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Long-term asymptomatic SARS-CoV-2 infection associated with deficiency on multiple immune cells

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ABSTRACT

The immune responses and the function of immune cells among asymptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection cases, especially in immuno-compromised individuals, remain largely unknown. Here we present a case of asymptomatic SARS-CoV-2 infection that lasted for at least 67 days. The patient has administrated Thymalfasin as 1.6 mg per dose every other day from Day 45 to 70, plus 200 mg per dose Arbidol antiviral therapy three doses per day from Day 48 to 57. Throughout the infection, no anti-SARS-CoV-2 specific IgM or IgG antibodies were detected. Instead, the patient showed either a low percentage or an absolute number of non-classical monocytes, dendritic cells (DCs), $CD4^+$ T cells, and regulatory T cells (Tregs), which may account for the clinical feature and absence of antibody response. This case may shed new light on the outbreak management related to control/prevention, treatment, and vaccination of SARS-CoV-2 and other virus infections in immunocompromised individuals.

1. Introduction

The coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the clinical manifestations were widely varied, ranging from asymptomatic to mild, moderate, and severe pneumonia, which frequently leads to death [1]. The variability of disease severity was closely related to the individual immune responses to SARS-CoV-2 after the first infection [2]. For example, Wong *et al.* reported that total lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, B cells, and natural killer (NK) cells decreased in COVID-19 patients, and severe cases had a lower level than mild cases [3]. And Zhou *et al.* found that acute SARS-CoV-2 infection resulted in broad immune cell reduction, including T cells, NK, monocyte, and DCs [4]. But most SARS-CoV-2 infected people, including asymptomatic individuals, developed virus-specific antibodies for up to months [5,6].

Immuno-compromised patients are prone to progress into severe or critical types underpinned by impaired immune function. However, in this case study, we present an asymptomatic COVID-19 patient who was initially diagnosed positive in Nigeria but negative in the following three tests before traveling to Guangzhou, China, where she was tested positive again. The patient was administrated Thymalfasin plus Arbidol antiviral therapy. The patient had been positive with real-time Reverse-transcription PCR (RT-PCR) for 67 days, but no SARS-CoV-2 specific IgM or IgG antibodies were detected in the sera. Flow cytometry analysis of the blood samples found that the patient had dysfunctions in immune response with a low percentage or an absolute number of non-classical monocytes, DCs, $CD4^+$ T cells, and Tregs.

2. Case presentation

A 33-year-old female overseas worker was diagnosed as SARS-CoV-2 nucleic acid positive during quarantine when entering Guangzhou, China. The patient was initially diagnosed but without any symptoms in early January of 2021 (Day 17) in Nigeria, where she had worked since 2019. But the tests performed on Day 13, 12, and 8 showed negative, and she took an airplane on Day 6 back to Guangzhou.

During quarantine time, real-time RT-PCR (Daan Gene, China) of nasal swabs were collected on Day 3 and 2, showed negative but posi-

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tive for both ORF1ab and N genes on Day 0, with C_T values of 33.62 and 37.31, respectively (Fig. 1). Then the patient was sampled every two days. On Day 2, the C_T values dropped to 23.57 and 18.53 for ORF1ab and N, respectively; then the CT values increased towards the detection limit, indicating virus clearance. However, the patient was negative for both genes until 69 and 70 days later, when she was discharged. No SARS-CoV-2 specific IgM or IgG antibodies were detected in the sera collected on Day 0, 28, 35, 41, 48, 55, 62, 69, 77 and 98 with SARS-CoV-2 IgM/IgG detection kit (Livzon, China, Fig. 1).

Although chest CT examination on Day 0 showed multiple small solid nodules in the bilateral oblique fissure of the left lung and lymph node, the left hilar showed calcification. The patient showed no other symptoms and had nothing noted about multiple tests, including BCA, liver function, biochemical tests, coagulation function, erythrocyte sedimentation rate, procalcitonin, C-reactive protein, myocardial enzymes, and myocardial injury markers. The patient was administrated Thymalfasin 1.6 mg per dose every other day from Day 45 to 70, plus Arbidol antiviral therapy 200 mg per dose three doses per day from Day 48 to 57, when the virus wasn't cleared yet.

