Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Case Report

Prolonged SARS-CoV-2 infection associated with long-term corticosteroid use in a patient with impaired B-cell immunity

Momoko Morishita a, Manabu Suzuki a, Akihiro Matsunaga b, Keishi Ishizhima c, Tsukasa Yamamoto c, Yudai Kuroda c, Takayuki Kanno a, Yoshie Tsujimoto a, Akane Ishida a, Masao Hashimoto a, Satoru Ishii a, Jin Takasaki a, Go Naka a, Motoyasu Ikura a, Shinyu Izumi a, Tadaki Suzuki d, Ken Maeda e, Yukihito Ishizaka b, Masayuki Hojo a, Haruhito Sugiyama a

a Department of Respiratory Medicine, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo, 162-8655, Japan
b Department of Intractable Diseases, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo, 162-8655, Japan
c Department of Veterinary Science, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan
d Department of Pathology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan

ARTICLE INFO

Keywords:
SARS-CoV-2 infection
Corticosteroids
COVID-19
Viral shedding
Immunocompromised
Rituximab

ABSTRACT

Corticosteroids are widely used to treat severe COVID-19, but in immunocompromised individuals, who are susceptible to persistent infection, long term corticosteroid use may delay viral clearance. We present a case of prolonged SARS-CoV-2 infection in a man with significantly impaired B-cell immunity due to non-Hodgkin lymphoma which had been treated with rituximab. SARS-CoV-2 shedding persisted, despite treatment with remdesivir. Viral sequencing confirmed the persistence of the same viral strain, ruling out the possibility of reinfection. Although SARS-CoV-2 IgG, IgA and IgM remained negative throughout the treatment period, after reduction of the corticosteroid dose, PCR became negative. Long-term corticosteroid treatment, especially in immunocompromised individuals, may result in suppression of cell-mediated immunity and prolonged SARS-CoV-2 infection.

1. Introduction

Corticosteroids are widely used to treat severe COVID-19, but in immunocompromised individuals, who are susceptible to persistent infection, long term corticosteroid use may delay viral clearance.

B-cell depletion caused by rituximab impairs the adaptive immune response and the ability to produce neutralizing antibodies, and long-term use of corticosteroids may weaken the cell-mediated immune response, resulting in persistent infection.

We present a case of prolonged SARS-CoV-2 infection in a patient with significantly impaired B-cell immunity due to non-Hodgkin lymphoma, which had been treated with rituximab, who recovered after the dose of corticosteroids was decreased.

2. Case report

On September 7, 2020, a 51-year-old man with a history of non-Hodgkin lymphoma presented to his doctor with fever and was hospitalized. He had been treated with rituximab, which had last been administered in June 2020 (Fig. 1). SARS-CoV-2 polymerase chain reaction (PCR) performed on a nasopharyngeal swab was positive, confirming the diagnosis of COVID-19. Twelve days after admission, he developed a high fever and hypoxia. More than 10 days had passed since the onset, so the symptoms were thought to be organizing pneumonia, and dexamethasone 6 mg/day was started. Temporarily, he needed 8 L of oxygen, but after starting steroid therapy his fever and respiratory status improved. After 10 days of dexamethasone administration, there was still a demand for oxygen, so 40 mg prednisone was restarted as an organized pneumoniae after COVID-19 infection. The prednisone dose was reduced to 30 mg, and he was discharged from hospital on October 24, 2020.

However, 5 days after discharge, he was readmitted due to a recurrence of fever. When the dose of prednisone was increased to more than 35 mg per day his fever declined, but with lower doses, the fever re-emerged. This pattern occurred repeatedly. Additionally, during the 3 months of treatment, SARS-CoV-2 PCR tests of nasopharyngeal swabs
remained positive. For further treatment, he was transferred to our hospital on November 30, 2020.

He had no fever on admission to our hospital on a dose of 35 mg of prednisone per day. We thought that the long-term use of corticosteroids may be delaying clearance of SARS-CoV-2 virus, so we reduced prednisone to 20 mg, and he was discharged on December 9, 2020, with no symptoms. In order to determine the amount of active virus, we submitted a sputum sample and nasopharyngeal swab for viral testing.

However, the day after discharge, he developed fever again and was readmitted to our hospital on December 16, 2020. Viral isolation testing revealed viable virus (with a cytopathic effect on Vero E6/TMPRSS2 cells) in both the nasopharyngeal swab and sputum samples from the previous admission. Therefore, to reduce the amount of virus, remdesivir was administered for 10 days. PCR testing of a nasopharyngeal swab was negative on December 24, 2020, and the patient was discharged again on December 28.

