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Original Article
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Rapid Recovery From SARS-CoV-2 Infection Among Immunocompromised Children Despite Limited Neutralizing Antibody Response: A Virologic and Sero-Immunologic Analysis of a Single-Center Cohort

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ABSTRACT

Background: Immunocompromised (IC) pediatric patients are at increased risk of severe acute respiratory syndrome coronavirus 2 infection, but the viral kinetics and sero-immunologic response in pediatric IC patients are not fully understood.
Methods: From April to June 2022, a prospective cohort study was conducted. IC pediatric patients hospitalized for coronavirus disease 2019 (COVID-19) were enrolled. Serial saliva swab and serum specimens were subjected to reverse transcription polymerase chain reaction assays with mutation sequencing, viral culture, anti-spike-protein, anti-nucleocapsid antibody assays, plaque reduction neutralization test (PRNT) and multiplex cytokine assays.
Results: Eleven IC children were evaluated. Their COVID-19 symptoms resolved promptly (median, 2.5 days; interquartile range, 2.0–4.3). Saliva swab specimens contained lower viral loads than nasopharyngeal swabs ($P = 0.008$). All cases were BA.2 infection, and 45.5% tested negative within 14 days by saliva swab from symptom onset. Eight (72.7%) showed a time-dependent increase in BA.2 PRNT titers, followed by rapid waning. Multiplex cytokine assays revealed that monocyte/macrophage activation and Th_1 responses were comparable to those of non-IC adults. Activation of interleukin (IL)-1Ra and IL-6 was brief, and IL-17A was suppressed. Activated interferon (IFN)-γ and IL-18/IL-1F4 signals were observed.
Conclusion: IC pediatric patients rapidly recovered from COVID-19 with low viral loads. Antibody response was limited, but cytokine analysis suggested an enhanced IFN-γ- and

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Disclosure

The authors have no potential conflicts of interest to disclose.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization: Ko JH, Kim YJ. Data curation: Kim DR, Park BK, Baek JY, Jeong CH, Ko JH. Formal analysis: Kim DR, Baek JY, Jeong CH, Koh JY, Ko JH. Funding acquisition: Ko JH. Investigation: Kim DR, Park BK, Shin A, Lee JW, Ju HY, Cho HW, Yoo KH, Sung KW, Kim YJ. Methodology: Park BK, Baek JY, Kim

IL-18-mediated immune response without excessive activation of inflammatory cascades. To validate our observation, immune cell-based functional studies need to be conducted among IC and non-IC children.

Keywords: SARS-CoV-2; Immunocompromised Children; Antibodies, Neutralizing; Cytokines

INTRODUCTION

The emergence of the omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in January 2022 led to the 5th and largest surge of coronavirus disease 2019 (COVID-19) in the Republic of Korea.[1-3] In 2022, about 80% of the pediatric population, including immunocompromised (IC) children, had been infected with SARS-CoV-2, and they remain exposed to unceasingly circulating omicron sublineages.[1-3] In adult patients, it is well-established that individuals with risk factors, including old age, obesity, smoking, underlying diseases, or genetic predispositions, are more likely to develop severe illness.[4-9] In particular, IC hosts exhibit prolonged viral shedding, with or without persistent symptoms or clinical deterioration.[10-12] However, children generally experience milder illness, possibly due to pre-existing immunity to other coronaviruses, fewer comorbidities, and/or lower expression of the angiotensin-converting enzyme 2 (ACE2) receptor, which is the binding site for SARS-CoV-2.[13]

While reports suggest that IC children may not have an increased risk of severe COVID-19, the viral kinetics and sero-immunologic responses in this population remain unclear.[14,15] To enhance our understanding of mild COVID-19 presentation in certain IC children, we conducted a comprehensive investigation of the virologic and sero-immunologic responses in this population.

