Robust long-term immunity to SARS-CoV-2 in patients recovered from severe COVID-19 after interleukin-6 blockade

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eBioMedicine 2022;82: 104153
Published online 8 July 2022
https://doi.org/10.1016/j.ebiom.2022.104153

Summary

Background Whether interleukin-6 (IL-6) blockade in patients with COVID-19 will affect the protective immunity against SARS-CoV-2 has become an important concern for anti-IL-6 therapy. We aimed to investigate the effects of IL-6 blockade on long-term immunity to SARS-CoV-2.

Methods Prospective, longitudinal cohort study conducted in patients hospitalized for severe or critical COVID-19 with laboratory confirmed SARS-CoV-2 infection. We assessed humoral (anti-S1 domain of the spike [S], anti-nucleocapsid [N], anti-trimeric spike [TrimericS] IgG, and neutralizing antibodies [Nab]) and T-cell (interferon-γ release assay [IGRA]) responses and evaluated the incidence of reinfections over one year after infection in patients undergoing IL-6 blockade with tocilizumab and compared them with untreated subjects.

Findings From 150 adults admitted with confirmed SARS-CoV-2 infection, 78 were 1:1 propensity score-matched. Patients receiving anti-IL6 therapy showed a shorter time to S-IgG seropositivity and stronger S-IgG and N-IgG antibody responses. Among unvaccinated subjects one year after infection, median (Q1-Q3) levels of TrimericS-IgG (295 vs 121 BAU/mL; $p = 0.011$) and Nab (74.7 vs 41.0 %IH; $p = 0.012$) were higher in those undergoing anti-IL6 therapy, and a greater proportion of them had Nab (80.6% vs 57.7%; $p = 0.028$). T-cell immunity was also better in those treated with anti-IL6, with higher median (Q1-Q3) interferon-γ responses (1760 [702−3992] vs 542 [35−1716] mIU/mL; $p = 0.013$) and more patients showing positive T-cell responses in the IGRA one year after infection. Patients treated with anti-IL6 had fewer reinfections during follow-up and responded to vaccination with robust increase in both antibody and T-cell immunity.

Interpretation IL-6 blockade in patients with severe COVID-19 does not have deleterious effects on long-term immunity to SARS-CoV-2. The magnitude of both antibody and T-cell responses was stronger than the observed in non-anti-cytokine-treated patients with no increase in the risk of reinfections.

Funding Instituto de Salud Carlos-III (Spain).

Keywords: SARS-CoV-2; COVID-19; Interleukin-6 blockade; Tocilizumab; Antibody responses; T-cell responses; Reinfection; Immunity

Introduction

Uncontrolled interleukin-6 (IL-6) release resulting from dysregulated host immune response is a distinctive feature of severe SARS-CoV-2 infections,[1] and serum levels of this cytokine correlate with disease severity.[2] Consistently, therapeutic strategies modulating IL-6 have shown to improve clinical outcomes of patients with COVID-19,[3] and tocilizumab, a monoclonal antibody that blocks IL-6 receptors effectively, is currently

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Research in context

Evidence before this study

Therapeutic strategies modulating interleukin-6 (IL-6) have shown to improve clinical outcomes of patients with COVID-19, and are currently recommended for the treatment of patients with severe disease. However, their effects on quality and durability of the immune response against SARS-CoV-2 remain scarce and inconclusive. Published data are limited and restricted to early stages after therapy. A previous study found that the use of anti-IL-6 agents did not affect the initial antibody response against SARS-CoV-2, but significant reductions in neutralizing activity of anti-SARS-CoV-2 antibodies have recently been reported in a small group of patients treated with anti-IL-6 receptor monoclonal antibodies, raising concerns on the risk of reinfection and suboptimal response to vaccination in patients treated with anti-IL-6 agents.

Added value of this study

This study has investigated the effects of IL-6 blockade with tocilizumab on humoral and cellular immunity to SARS-CoV-2 and analyzes the incidence of reinfections over one year after hospital admission for COVID-19. The study shows that blocking IL-6 signaling does not impair long-term immunity to SARS-CoV-2. The magnitude of both antibody and T-cell responses were above the observed in non-anti-cytokine-treated patients and remained significantly stronger one year after recovering from COVID-19. Patients treated with anti-IL6 also had fewer reinfections and responded to vaccination with robust increase in antibody and T-cell immunity.

Implications of all the available evidence

Immunomodulatory therapy based on IL-6 blockade in patients with severe COVID-19 does not have deleterious effects on the development of long-term immunity to SARS-CoV-2. Our data support the long-term safety of this therapeutic strategy from a virological and immunological perspective. The results can also be extrapolated to patients receiving other anti-IL-6 blockers for rheumatologic diseases who acquire SARS-CoV-2 and potentially other acute viral infections and warrant additional studies to understand the role of IL-6 during viral diseases.

recommended for the treatment of patients with severe disease.[4]

Whether IL-6 blockade will affect the antiviral immune response against SARS-CoV-2 has become an important concern for anti-IL-6 therapy in this setting.[5] IL-6 is a multifunctional cytokine that regulates many aspects of innate and adaptive immunity, including the differentiation of B cells, cytotoxic T-lymphocytes, and macrophage/monocyte functions.[6,7] Consequently, therapy directed against IL-6 might prevent mounting a proper immune response when administered to patients with a viral infection. On the other hand, experimental evidence suggests that overexpression of IL-6 might be detrimental and could have negative consequences on the viral immune response by impairing the polarization and functionality of Th1 cells and the lytic capacity of CD8 T-cells through different mechanisms.[8,9] In addition, excessive IL-6 levels could contribute to lymphocytopenia,[10] a striking feature of full-blown COVID-19.[1]