On January 19, 2021, the patient returned to the hospital with anuria and a SARS-CoV-2 PCR test of a nasopharyngeal swab and viral culture were positive. We conducted SARS-CoV-2 whole-genome viral sequencing of virus isolated from sputum specimens collected on December 3, 2020 (during the first admission to our hospital) and on January 19, 2021. Both viral isolates had the same mutation in common, indicating persistent infection with the same viral strain rather than reinfection (Fig. 2A).

At this time, the patient had no fever or hypoxia so we did not provide any treatment. A PCR test was negative a week later. The last administration of rituximab had been in June 2020. The effect of rituximab is thought to last about 6 months, so we assumed that the effect of rituximab had worn off and that his humoral immunity had recovered and cleared the virus. However, anti-SARS-CoV-2 IgG, IgA and IgM were all negative throughout his treatment, even after his SARS-CoV-2 PCR results became negative again. Our patient differs from the patients with persistent infection.

In this case, it could be concluded that cell-mediated immunity

3. Discussion

We encountered a case of prolonged SARS-CoV-2 infection in a patient with significantly impaired B-cell immunity due to non-Hodgkin lymphoma treated with rituximab. SARS-CoV-2 PCR and virus culture remained positive 133 days after the first PCR test. The ability to produce neutralizing antibodies was evaluated repeatedly, but no IgG, IgA, or IgM production was observed, indicating probable rituximab-induced B cell dysfunction. Moreover, phylogenetic analysis indicated that viruses in the specimens collected on December 3, 2020 and January 19, 2021 were identical and consistent with persistent infection. In some cases, SARS-CoV-2 PCR positivity is known to persist longer than a few weeks, but “positive” PCR results does not necessarily mean presence of viable virus [1,2]. Therefore, we at first thought this theory was also true to our case, meaning that there was no longer viable virus at the first discharge. Then we permitted him to discharge, but we could not confirm the PCR test negative, so we asked him to refrain from going to work. However, it revealed that virus culture was also positive. Thus, we tried to reduce the amount of viable virus with remdesivir and decrease of corticosteroids, and moreover, the next time of discharge, we confirmed PCR negative twice.

As to transmission of the virus, throughout our treatment and follow-up period, there was no episode of infection of the people around the patient. He lived with his wife, who transmitted the virus to the patient, and she never got reinfection.

Considering the theory above, there was some risk of transmission at the first discharge. Presence of symptoms such as cough is also known to affect transmission risk [3], and in this case the only symptom was fever, which might be one of the factors that prevented the transmission.

A literature review revealed other 21 cases of persistent SARS-CoV-2 infection in immunocompromised hosts [4–10] (Table 1). In the 14 cases in which the treatment prior to developing SARS-CoV-2 infection was specified, nine patients had been treated with anti-CD20 monoclonal antibody (seven used rituximab, one used obinutuzumab and one used mosunetuzumab). Thus, it appears that anti-CD20 treatment is a risk factor for prolonged SARS-CoV-2 infection.

In the nine cases in which treatment after developing SARS-CoV-2 infection was reported, six patients were treated with remdesivir. In all six cases, the patient’s symptoms recovered, but the infection subsequently relapsed. Therefore, remdesivir may play a role in decreasing viral load, but is insufficient to clear infection, especially in immunocompromised patients. Four of the six patients transfused with convalescent plasma were reported to have recovered, so insertion of SARS-CoV-2 antibody from outside such as convalescent plasma or monoclonal antibodies may be an option for treating immunocompromised patients with persistent infection.

In our patient, as in previous reports, symptoms decreased after administration of remdesivir, but he relapsed and his SARS-CoV-2 PCR result became positive again. Our patient differs from the patients described in previous case reports in that he recovered without any antibody treatment.

In this case, it could be concluded that cell-mediated immunity
cleared virus in the absence of anti-SARS-CoV-2 antibodies. Coordinated immune responses of humoral and cell-mediated immunity are important for SARS-CoV-2 recovery [11], and CD8 T-cell responses are thought to be an important determinant of persistence [12].