METHODS

A detailed description of the study population, laboratory procedures, and statistical analyses is provided in **Supplementary Material 1**. In brief, we conducted a prospective cohort study from April to June 2022 during the 5th Korean domestic outbreak wave.[1-3] We screened and enrolled IC pediatric patients hospitalized for COVID-19 management at a 2,000-bed tertiary care hospital. Remdesivir was available and tixagevimab/cilgavimab (Evusheld™; AstraZeneca, London, UK) was not available during this study period in Korea. Detailed information on adult sera for comparing antibody responses and cytokines is provided in the **Supplementary Material 1**.[16,17] Serial saliva swab samples from the enrolled children were used in reverse transcription polymerase chain reaction (RT-PCR) assays and viral culture.[18-23] The initial specimen from each patient was also tested to identify the specific strain using a mutation detection kit. Anti-spike protein antibody (Sab) titers were quantitatively measured using the Elecsys® Anti-SARS-CoV-2 S kit (Roche Diagnostics, Switzerland) and adjusted to binding antibody units (BAU).[24-27] Nucleocapsid protein antibody (Nab) titers were measured using the Elecsys® Anti-SARS-CoV-2 kit from Roche Diagnostics.[16,28-30] A plaque reduction neutralization test (PRNT) was conducted against wild-type SARS-CoV-2 (WT) and omicron sublineages, including BA.1, BA.2, BA.5, and BA.2.75.[2,17,24] The 50% neutralizing dose (ND_{50}) titer was calculated using the Karber formula.[31] A PRNT $ND_{50} \geq 20$ was considered positive, and a previous publication estimated that a WT PRNT ND_{50} of 118.25 indicated a 50% protective value.[17] Twenty-five cytokines were measured using Luminex multiplex assay

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kits.[32] Measured cytokines include chemokines (CCL2/monocyte chemoattractant protein (MCP)-1, CCL3/macrophage inflammatory protein (MIP)-1α, CCL4/MIP-1β), and cytokines associated with cell proliferation (GM-CSF, interleukin (IL)-3, IL-7, IL-8, IL-15, and CXCL-10), inflammatory responses (IL-1β, IL-1Ra, IL-6, and interferon-gamma (IFN-γ), Th_1 responses (IL-18, TNF-α, IL-2, IL-12p70, and TNF-β), Th_2 responses (IL-4, IL-5, and IL-17E/IL-25), Th_{17} responses (IL-17A and IL-23), and Treg responses (IL-10 and TGF-β). Statistical analysis is described in detail in the **Supplementary Material 1**.

Ethics statement

This study was approved by the Institutional Review Board of Samsung Medical Center (SMC 2020-03-113), and written informed consent was obtained from the participants themselves (adults) or their parents (children).

RESULTS

Baseline characteristics of IC children and non-IC adult comparators

Eleven IC children with a median age of 8.3 years (interquartile range [IQR], 3.9–11.1) were enrolled, eight of whom (72.7%) were male. Seven (63.6%) had malignancy and four (36.4%) had inborn errors of immunity (IEI), three of which belonged to the 'Predominantly antibody deficiencies (PAD)' category.[33] Detailed information of the enrolled children are presented in **Supplementary Table 1**. Symptoms resolved in a median of 2.5 days (IQR, 2–4.3) and remdesivir was administered to five patients for a median of three days, with no difference in time to symptom resolution between the remdesivir-treated and non-treated patients (P = 0.995). None of the patients experienced pneumonia progression or fatalities. Characteristics of non-IC adult comparators are presented in **Supplementary Material 1**.

Salivary viral load and culture results

We collected 36 saliva swabs from 11 IC children. Initial samples were obtained at a median of three days (IQR, 2–5) from symptom onset, with a median ORF1ab gene cycle threshold (Ct) value of 31.9 (range, 22.2–40.0). Comparing the Ct values of the first saliva specimen for each patient with nasopharyngeal (NP) swabs (collected on the same day or within ± two days), we found that saliva specimens had significantly higher Ct values than NP swabs (9 paired samples, median 31.0 vs. 22.9, P = 0.008, **Fig. 1A**) indicating lower viral loads in saliva. The serial viral load kinetics of saliva specimens were presented in **Fig. 1B**. By day 14 after symptom onset, five patients (P01, P02, P05, P09, and P10; 45.5%) achieved negative conversion. Only two of the 36 saliva samples from P06 and P07 were positive in culture. One was obtained on day 8 from symptom onset (P07), and the other on day 14 (P06). These two saliva samples had the lowest Ct values among the 36 samples, at 17.2 and 17.8, respectively. Notably, both patients had mild symptoms and recovered without complications. Mutation sequencing of the spike gene confirmed that all IC pediatric patients were infected to the omicron BA.2 variant.
In summary, PCR studies indicated that saliva specimens from omicron BA.2-infected IC pediatric patients contained a low viral load with a short duration of viral shedding, regardless of remdesivir treatment, compared to adult specimens from the previous study.[34]