Published data on the effects of therapeutic use of IL-6 blockade on the immune response against SARS-CoV-2 are scarce and restricted to early stages after therapy.[11-13] Whilst we found that the use of anti-IL-6 agents did not affect the initial antibody response against SARS-CoV-2,[11] significant reductions in neutralizing activity of anti-SARS-CoV-2 antibodies have recently been reported in a small group of patients treated with anti-IL-6 receptor monoclonal antibodies, raising concerns on the risk of reinfection and suboptimal response to vaccination in patients treated with anti-IL-6 agents.[13]

Long-term data on host immune response to SARS-CoV-2 and clinical outcome of patients recovered from COVID-19 after treatment with IL-6 inhibitors are crucial to understand the potential impact of this therapy on the protective immunity against SARS-CoV-2 and might provide insight into the role of this cytokine during viral infections. In this work, we comprehensively investigated the effects of IL-6 blockade on both humoral and cellular immunity to SARS-CoV-2 in a cohort of patients admitted with severe or critical COVID-19 treated with tocilizumab and compared them with matched untreated subjects. In both groups, we measured anti-SARS-CoV-2 specific antibodies, serum neutralizing activity and T-cell responses, and analyzed the incidence of reinfections over one year after hospital admission.

Methods

Setting and study subjects

This prospective study was conducted in all the patients hospitalized with confirmed COVID-19 during the first wave of the pandemic (between March 10th and June 30th, 2020) at Hospital General Universitario de Elche (Spain), who were longitudinally followed-up for 12 months. Blood samples for routine lab tests, plasma biomarkers, serologic tests, and nasopharyngeal samples for SARS-CoV-2 were serially obtained at different timepoints during hospital stay, and at 1, 2, 6 and 12 months after patients' discharge. Serum samples for the measurement of the levels of antibodies to SARS-CoV-2 were collected and frozen at −80 °C.

Details of the medical management during hospital stay with preliminary results are provided elsewhere.[11,14] Therapy for COVID-19 was given following institutional

guidelines. Tocilizumab was administered at a dose of 600 mg intravenously if the weight was ≥75 kg or 400 mg when the weight <75 kg if any of the following pre-established criteria were met: a CURB-65 >2; oxygen saturation <93%; respiratory frequency >30 per min; a chest radiograph with bilateral multilobar infiltrates; one of the following biological markers: D-dimer ≥0.7 μg/L, IL-6 ≥40 pg/ml, lymphocytes $<800 \times 10^9$/L, ferritin ≥700 μg/L, fibrinogen >700 mg/dl or C-reactive protein (CRP) >25 mg/L. Patients were reevaluated on the following 24 h. Response to therapy was defined as resolution of fever, improvement in tachypnea, improvement in oxygen saturation by at least 5%, decrease in CRP of at least 25%, or no radiological progression 24 h after tocilizumab administration. Non-response was defined by the absence of any of 24 h response criteria, or an increase in the SOFA score >2 measured at 48–72 h or at day 7 after tocilizumab, ICU admission or death. If no clinical response was achieved at 24 h after tocilizumab administration, defined as persistence of fever, no improvement in tachypnea, no improvement in oxygen saturation by at least 5%, no decrease in CRP of at least 25%, or radiological progression, a second dose of tocilizumab (400 mg) was administered.

Ethics

The protocol (PI19/2021) was approved by the Ethical Committee of the Hospital General Universitario de Elche as part of the COVID-19@Spain study. Informed consent was obtained from all subjects.

Blood collection and processing

Serum, EDTA plasma and whole blood specimens were obtained for measuring SARS-CoV-2–specific antibodies, neutralizing antibodies and interferon-γ (IFN-γ) release assays (IGRA), respectively. Blood was collected in serum tubes, lithium heparin tubes and K2-EDTA tubes, consecutively. Serum tubes were centrifuged, and serum used to perform the anti-spike antibody immunoassay. Whole blood from lithium heparin tube was used for IGRA incubation within 4 h. K2-EDTA tube was centrifuged, and plasma was then aliquoted and stored at −80 °C prior to performing the IgG and neutralizing antibodies ELISA assays.

Antibody responses to SARS-CoV-2

Detection of SARS-CoV-2–specific antibodies was performed with four different immunoassays to detect IgG against SARS-CoV-2 spike and internal nucleocapsid protein, IgG against SARS-CoV-2 trimeric spike protein, and neutralizing antibodies.

IgG against SARS-CoV-2 spike and internal nucleocapsid protein

IgG against the surface S1 domain of the spike protein (S-IgG) and the internal nucleocapsid (N) protein (N-IgG) (Euroimmun, Lubeck, Germany) were measured (in EDTA plasma samples) at hospital admission and at 1, 2, 6 and 12 months after patients' discharge, using commercial semi-quantitative EIA kits in an automated instrument (Dynex DS2® ELISA system) following the manufacturer instructions. Antibody levels were evaluated by calculating the ratio of the optical density (OD) of the patient sample over the OD of the calibrator (sample OD/calibrator OD = S/CO [absorbance/cut-off]). Results were interpreted according to the following criteria: ratio <1.1 was defined as negative and ratio ≥1.1 as positive.

IgG against SARS-CoV-2 trimeric spike protein

IgG antibody serum levels against the trimeric spike protein (TrimericS-IgG) were quantified at the 12-month visit using commercial quantitative immunoassay kits (LIAISON® SARS-CoV-2 TrimericS IgG assay, DiaSorin, Saluggia, Italy) in an automated platform (LIAISON® XL Analyzer) following the manufacturer's instructions. Results were expressed as Binding Antibody Units (BAU) and interpreted according to the following criteria: ≥33.8 BAU/mL was considered positive with a numeric value for quantitative measurement.