Blood was sampled on Day 77 and Day 98 and subjected to flow cytometry tests on various cell populations. As shown in Table 1, total monocytes were normal, with a slight increase of classical monocytes on Day 98 and a decrease of intermediate monocytes on Day 77, both in percentage but not in absolute number. Still, compared to the reference value, the rate of non-classical monocytes in monocytes was lower on Day 77 and dropped almost 50% in both percentage and absolute number on Day 98. But DCs were reversed for both days, increasing from 0.15% to 0.27%, though the total number was significantly low. Among DCs, myeloid DCs (mDCs) showed a similar trend as DCs; however, type 1 and type 2 mDCs were relatively high in percentage. A low rate was also found for $CD4^+$ T cells but didn't vary much on both days. Like non-classical monocytes, Tregs were deficient and significantly decreased from Day 77 to Day 98, while Naïve Tregs had some increase but were still low on both days. $CD8^+$ T cells and NK cells were normal in percentages and absolute numbers. The patient had slightly low B cells on Day 77 in total number, but not in rate.

3. Discussion

In severe and critical cases, acute SARS-CoV-2 infection could reduce broad immune cells, including $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and DCs in severe and critical cases [3,4]. Benjamin *et al.* found high proportions of SARS-CoV-2-reactive cytotoxic $CD4^+$ T cells and a reduced proportion of SARS-CoV-2-reactive Tregs in hospitalized patients [7]. But SARS-CoV-2 infection led to diverse effects on monocytes, with reduction of non-classical monocytes and accumulation of classical monocytes in severe patients [8]; Gatti *et al.* also reported an increase of non-classical and intermediate monocytes in patients with moderate symptoms [9]. In addition, non-classical monocytes increased in patients with infectious diseases, and *in vitro* cultured non-classical monocytes exhibit phenotypic and functional dendritic cell-like characteristics [10,11], indicating they play essential roles in the immune response against pathogens.

Long *et al.* reported that 81% and 62% of asymptomatic patients tested positive for IgG and IgM after exposed for 3 to 4 weeks, respectively [6]. Although the low percentage or an increase in absolute number of multiple immune cells of the case might result from SARS-CoV-2 infection, the absence of SARS-CoV-2 specific antibody indicated that the patient might have dysfunctions of the immune system response. Supporting, the patient complained of frequent cough and cold, and no IgG antibody was detected against yellow fever virus (YFV) when the patient was vaccinated before she went to Nigeria in 2019 (data not shown).

Interestingly, SARS-CoV-2 RNA was detected up to 105 days after initial diagnosis in an immunocompromised female individual with chronic lymphocytic leukemia who finally cleared the virus after two doses of convalescent plasma transfusion [12]. Our patient was diagnosed positive for 67 days. Thymalfasin was administered as 1.6 mg per dose every other day from Day 45 to 70, plus Arbidol antiviral therapy as 200 mg per dose three doses per day from Day 48 to 57, when the virus wasn't cleared yet. Our patient was initially diagnosed positive in Nigeria but became negative in the following three tests before traveling to Guangzhou, China, where she became positive again. At least 13 days when the virus was cleared between the two diagnostic results. It's interesting to investigate how the virus was removed twice in such an individual with a dysfunctional immune response.

It's not clear whether the second result was the reoccurrence of the first one or from another infection. However, since the patient developed no antibodies against SARS-CoV-2 and YFV, it's possible that the second was from reinfection; in line with it, one of her colleagues was diagnosed on the day before leaving Nigeria SARS-CoV-2 with fever.

In summary, we report a case of asymptomatic SARS-CoV-2 infection up to 67 days with a low percentage or an absolute number of non-classical monocytes, DCs, $CD4^+$ T cells, and Tregs. In addition, the patient generated no antibodies against SARS-CoV-2 or YFV despite being vaccinated. These results indicated that the patient

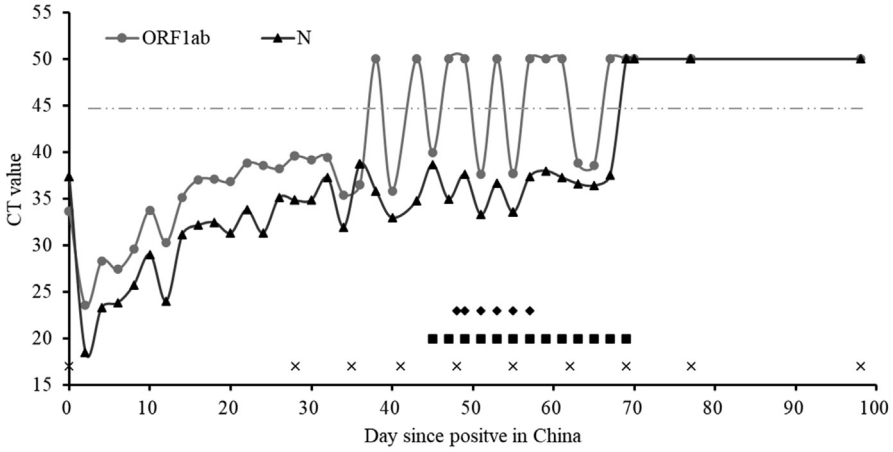

Fig. 1. The C_T value of real-time Reverse-transcription PCR (RT-PCR) detecting ORF1ab and N genes. The dotted line indicates the detection limit, and the negative detection is given a C_T value of 45. × indicates the negative result of SARS-CoV-2 specific IgM and IgG antibodies, ■ indicating the treatment of Thymalfasin as 1.6 mg per dose every other day, and ◆ Arbidol therapy as 200 mg per dose, three doses a day.