Sab, Nab, and neutralizing antibody kinetics in IC pediatric patients

We measured Sab and Nab concentrations in 39 specimens collected from 11 IC pediatric patients and compared them with those in 33 serum samples obtained from 22 non-IC adult patients (**Fig. 2A and B**). In contrast to non-IC comparators, IC pediatric patients exhibited

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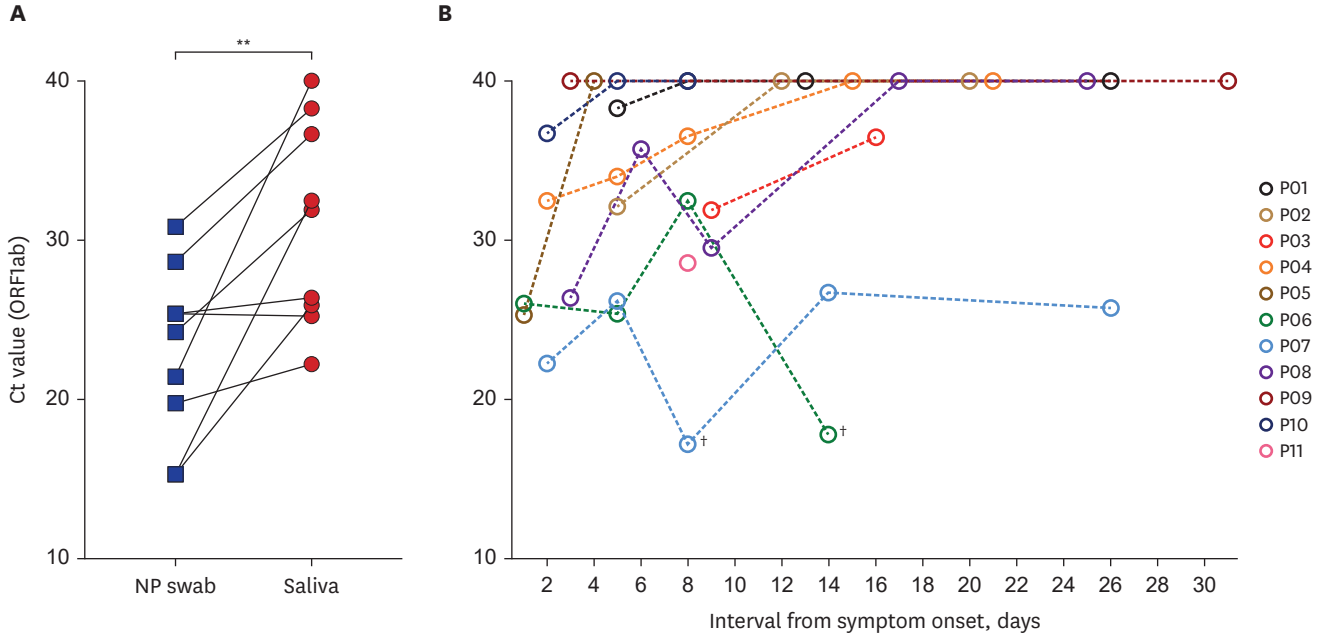

Fig. 1. SARS-CoV-2 viral kinetics of IC pediatric patients. (**A**) Comparison of Ct values of initial NP swab and saliva specimens. (**B**) Kinetics of Ct values of serial saliva specimens in IC pediatric patients.
Ct = cycle threshold, NP = nasopharyngeal, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, IC = immunocompromised.
Statistical comparisons are represented by the following symbols: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; and $^{****}P \leq 0.0001$. †Culture-positive specimens.