Neutralizing antibodies against SARS-CoV-2

Detection of neutralizing antibodies against SARS-CoV-2 (Nab) was performed at the 12-month visit in an automated instrument (Dynex DS2® ELISA system) by means of a surrogate neutralizing antibody test (SARS-CoV-2 NeutraLISA, Euroimmun, Lübeck, Germany), that determines the inhibitory effect of antibodies that can compete with the biotinylated host-cell receptor (ACE2) for the binding to the receptor-binding domain (RBD) of the S1 subunit of SARS-CoV-2 spike protein (inhibition percentage, %IH). Results were interpreted as follows: %IH <20 was considered negative, %IH ≥20 to <35 was considered borderline, and %IH ≥35 was considered positive.

Cellular response to SARS-CoV-2

SARS-CoV-2 cellular response was measured at the 12-month visit using a specific quantitative IFN-γ release assay in whole blood following the manufacturer's instructions (SARS-CoV-2 IGRA stimulation tube set, Euroimmun, Lübeck, Germany). Details are provided elsewhere.[15] Briefly, lithium heparinized blood from each patient was incubated 21 h at 37 °C in the three tubes supplied: blank tube for the individual IFN-γ background and, mitogen tube for unspecific IFN-γ secretion as controls, and stimulation tube coated with antigens of the SARS-CoV-2 spike protein for specific

IFN-γ secretion. The IFN-γ concentration released in the plasma fraction obtained after centrifugation of the three tubes was then measured by an enzyme-linked immunosorbent assay (Human interferon-gamma ELISA, Euroimmun, Lübeck, Germany) with an automated instrument (Dynex DS2® ELISA system) in international units per milliliter (IU/mL). IFN-γ response was defined as stimulated minus unstimulated. Results were interpreted as follows: IFN-γ[SARS-CoV-2] − IFN-γ[blank] <100 mIU/mL was considered negative, 100−200 was considered borderline, and >200 was considered positive. Upper limit of quantification achieved was 5000 mIU/mL. Concentrations of IFN-γ above the calibration curve were defined as >5000 mIU/mL.

Investigation of SARS-CoV-2 reinfections

We evaluated the incidence of late re-infections and recurrences occurring in tocilizumab treated and untreated subjects during the 12-month follow-up period using genomic sequencing to confirm SARS-CoV-2 reinfections. Suspected reinfection was defined according to the CDC criteria.[16] Briefly, subjects with detected SARS-CoV-2 RNA more than 90 days after the first detection of SARS-CoV-2 RNA, whether or not symptoms were present, were considered suspected reinfections. RT-PCR analysis for SARS-CoV-2 was performed by means of a commercially available kit (AllplexTM 2019-nCoV Assay, Seegene, Seoul, Korea) which targeted the E, RdRP, and N genes. Identification of paired specimens from distinct lineages was considered as confirmed SARS-CoV-2 reinfection. Genome sequencing of SARS-CoV-2 was performed on nasopharyngeal samples following ARTIC amplicon sequencing protocol for MinIon version V3.[14,17] Additionally, when SARS-CoV-2 RNA detection or paired specimens for genome sequencing testing were not available, we used serologic testing as a proxy to determine the immunologic response to initial infection and to suspected reinfection, and cases with positivization of antibody to nucleocapsid protein were considered as possible SARS-CoV-2 new cases or reinfections.[16]

Statistics

Continuous data are reported as median ± 25th and 75th percentiles (Q1, Q3), and categorical variables as percentages. Mann-Whitney-Wilcoxon or Student's t-test were used to compare continuous variables, according to the result of Shapiro Wilks' contrast of normality. For categorical variables comparison among tocilizumab treated and untreated patients, the chi-square and Fisher's exact test were used for more of 2 categories and dichotomous variables, respectively.

To balance treatment groups, a propensity score matching logistic regression model was fitted with a 1:1 ratio among groups to compare patients receiving tocilizumab with patients not receiving tocilizumab. Covariates with a p-value <0.05 in the crude comparison between treatment groups were used for matching. Matching variables were relevant baseline data that might have affected treatment decisions: sex, age, Charlson comorbidity index, WHO COVID-19 severity ordinal scale, presence of pneumonia, bilateral pulmonary infiltrates on chest x-ray, severe chronic kidney disease (CKD-EPI creatinine-based estimation equation for glomerular filtration rate <30 mL/min), and CRP levels. Standardized mean differences (SMD) were calculated to examine the balance of covariate distribution between treatment groups. Because SMD is independent of the unit of measurement, it allows comparison between variables with different units of measurement. Matched patients, after propensity score adjustment, were compared for the variables of interest, i.e. S/N-IgG levels throughout the year of follow-up, and anti-trimeric spike IgG, neutralizing antibodies and SARS-CoV-2 IGRA at the 12-month visit. To represent the temporal changes throughout the follow-up of the S/N-IgG levels, local polynomial regression models were employed using weighted least squares to estimate the performance of each antibody since the hospital admission day. Differences in temporal trends were analyzed through linear mixed models. Statistical analyses were performed by R software (R Core Team 2021. R-4.1.0).

Role of the funding source

The funder of the study had no direct role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During the study period, 166 adult patients were admitted with confirmed SARS-CoV-2 infection by RT-PCR, and 150 (90.4%) subjects with available blood samples during the follow-up were finally included for analyses (see Supplementary Figure S1). Of them, 78 (52%) were treated with tocilizumab (69 and 9 received 1 and 2 doses, respectively). Characteristics of the patients before propensity score-matching are shown in Supplemental Table S1. Among patients undergoing IL-6 blockade there were more males, and they had more frequently pneumonia and bilateral infiltrates on chest x-ray. Regarding concomitant treatment, they were more frequently treated with lopinavir/ritonavir and azithromycin, and received more frequently concomitant therapy with corticosteroids (Supplemental Table S1). Their baseline concentrations (median [Q1−Q3]) of CRP (63.1 [32.3−100.3] vs 28.1 [3.3−49.6] mg/L, p<0.001), IL-6 (104.7 [46.9−255.8] vs 13.7 [6.7−31.5] pg/mL, p<0.001)

