-infected patients not receiving antiretroviral therapy: a randomized controlled trial. JAMA **2012**; 308:353–61.
22. Roques P, Thiberville S-D, Dupuis-Maguiraga L, et al. Paradoxical effect of chloroquine treatment in enhancing chikungunya virus infection. Viruses **2018**; 10:268.
23. Pujadas E, Chaudhry F, McBride R, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. Lancet Respir Med **2020**; 8:e70.
24. Imai M, Iwatsuki-Horimoto K, Hatta M, et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. Proc Natl Acad Sci U S A **2020**; 117:16587–95.
25. Guallar MP, Meiriño R, Donat-Vargas C, et al. Inoculum at the time of SARS-CoV-2 exposure and risk of disease severity. Int J Infect Dis **2020**; 97:290–2.
26. RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med **2021**; 384:693–704.
27. Buchholz UJ, Bukreyev A, Yang L, et al. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci U S A **2004**; 101:9804–9.
28. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2—preliminary report. N Engl J Med **2020**; 383:1920–31.