increased Sab titers during the 1st week of illness, with a median of 169.8 BAU/mL (IQR, 50.0–1,077.0). These titers were higher in the 2nd week of illness (median, 212.4 BAU/mL; IQR, 47.5–1,162.0) but declined in the 3rd and 4th weeks. In contrast, non-IC comparators initially had no detectable Sab, but concentrations progressively increased from the 2nd week of illness, as previously reported.[30] Serum Nab in IC pediatric patients remained at lower concentrations during the follow-up periods, while non-IC patients showed a progressive increase, becoming significantly greater than in IC patients in the 3rd and 4th weeks of illness (both $P < 0.05$). When examining Sab kinetics individually, nine (P01-02, P04-P08, P10-11) (81.8%) IC pediatric patients exhibited detectable Sab in the initial specimens, with titers declining during follow-up (indicated by dashed lines, **Fig. 2C**). Only two IC patients (P02 and P10, represented by solid lines) demonstrated a substantial increase in Sab titers, 10-fold (from 11 to 116 BAU/mL) and 20-fold (from 89 to 1,768 BAU/mL), respectively). However, the Sab concentrations in P10 showed a rapid waning of antibody titers. Among the seven patients with detectable Sab at baseline in the 1st week (P01, P04-08, P10), one (P06, 14.3%) exhibited a simultaneous positive Nab response, and another one (P01, 14.3%) exhibited a conversion from negative to positive during follow-up (**Fig. 2D**). None of the three IEI patients with PAD (P03, P05, P09) exhibited an adequate antibody response to either Sab or Nab.

To investigate neutralizing antibody responses specific to the infecting strain and cross-reactivity to other omicron sublineages, we conducted PRNT against the BA.1, BA.2, BA.5, and BA.2.75 variants for 39 serial serum samples. The median value of PRNT against the BA.2 increased tenfold in the 3rd week, indicating an antibody response specific to the infecting strain (**Fig. 3C**). In the 1st week, eight IC patients exhibited positive When assessing BA.2 PRNT kinetics individually, eight IC pediatric patients (P01-P05, P08-09, P11) (72.7%) demonstrated progressively increasing antibody titers after symptom onset (indicated by solid lines, **Fig. 3D**). Among these, two patients (P01 and P04) showed an early peak response

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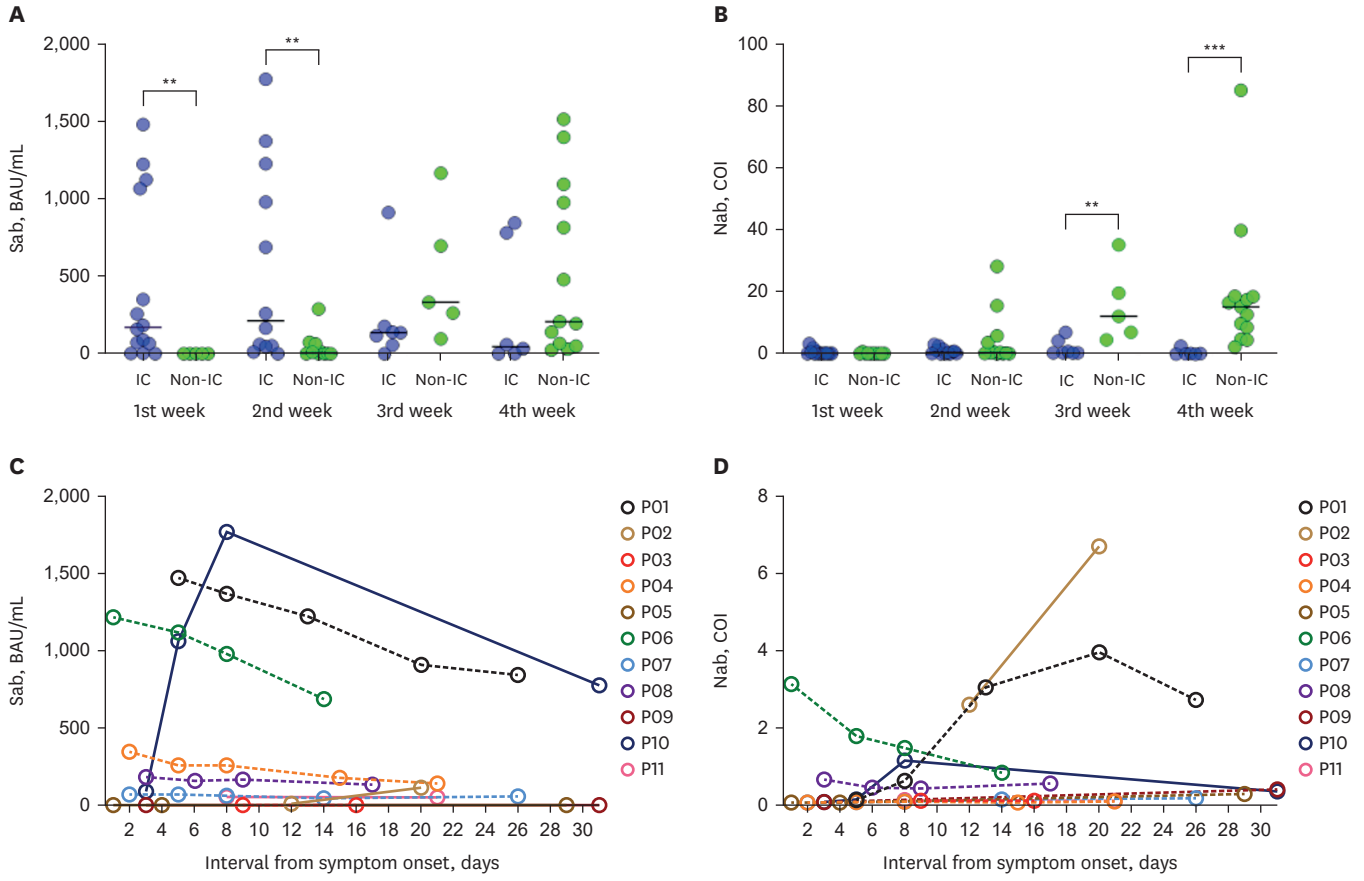