Variable	Non-Tocilizumab N = 39	Tocilizumab N = 39	Total N = 78	P
Sex, male	22 (56.4)	22 (56.4)	44 (56.4)	1.000
Age, years	66 (56–74)	62 (57–74)	64 (56–74)	0.803
Smoking	5 (12.8)	3 (7.7)	8 (10.3)	0.554
Charlson comorbidity index	3 (1–5)	3 (1–5)	3 (1–5)	0.582
Comorbidities				
Diabetes	13 (33.3)	5 (12.8)	18 (23.1)	0.058
Congestive heart failure	1 (2.6)	3 (7.7)	4 (5.1)	0.615
Previous AMI	3 (7.7)	2 (5.1)	5 (6.4)	1.000
Stroke	4 (10.3)	0 (0)	4 (5.1)	0.115
Respiratory disease	4 (10.3)	9 (23.1)	13 (16.7)	0.224
Renal disease	2 (5.1)	5 (12.8)	7 (9.0)	0.431
Peripheral arterial disease	1 (2.6)	1 (2.6)	2 (2.6)	1.000
Clinical status				
Days from symptom onset to admission	6 (3–11)	6 (4–10)	6 (3–10)	0.976
WHO severity score	4 (4–4)	4 (4–4)	4 (4–4)	0.256
Pneumonia	37 (94.9)	37 (94.9)	74 (94.9)	1.000
Bilateral infiltrates on chest x-ray	24 (61.5)	30 (76.9)	54 (69.2)	0.338
Severe CKD[a]	1 (2.6)	1 (2.6)	2 (2.6)	1.000
C-reactive protein, mg/L	40.7 (18.9–58.8)	54.8 (18.4–72.4)	45.8 (18.6–69.5)	0.441
Microbiological data				
SARS-CoV-2 RNA, log10 copies/sample	4.1 (3.1–4.6)	4.2 (3.2–5.1)	4.2 (3.1–4.8)	0.445
Concomitant antimicrobial/ immunomodulatory drugs				
HCQ-based combinations	38 (97.4)	39 (100)	77 (98.7)	1.000
Azithromycin	36 (92.3)	37 (94.9)	73 (93.6)	1.000
Lopinavir/ritonavir	35 (89.7)	39 (100)	74 (94.9)	0.115
Remdesivir	1 (2.6)	0 (0)	1 (1.3)	1.000
Interferon-β-1b	8 (20.5)	11 (28.2)	19 (24.4)	0.599
Corticosteroids[b]	10 (25.6)	15 (38.5)	25 (32.1)	0.332
Outcomes				
Death	3 (7.7)	2 (5.1)	5 (6.4)	1.000
ICU admission	8 (20.5)	4 (10.3)	12 (15.4)	0.347
Suspected reinfections (table S3)	6 (15.4)	2 (5)	8 (10.3)	0.263

***Table 1*: Characteristics of persons with COVID-19 admitted to hospital by tocilizumab treatment after propensity score matching.**
Categorical variables are expressed as no. and %, and continuous variables as median (Q1-Q3). AMI, acute myocardial infarction; CKD, chronic kidney disease; HCQ, hydroxychloroquine; ICU, Intensive Care Unit.
[a] Glomerular filtration rate <30 mL/min.
[b] Either oral or intravenous dexamethasone or short course methylprednisolone 0.5–1 mg/kg/day divided in 2 intravenous doses for 3 days.

and ferritin (488 [345–710] vs 224 [105–357] ng/mL, $p<0.001$) were significantly higher whereas the lymphocyte count was lower (1.0 [0.8–1.4] vs 1.4 [1.0–1.7] x10^3/μL, $p = 0.002$).

IL-6 blockade was associated with deeper declines in the levels of inflammatory biomarkers and greater increases in lymphocyte counts (Supplemental Figure S2). Median (Q1–Q3) reduction at week 2 for CRP in patients who received tocilizumab was −55.7 (−96.6 to −24.1) vs −10.6 (−41.0 to −0.4) mg/L ($p<0.001$) in untreated patients; for IL-6, −50.3 (−168.2 to −1.1) vs −7.0 (−22.6 to 0.0) pg/mL ($p = 0.009$), and for ferritin, −58.0 (−147.5 to −1.8) vs 0.0 (−8.0 to 14.0) ng/mL ($p<0.001$). Patients undergoing IL-6 blockade showed higher median (Q1–Q3) increases in lymphocyte counts at week 4 (0.2 [0.0 to 0.6] vs 0.2 [−0.1 to 0.6] x10^3/μL, $p = 0.020$), week 8 (0.6 [0.3 to 0.9] vs 0.3 [0.0 to 0.7] x10^3/μL, $p = 0.010$), and week 12 (0.8 [0.4 to 1.3] vs 0.3 [−0.2 to 0.7], $p<0.001$).

After 1:1 propensity score-matching, 39 patients in the tocilizumab group (3 [7.7%] with two doses) and 39 in the control group were compared for the variables of interest. Their baseline characteristics according to study group after matching are presented in Table 1. Absolute standardized mean (ASM) differences between the two study groups diminished compared to those previous to propensity matching, and *p*-values for ASM tests were above 0.05 for all relevant baseline characteristics, including demographics, comorbidities, clinical status, concomitant therapy with corticosteroids and other drugs, and clinical outcomes, reflecting adequate balance between the two groups.