In this case, use of rituximab is likely to have been the main cause of persistent infection, but decreasing the dose of corticosteroids might have helped to stimulate cell-mediated immunity. Actually, as the dose of corticosteroids was decreased, a slight upward trend in absolute lymphocyte counts was observed. T cells are affected more than B cells by corticosteroids [13], so in this case, we could assume that the decrease in lymphocyte counts reflected the decrease in T cells, and as the T cell counts recovered, cell-mediated immunity also recovered. Recently, the RECOVERY trial showed the effectiveness of corticosteroids in severe cases [14] and corticosteroids are now widely used. However, corticosteroids might be associated with a risk of prolonging SARS-CoV-2 shedding, especially in immunocompromised patients. Tang et al. [15] suggested that the early use of corticosteroids is a risk factor for prolonged virus shedding. This case supports this theory.

In conclusion, immunocompromised individuals are at risk of prolonged SARS-CoV-2 infection, and long-term use of corticosteroids, may aggravate this risk. Therefore, as the prolonged use of corticosteroids not only provides anti-inflammatory effects, but is also associated with delayed viral clearance due to cellular immunosuppression, it is important to consider early reduction of corticosteroid use in patients with SARS-CoV-2 infection, especially in immunocompromised patients.

**Author statement**

All authors meet the ICMJE authorship criteria; Momoko Morishita and Manabu Suzuki managed the patient. Manabu Suzuki was responsible for the conception of the work and Morishita Momoko was responsible for the interpretation of data and draft of the work. Yoshie Tsujimoto, Akane Ishida, Masao Hashimoto, Satoru Ishii,
Table 1

21 cases of persistent SARS-CoV-2 infection in immunocompromised individuals.

| Age  | Sex | Baseline                          | Baseline-Treatment                | Treatment for COVID19 |
|------|-----|-----------------------------------|-----------------------------------|-----------------------|
| 70   | M   | Mantle cell lymphoma              | Rituximab, Bendamustine, Cytarabine | NA                    |
| 50   | F   | Neuronelytis Optica               | Rituximab                         | NA                    |
| 47   | F   | MM                                | Dexamethasone, Cisplatin, Doxorubicin, Cyclophosphamide | NA                    |
| 70   | M   | Mantle cell lymphoma              | Mosanetzumab                      | Remdesivir, convalescent plasma |
| 71   | M   | CLL, hypogammaglobulinemia        | IVIG                              | convalescent plasma   |
| 62   | M   | Heart transplantation             | MMF, steroid, Cyclophosphamide    | NA                    |
| 71   | M   | CLL, lymphocytic stem cell        | NA                                | NA                    |
| 71   | M   | CLL, hypogammaglobulinemia        | NA                                | NA                    |
| 71   | M   | CLL, hypogammaglobulinemia        | NA                                | NA                    |
| 71   | M   | CLL, haematological malignancy    | NA                                | NA                    |
| 47   | M   | Follicular lymphoma               | Obinutuzumab                      | Favipiravir            |
| 71   | F   | CLL, hypogammaglobulinemia        | IVIG                              | convalescent plasma   |
| 62   | M   | CLL, hypogammaglobulinemia        | IVIG                              | convalescent plasma   |
| 56   | F   | Follicular Lymphoma               | Rituximab                         | Remdesivir, convalescent plasma |
| 50   | M   | CLL, hypogammaglobulinemia        | Rituximab                         | Remdesivir, convalescent plasma |
| 70   | M   | CLL, hypogammaglobulinemia        | Rituximab                         | Remdesivir, convalescent plasma |
| 73   | M   | MM                                | CAR-T cell therapy                | Remdesivir            |
| 66   | M   | HIV                               | NA                                | NA                    |
| 71   | M   | Cardiac transplantation           | steroid, Mycophenolic acid, Belatacept | NA                    |
| 71   | M   | RA                                | NA                                | NA                    |

Abbreviations: NA; Not Available, MM; Multiple Myeloma, CLL; Chronic Lymphocytic Leukemia, HIV; Human Immunodeficiency Virus, RA; rheumatoid arthritis, IVIG; intravenous immunoglobulin, MMF; mycophenolate mofetil, CAR; chimeric antigen receptor, COVID-19; coronavirus disease 2019.

Jin Takasaki, Go Naka, Motoyasu Ikura, Shinyu Izumi were participated in the discussion for the treatment and gave the important suggestion. Masayuki Hojo and Haruhito Sugiyama reviewed and supervised the manuscript. Akihiro Matsunaga and Yukihito Ishizaka reviewed and supervised the discussion for the treatment and gave the important suggestion. All authors have contributed significantly to the work and approved the final version of the manuscript.