Fig. 2. SARS-CoV-2 Sab and Nab kinetics of IC pediatric patients. (**A**) Sab titers for the first to fourth weeks after the onset of illness, compared with those of non-IC adult comparators. (**B**) Nab titers for the first to fourth weeks after the onset of illness, compared with those of non-IC adult comparators. (**C**) Sab kinetics presented as paired specimens from each patient. (**D**) Nab kinetics presented as paired specimens from each patient.
SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, Sab = anti-spike antibody, Nab = anti-nucleocapsid antibody, IC = immunocompromised, BAU = binding antibody unit, COI = cut-off index.
Statistical comparisons are represented by the following symbols: $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

around two weeks after symptom onset and waning from the 3rd week. Three patients exhibited delayed and minimal increases (P05, P09, and P11), and two of them (P05 and P09) had IEI associated with PAD. Three IC patients (P02, P03, and P08), including one (P03) with PAD, had limited serial samples for evaluation. When assessing responses to other omicron sublineages, fewer patients had detectable PRNTs in the 1st week (9.1% against BA.1, 9.1% against BA.5, and 18.2% against BA.2.75; **Fig. 3A-B, E–H**). In the individual plotting of PRNT titers, four patients (P01, P02, P04, and P11) demonstrated increasing BA.1 PRNT titers over time. For BA.5 and BA.2.75 PRNTs, only two patients each (P01 and P08 for BA.5; and P01 and P04 for BA.27.5) showed rising titers. Patients with IEI associated with PAD (P03, P05, P09) did not show PRNT response to strains other than BA.2. When comparing individual Sab kinetics, three IC patients (P01, P06, and P10) exhibited high Sab concentrations (> 1,000 BAU/mL, **Fig. 2C**), but only P01 showed a time-dependent PRNT response.

The correlation between Sab and BA.2 PRNT titers was not statistically significant (**Supplementary Fig. 1**), with low correlation coefficients (all $R^2 < 0.2$). In summary, the investigation of Sab, Nab, and neutralizing antibodies suggested that IC pediatric patients may have had previous subclinical SARS-CoV-2 infections, and the overall antibody response to the current BA.2 infection was weak and suppressed.