Antibody responses to SARS-CoV-2

IgG against SARS-CoV-2 spike and internal nucleocapsid protein. Figures 1 and 2 and Table S2 show the S-IgG and N-IgG S/CO values of all measurements over time, by anti-IL6 therapy and vaccination, after propensity score matching. Median (Q1–Q3) time from the onset of symptoms to seropositivity for S-IgG in patients who received tocilizumab was 16 (12–20) vs 18 (15–30) days (p = 0.046) in untreated patients; and 16 (12–20) and 18 (15–30) days, respectively, for N-IgG (p = 0.050). Patients receiving anti-IL6 therapy had a stronger S-IgG (peak S-IgG 6.7 [5.2–12.1] S/CO and 4.9 [0.2–7.4] S/CO in patients with or without anti-IL6 therapy, respectively [p = 0.003]), and N-IgG antibody response (peak N-IgG 4.3 [3.5–6.0] and 3.6 [0.1–4.8] in patients with or without anti-IL6 therapy, respectively [p = 0.053]).

As expected, in patients who were not vaccinated during follow-up, antibody titers gradually waned during the 12-month follow-up. Among those who remained unvaccinated at the 12-month visit after COVID-19 diagnosis, the proportion of patients treated with tocilizumab with positive S-IgG antibodies was 90.3% (n = 28) after a median (Q1–Q3) of 364 (361–367) days from the onset of symptoms vs 65.4% (n = 17) (p = 0.016) after 364 (357–368) days in untreated patients. A strong S-IgG antibody response was seen in tocilizumab treated and untreated subjects after vaccination (Figure 2C and Supplemental Table S3).

IgG against SARS-CoV-2 trimeric spike protein and neutralizing antibodies. IgG antibody serum levels against trimeric spike protein and neutralizing antibodies at the 12-month visit after COVID-19 diagnosis were higher in patients treated with anti-IL-6 therapy (Figure 3A and 3B and Supplemental Table S4). Among unvaccinated subjects, the proportion of patients treated with tocilizumab with positive TrimericS-IgG and neutralizing antibodies above the cut-off point, respectively, was 93.5% (n = 29) and 80.6% (n = 25) after a median (Q1–Q3) of 364 (361–367) days from the onset of symptoms vs 65.4% (n = 17) and 57.7% (n = 15) (p = 0.016 and 0.028) after 365 (361–370) days in untreated patients. TrimericS-IgG levels and neutralizing activity were similar after vaccination in tocilizumab treated and untreated patients (Figure 3A and 3B and Supplemental Tables S3 and S4).

T-cell responses to SARS-CoV-2

T-cell responses at the 12-month visit after COVID-19 diagnosis were stronger in patients treated with anti-IL6 therapy (Figure 3C and Supplemental Tables S3 and S4). Compared with untreated patients, those receiving tocilizumab had significantly better IFN-γ responses (median [Q1–Q3], 1760 [702–3992] vs 542 [35–1716] mIU/mL; p = 0.013). Among those who remained unvaccinated at the 12-month visit after COVID-19 diagnosis, the proportion of patients treated with tocilizumab with positive T-cell response in the IGRA was 87.1% (n = 27) vs 62.5% (n = 15) (p = 0.079) in untreated patients, with better IFN-γ responses (1458 [658–2575] vs 501 [34–1429] mIU/mL, p = 0.008). The magnitude of T-cell responses to vaccination was similar in subjects treated and untreated with tocilizumab, (median [Q1–Q3], 4757 3825->5000] vs 4255 [2633->5000] mIU/mL; p = 0.878) (Supplemental Table S2).

SARS-CoV-2 RNA reinfections

Among the entire cohort of 150 subjects, there were 11 cases that met the CDC criteria for suspected reinfection over the 12-month follow-up period. Following serological criteria (i.e. positivization of N-IgG) there were 5 additional cases of suspected reinfection. Virological and serological details of the patients are shown in Supplemental Table S3. Of those 16 suspected cases, in 3 subjects reinfection was ruled out after sequencing of paired stored samples, leaving a total of 13 cases of possible reinfection (3 cases among the 78 patients treated with tocilizumab [3.8%] vs. 10 cases among the 72 patients not receiving tocilizumab [13.9%]; p = 0.041). Among the 78 propensity score-matched patients there were 8 cases of possible reinfection (2 in patients with tocilizumab and 6 in those not receiving tocilizumab; p = 0.263)

Discussion

Despite the increasing use of IL-6 inhibitors during the COVID-19 pandemic, the impact of IL-6 blockade on immunity to SARS-CoV-2 remained largely unknown. Here we show that the use of tocilizumab to block IL-6 signaling in patients with severe or critical disease does not impair long-term immunity to SARS-CoV-2. Moreover, in our study, the magnitude of both antibody and T-cell responses were well above the observed in non-anti-cytokine-treated patients and remained significantly stronger one year after recovering from COVID-19. Noteworthy, neutralizing antibody titers, a predictor of protection from SARS-CoV-2 infection,[18] were significantly higher one year after treatment with tocilizumab compared to untreated individuals, in contrast to a recent study reporting reduced neutralizing activity in sera from 10 patients treated with IL-6 inhibitors (5 tocilizumab, 5 sarilumab) in the previous 60 days.[13] Consistently, we did not observe an increase in the incidence of reinfections during the 12-month study period. Additionally, subjects who received one or two vaccine doses during the follow-up period showed strong humoral and cellular responses with median TrimericS-IgG and