Declaration of competing interest

There are no conflicts of interest in this study.

Acknowledgement

This work was supported by Japan Agency for Medical Research and Development, Japan [grant numbers JP19fk018163, JP20fk108262 and JP20fk0108104], and the National Center for Global Health and Medicine Intramural Research Fund, Japan [grant number 20A2010].

References

[1] Wolff R, Corman VM, Guggemos W, Michael S, Sabine Z, Marcel AM, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581:465–9. https://doi.org/10.1038/s41586-020-2196-x.
[2] Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA 2020;323(22):2249–51. https://doi.org/10.1001/jama.2020.8259.
[3] Madewell ZJ, Yang Y, Longini IM, Halloran ME, Dean NE. Household Transmission of SARS-CoV-2: A Systematic Review and Meta-analysis. JAMA Netw Open 3, e2011756-e2031756. https://doi.org/10.1001/jamanetworkopen.2020.31756.
[4] Taramasso L, Sepulcre C, Mikuliska M, Magnasco L, Lai A, Bruzzone B, et al. Duration of isolation and precautions in immunocompromised patients with COVID-19. J Hosp Infect 2021;111:202–4. https://doi.org/10.1016/j.jhin.2021.02.014.
[5] Camprubi D, Gaya A, Marcos MA, Martí-Soler H, Soriano A, Mosquera MDM, et al. Persistent replication of SARS-CoV-2 in a severely immunocompromised patient treated with several courses of remdesivir. Int J Infect Dis 2021;104:379–81. https://doi.org/10.1016/j.ijid.2020.12.050.
[6] Reuken PA, Stallmach A, Pletz MW, Brandt C, Andrews N, Hahnfeld S, et al. Severe clinical relapse in an immunocompromised host with persistent SARS-CoV-2 infection. Leukemia 2021;35:920–3. https://doi.org/10.1038/s41375-021-0175-8.
[7] Helleberg M, Niemann CU, Moestrup KS, Kirk O, Lebech AM, Lane C, et al. Persistent COVID-19 in an immunocompromised patient temporarily responsive to two courses of remdesivir therapy. J Infect Dis 2020;222:1103–7. https://doi.org/10.1093/infdis/jjaa072.
[8] Abbasi J. Researchers tie severe immunosuppression to chronic COVID-19 and virus variants. JAMA 2021;325:2033–5. https://doi.org/10.1001/jama.2021.7212.
[9] Hensley MK, Bain WG, Jacobs J, Nambulli S, Parihk U, Gillo A, et al. Intractable coronavirus Disease 2019 (COVID-19) and prolonged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication in a chimeric antigen receptor-modified T-cell therapy recipient: a case study. Clin Infect Dis 2021;73:e815–21. https://doi.org/10.1093/cid/ciaa072.
[10] Tarhini H, Recoing A, Bridier-Nahmias A, Rahi M, Lambert C, Martres P, et al. Long-term severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectiousness among three immunocompromised patients: from prolonged viral shedding to SARS-CoV-2 superinfection. J Infect Dis 2021;223:1522–7. https://doi.org/10.1093/infdis/jiaa075.
[11] Rydzynski-Moderhacher C, Ramirez SI, Dan JM, Grifoni A, Hasting KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell 2020;183:996–1012. https://doi.org/10.1016/j.cell.2020.09.038. e19.
[12] Vibhlok LK, Nielsen SF, Pahus MH, Frattari GS, Olsen R, Andersen R, et al. SARS-CoV-2 persistence is associated with antigen-specific CD8 T-cell responses. ElBioMedicine 2021;64:103230. https://doi.org/10.1016/j.ebiomed.2021.103230.
[13] Matthew JO, Yuri K, Angelique B, Foo C, Jinguo C, Rongye S, Huizhi Z, Ena W, John ST, Robert N, CHI Consortium, et al. Effects of Systemically Administered Hydrocortisone on the Human Immunome. Sci Rep 2016:6:23002. https://doi.org/10.1038/srep23002.
[14] RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med 2021;384:693–704. https://doi.org/10.1056/nejmoa2023127.
[15] Tang X, Feng YM, Ni JX, Zhang JY, Liu LM, Hu K, et al. Early use of corticosteroids may prolong SARS-CoV-2 shedding in non-intensive care unit patients with COVID-19 pneumonia: a multicenter, single-blind, randomized control trial. Respiraion 2021;100:116–26. https://doi.org/10.1159/000512063.