Table 1
Summary of flow cytometry analysis of immune cells in the whole blood.

Cell population	Markers	Day 77		Day 98		Reference value
		Percentage	Indi.*	Percentage	Indi.	Percentage/denominator
		Absolute number/μL		Absolute number/μL		Absolute number/μL
Monocytes	$CD14^+$	9.51		9.81		3.00–10.90/leukocytes
		360.00		370.00		144.00–702.00
Classical monocytes	$CD14^{high}$ $CD16^-$	92.02		93.88	↑	68.44–93.40/monocytes
		332.00		347.00		114.00–589.00
Intermediate monocytes	$CD14^{high}$ $CD16^+$	2.02	↓	3.83		2.60–15.80/monocytes
		7.00		14.00		7.00–70.00
Non-classical monocytes	$CD14^+$ $CD16^{high}$	1.97	↓	1.00	↓	2.20–16.70/monocytes
		7.00		4.00	↓	7.00–86.00
Dendritic cells	Lin^- $HLA\text{-}DR^+$	0.15	↓	0.27		0.20–1.90/leukocytes
		6.00	↓	10.00	↓	20.00–121.00
myeloid DC (mDC)	Lin^- $HLA\text{-}DR^+$ $CD11c^+$	0.09	↓	0.16		0.10–1.70/leukocytes
		3.00	↓	6.00	↓	10.00–107.00
$CD16^+$ mDC	$HLA\text{-}DR^+$ $CD11c^+$ $CD16^+$	15.54	↓	15.17	↓	33.90–98.20/mDC
		1.00	↓	1.00	↓	5.00–95.00
mDC1	Lin^- $HLA\text{-}DR^+$ $CD11c^+$ $CD16^-$ $CD1c^+$ $Clec9A^-$	60.33		73.12	↑	1.70–61.60/mDC
		2.00		4.00		1.00–22.00
mDC2	Lin^- $HLA\text{-}DR^+$ $CD11c^+$ $CD16^-$ $CD1c^-$ $Clec9A^+$	16.97	↑	5.75	↑	0.10–4.50/mDC
		1.00	↑	0.00		0.00–0.90
$CD4^+$ T cells	$CD3^+$ $CD4^+$	39.37	↓	44.07	↓	46.20–78.00/T cells
		300.00		398.00		199.00–1414.00
$CD8^+$ T cells	$CD3^+$ $CD8^+$	43.45		43.30		14.80–48.40/T cells
		331.00		391.00		61.00–1,118.00
NK cells	$CD3^-$ $CD56^+$	13.55		7.64		3.30–32.90/lymphocytes
		135.00		90.00		53.00–569.00
Regulatory T cell (Treg)	$CD3^+$ $CD4^+$ $CD25^{high}$ $FoxP3^+$ $CD4^+$	6.94		3.13	↓	5.10–12.70/T cells
		21.00	↓	12.00	↓	28.00–142.00
Naïve Treg	$CD3^+$ $CD4^+$ $CD25^{high}$ $FoxP3^+$ $CD45RA^+$	6.49		8.09		3.50–77.30/Treg
		1.00	↓	1.00	↓	4.00–68.00
B cells	$CD19^+$ $CD3^-$	4.94		7.14		3.80–21.50
		49.00	↓	84.00		51.00–728.00

* ↑ and ↓ indicate increase and decrease compared to the reference value, respectively.

had dysfunctions in the immune response. This case may shed new light on the outbreak management related to control/prevention, treatment, and vaccination of SARS-CoV-2 and other virus infections in immunocompromised individuals.

Ethics statement

This study has been approved by Ethic Committee of the Jiangmen Hospital (approval serial number 2020139). The patient has signed informed consent to participate in this study.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Gang He: Investigation, Funding Acquisition. **Xia Chuai:** Data Curation, Funding Acquisition, Writing – Review & Editing. **Dan Liang:** Investigation. **Chunyu Chen:** Resources. **Changzheng Hu:** Resources. **Changwen Ke:** Supervision. **Bixia Ke:** Conceptualization. **Peilin Zhen:** Conceptualization, Project Administration. **Huajun Zhang:** Conceptualization, Writing – Original Draft.

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