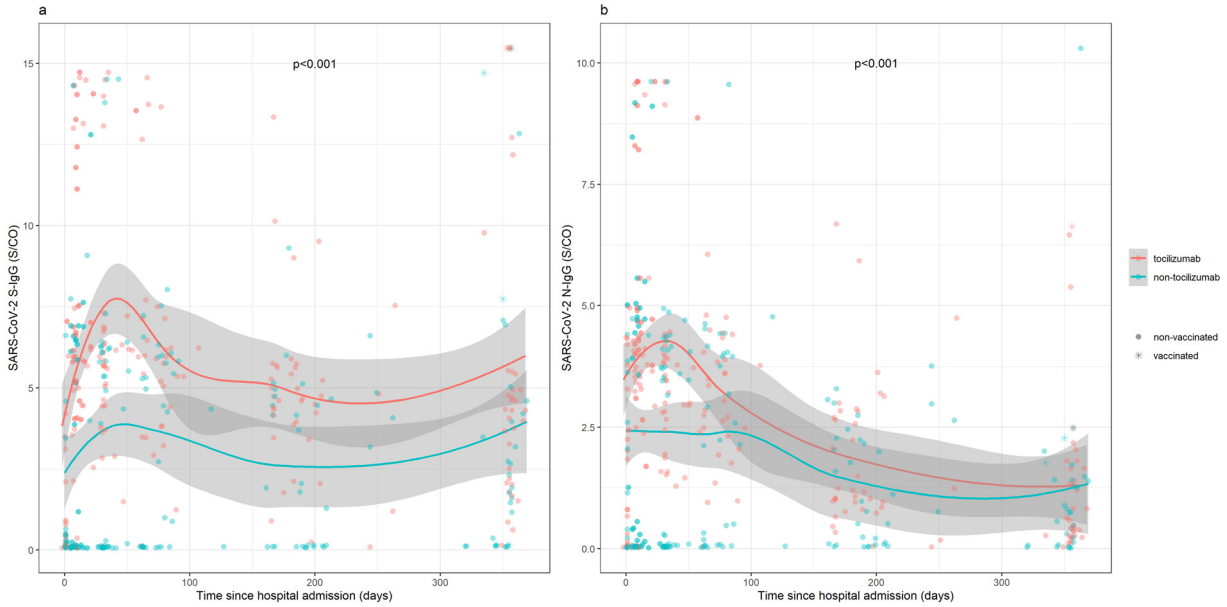

Figure 1. SARS-CoV-2 serological changes during follow-up according to therapy with tocilizumab after propensity score matching. Statistical analyses were performed using t-student test (to contrast the significance of interaction term in the linear mixed-effects model). (a) Changes of S-IgG titers during follow-up, with interpolation line and 90% confidence interval. (b) Changes of N-IgG. S-IgG, antibody against the SARS-CoV-2 surface S1 domain of the spike protein; N-IgG, antibody against the SARS-CoV-2 internal nucleocapsid protein; S/CO, absorbance/cut-off; positive S/CO $\geq$1·1.

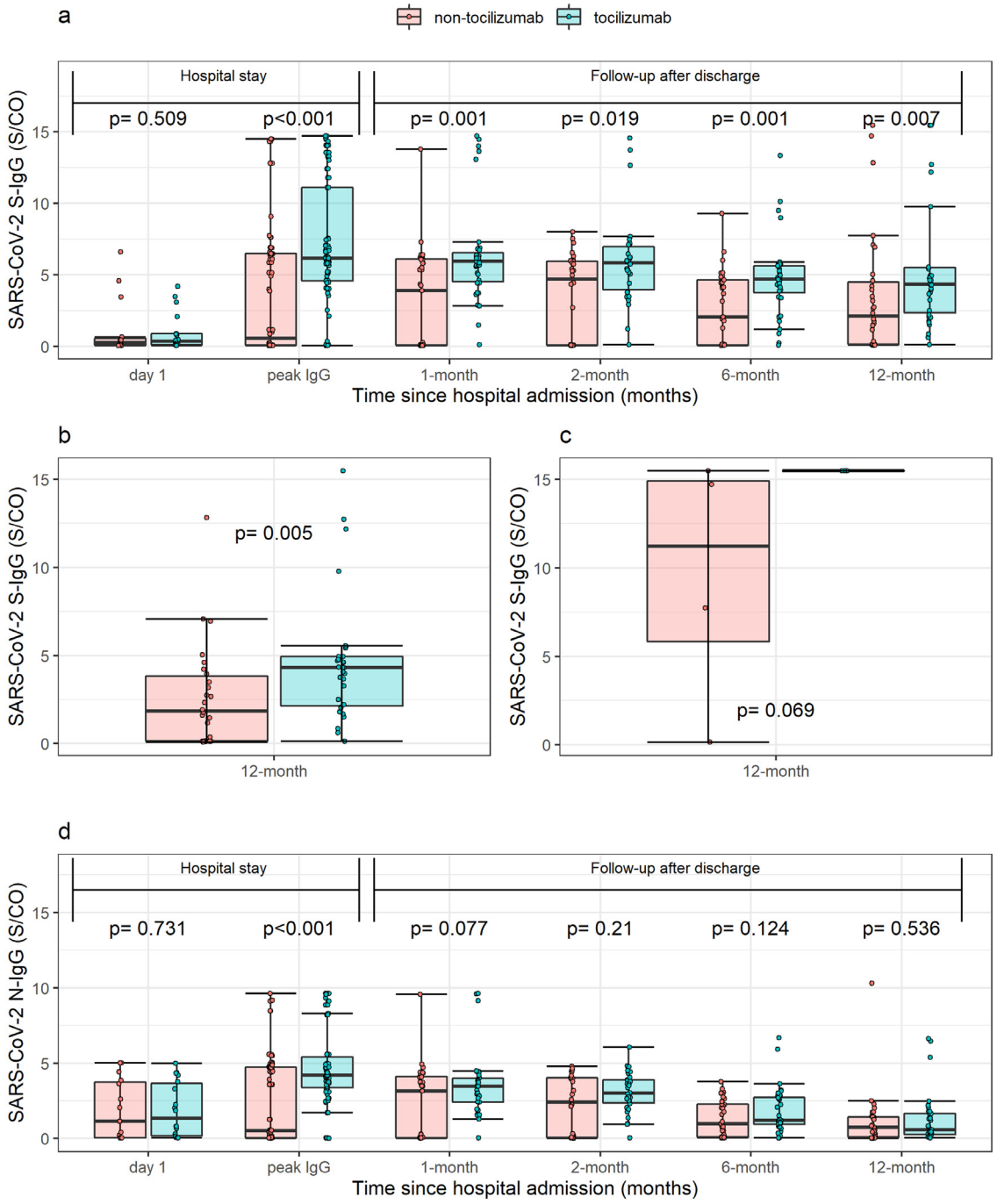

Figure 2. SARS-CoV-2 IgG antibody levels during hospital stay and at 1-, 2-, 6- and 12-month follow-up visits according to therapy with tocilizumab after propensity score matching. Statistical analyses were performed using Mann–Whitney–Wilcoxon test. (a) S-IgG titers in all patients. (b) S-IgG titers in non-vaccinated patients at 12-month visit. (c) S-IgG titers in vaccinated patients at 12-month visit. (d) N-IgG titers in all patients.