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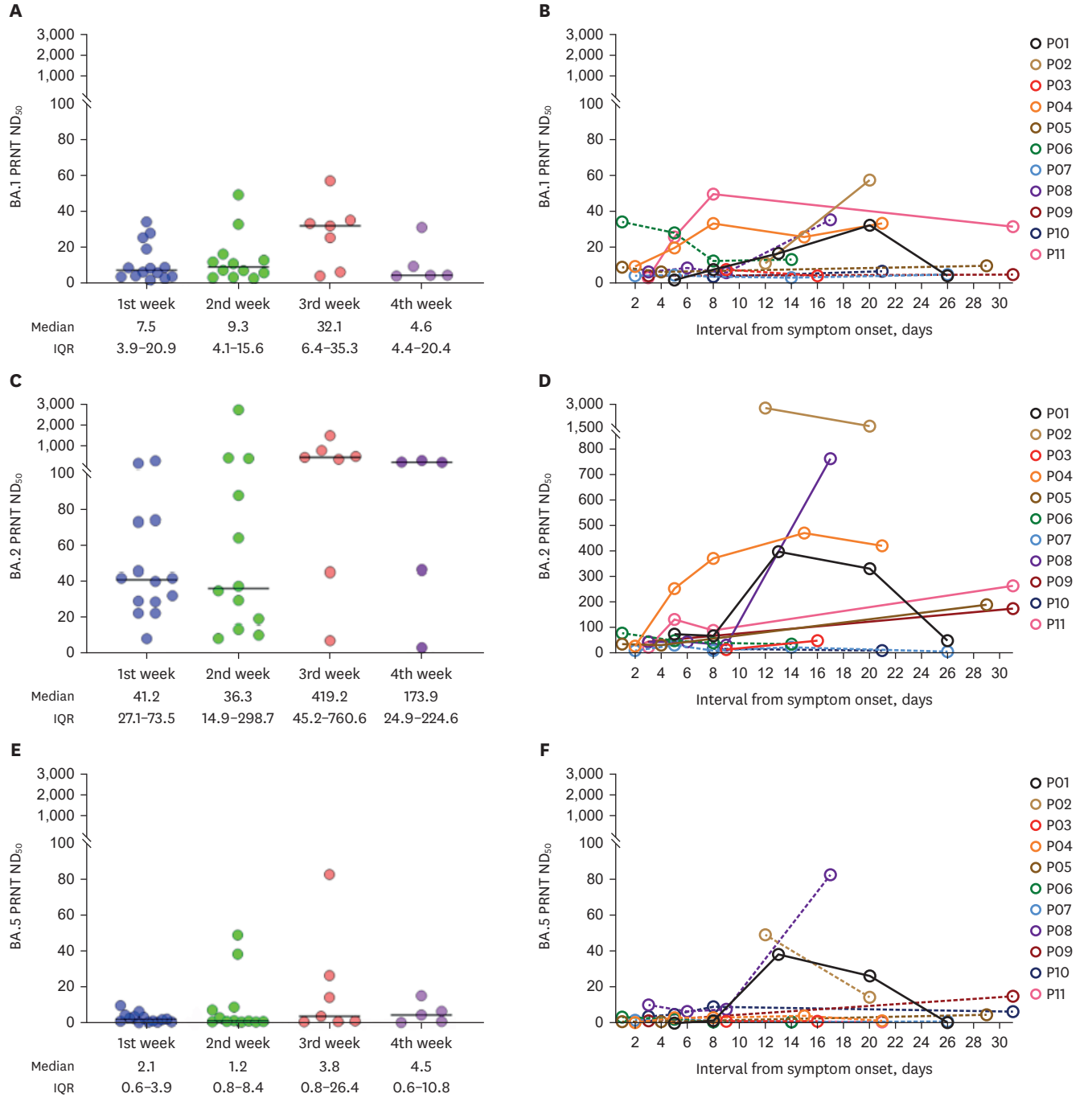

Fig. 3. Kinetics of neutralizing antibodies against BA.1, BA.2, BA.5, BA.2.75 in IC pediatric patients. (**A**) BA.1 PRNT titers for the first to fourth weeks after the onset of illness. (**B**) BA.1 PRNT ND_{50} kinetics presented as paired specimens from each patient. (**C**) BA.2 PRNT titers for the first to fourth week after the onset of illness. (**D**) BA.2 PRNT ND_{50} kinetics presented as paired specimens from each patient. (**E**) BA.5 PRNT titers for the first to fourth weeks after the onset of illness. (**F**) BA.5 PRNT ND_{50} kinetics presented as paired specimens from each patient. (**G**) BA.2.75 PRNT titers for the first to fourth weeks after the onset of illness. (**H**) BA.2.75 PRNT ND_{50} kinetics presented as paired specimens from each patient.
IC = immunocompromised, PRNT = plaque reduction neutralization test, ND_{50} = 50% neutralizing dose.