IFN-γ concentrations comparable to those seen in the control group.

These results expand upon our previous findings in the same cohort showing that IL-6 blockade does not impair either viral clearance or early antibody response to SARS-CoV-2.[11] In fact, in that analysis, patients receiving tocilizumab tended to have a better initial antibody response with shorter time to seropositivity.

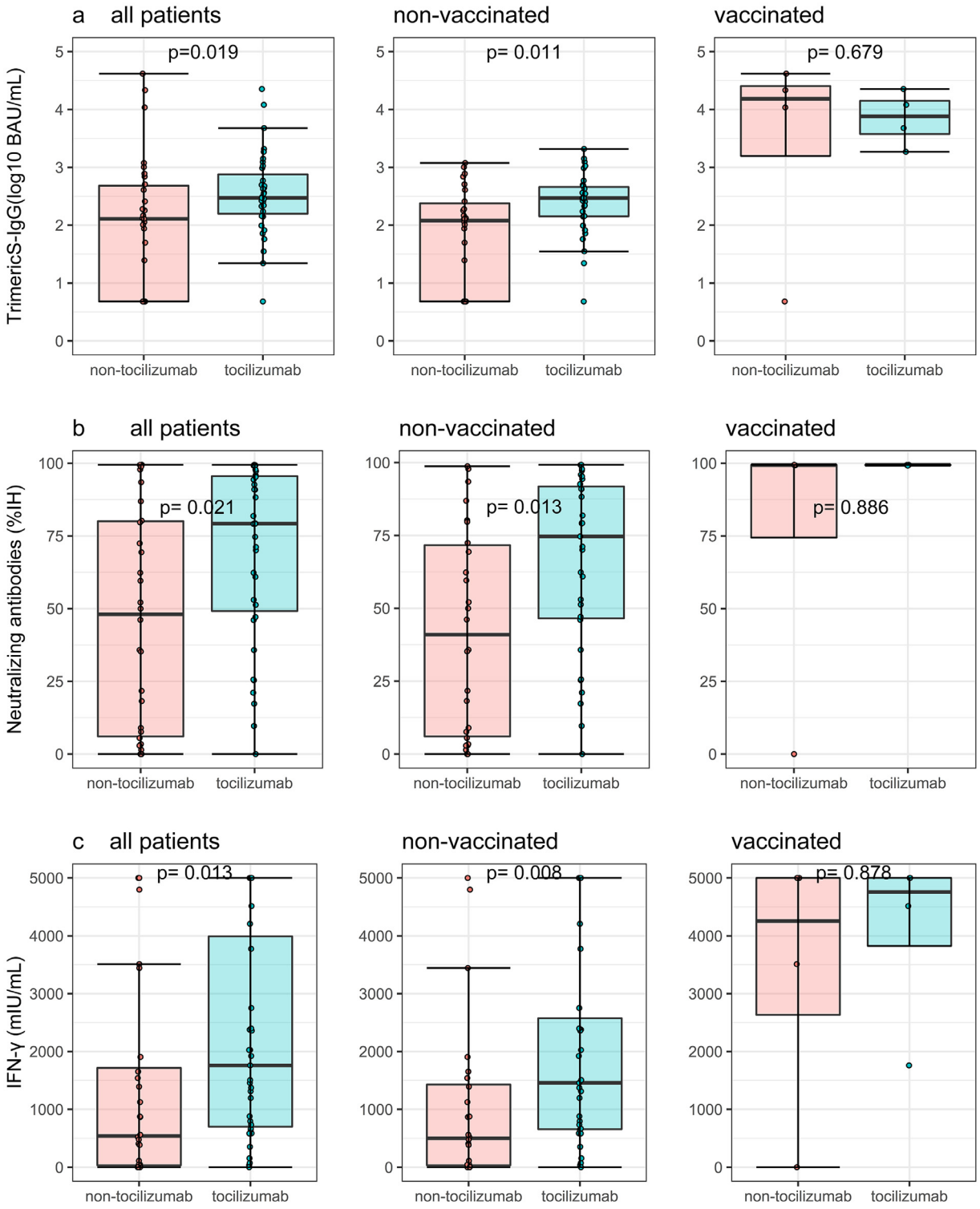

Figure 3. SARS-CoV-2 humoral and cellular response at 12-month visit according to therapy with tocilizumab and vaccination, after propensity score matching. Statistical analyses were performed using Mann–Whitney–Wilcoxon test. (a) Levels of SARS-CoV-2 IgG anti trimeric spike protein (TrimericS-IgG) in all patients, and separately non-vaccinated and vaccinated. (b) Levels of neutralizing antibodies in all patients, and separately non-vaccinated and vaccinated. (c) Concentration of interferon-γ (IFN-γ) released in the SARS-CoV-2 IGRA in all patients, and separately non-vaccinated and vaccinated.

Although patients undergoing IL-6 blockade exhibited prolonged viral shedding in the crude analyses, they had clinical and biological data reflecting the greater disease severity of candidates to anti-cytokine therapy, and the association of tocilizumab treatment with delayed viral clearance did not remain significant in the adjusted model.[11] The long-term data presented in this report confirm that immunomodulatory therapy with tocilizumab in COVID-19 patients admitted to hospital with severe or critical disease is associated with better immunity to SARS-CoV-2. The current research adds to previous report the longitudinal evaluation during one year, with consecutive sampling, close monitoring, and thorough investigations conducted in the patients to measure humoral and cellular immune responses, and to detect and characterize reinfections.

The superior immune responses to SARS-CoV-2 in patients undergoing IL-6 blockade coincided with significant increases in the absolute lymphocyte blood count. This is in line with previous investigations showing that IL-6 blockade in patients with COVID-19 can enhance circulating lymphocyte counts[19] and suggests that overproduction of IL-6 contributes to SARS-CoV-2-associated lymphocytopenia. A number of studies have shown that exuberant synthesis of pro-inflammatory cytokines like IL-6 and IL-1β can induce cell apoptosis and pyroptosis,[20] which might directly decimate lymphocytes following infection in vivo. IL-6 is known to suppress lymphopoiesis[21] and serum concentrations of this cytokine have been correlated with the degree of lymphopenia[10] and with circulating CD4+ and CD8+ T cell counts in COVID-19 patients,[22] with fatal cases showing usually very high levels of IL-6 and severe peripheral lymphopenia.[23]

In addition to low lymphocyte blood count, carefully conducted autopsy studies of human thoracic lymph nodes from severe COVID-19 cases have revealed a significant degree of lymphocyte death in lymph follicles and paracortical areas of lymph nodes with prominent loss of germinal centres associated with a marked reduction of germinal centre B cells[24] and accumulation of non-germinal-centre-derived activated B cells. These findings have been explained by a specific block in germinal centre type Bcl-6$^+$ T follicular helper cell differentiation.[24] Data obtained from animal models[25,26] and *post mortem* studies[24] suggest that those changes may be potentially mediated, among other mechanisms, by dramatic variations in the extra-follicular cytokine milieu[23] with very high local levels of TNF and possibly other cytokines potentially involved in blocking the final step in T follicular helper cell differentiation and attenuation of CD8+T cell immunity.[24] While there is general agreement that IL-6 signals affect germinal centre biology, the context of antigen engagement and the magnitude of expression of this multifunctional cytokine may influence the effects of IL-6 in promoting T follicular helper differentiation and germinal centre development. Interestingly, in a mice experimental model of acute viral infection with murine leukemia virus, in vivo blockage of IL-6 using a monoclonal antibody resulted in reduced viral loads and increased production of IFN-γ, indicating that some of the negative effects of IL-6 on immune responses might be restored with IL-6 blockade.[9]

Collectively, these data suggest that dysregulated IL-6 following SARS-CoV-2 infection may be involved in inducing lymphocytopenia and might aggravate defective lymphocyte functions. The observed improvement in SARS-CoV-2 immune response after blockade of the disproportionate IL-6 signal might be related in part to the alleviation of the effects of a hyper inflammatory milieu, eventually lifting restrictions on lymphoid CD4 + T follicular helper cells differentiation and germinal-centre formation and breaking the vicious cycle of aberrant cytokine release and lymphocyte loss and dysfunction.[5,24] Therefore, the potential mechanisms for the long-term immune advantage of tocilizumab treatment might include a prompt alleviation of SARS-CoV-2-associated lymphocytopenia and restoration of germinal centre B cells by boosting T follicular helper cell differentiation. The contribution of a longer period of infection during the acute phase to the strength of adaptive immune response cannot be excluded.

We acknowledge that our study has limitations. The observational nature of the study is a weakness. To balance treatment groups, we used a propensity score-matching including relevant baseline data that might have affected treatment decisions, including disease severity, but residual confounding by tocilizumab indication cannot be excluded. This is a single centre study, and the results may not therefore be generalizable to the wider population.

In addition to the longitudinal design with close and long follow-up, the study has several notable strengths, including the utilization of validated serological platforms, measuring the neutralizing activity of antibodies and T-cell immunity to SARS-CoV-2, to accurately assess humoral and cellular immune response. To our knowledge this is the first study to date to evaluate comprehensively any potential effect of IL-6 inhibitors on the development of long-term immunity to SARS-CoV-2.

In conclusion, immunomodulatory therapy based on IL-6 blockade in patients with severe COVID-19 does not have deleterious effects on the development of long-term immunity to SARS-CoV-2. On the contrary, the magnitude of both antibody and T-cell responses in patients receiving tocilizumab in this study were above the observed in non-anti-cytokine-treated patients and remained significantly stronger one year after recovering from COVID-19, with no increase in the risk of reinfections observed. This investigation supports the safety of this anti-cytokine therapeutic strategy for COVID-19 from a virological and immunological perspective.

Contributors

FG and MM conceived and designed the study. MFG, JGA, SP and VA collected and were responsible for the data. JAG performed the statistical analysis. MM, FG, JGA, SP, AB, and PM participated in patient care, investigation, and data collection. MFG and VA performed the microbiology analyses. FG, MM and MFG wrote the first draft of the manuscript. All authors drafted the manuscript for important intellectual content, contributed to the revision of the final version of the manuscript, and approved the final version submitted.

Data sharing statement

The data that support this work are available from the corresponding author upon reasonable request.

Declaration of interests

The authors declare no competing interests.

Acknowledgments

This work was supported by the RD16/0025/0038 and CB21/13/00011 grants of the Spanish National Plan for Scientific and Technical Research and Innovation and cofinanced by Instituto de Salud Carlos III—Subdirección General de Evaluación y Fondo Europeo de Desarrollo Regional (grant numbers PI16/01740, PI18/01861, CM19/00160, CM20/00066, and COV20/00005).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104153.

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