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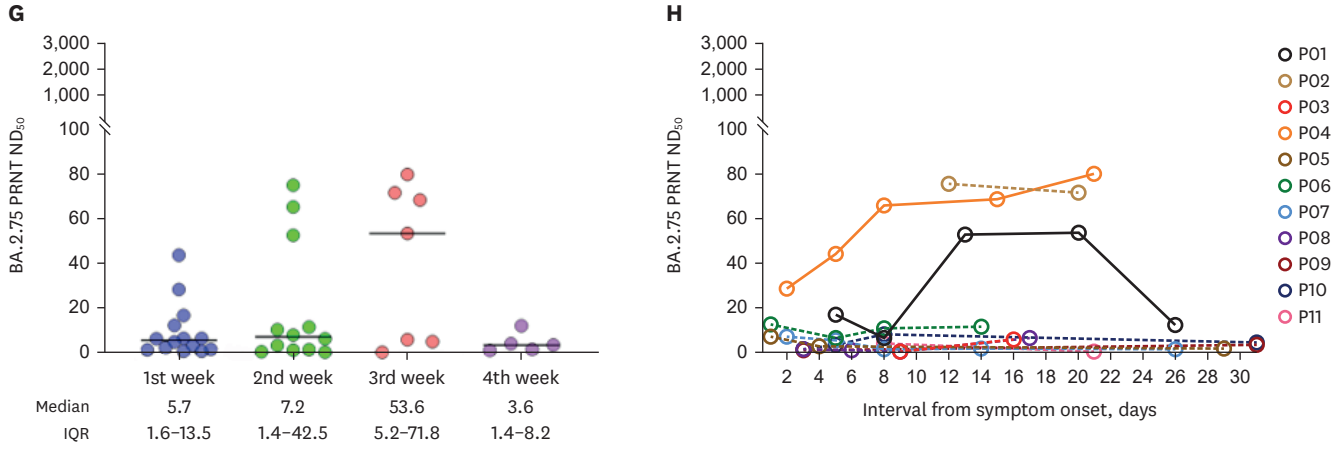

Fig. 3. (Continued) Kinetics of neutralizing antibodies against BA.1, BA.2, BA.5, BA.2.75 in IC pediatric patients. (**A**) BA.1 PRNT titers for the first to fourth weeks after the onset of illness. (**B**) BA.1 PRNT ND_{50} kinetics presented as paired specimens from each patient. (**C**) BA.2 PRNT titers for the first to fourth week after the onset of illness. (**D**) BA.2 PRNT ND_{50} kinetics presented as paired specimens from each patient. (**E**) BA.5 PRNT titers for the first to fourth weeks after the onset of illness. (**F**) BA.5 PRNT ND_{50} kinetics presented as paired specimens from each patient. (**G**) BA.2.75 PRNT titers for the first to fourth weeks after the onset of illness. (**H**) BA.2.75 PRNT ND_{50} kinetics presented as paired specimens from each patient.
IC = immunocompromised, PRNT = plaque reduction neutralization test, ND_{50} = 50% neutralizing dose.

Cytokine response of IC pediatric patients, in comparison with non-IC adult comparators

We conducted a multiplex cytokine assay on 35 serum samples from 11 IC pediatric patients and compared them to 44 serial serum samples from 22 non-IC adult comparators. We also used 21 serum samples from 16 non-IC adult patients as the convalescent status reference. Among chemokines, CCL2/MCP-1 concentrations remained similar to those in the convalescent sera, while CCL4/MIP-1β concentrations increased in IC patients (**Supplementary Fig. 2A** and **Fig. 4A**). CCL3/MIP-1α concentrations were not significantly increased in either IC and non-IC patients (**Supplementary Fig. 2B**). For cytokines related to cell proliferation, we found serum CXCL10/IP10 concentrations were elevated in the 1st week after the onset of illness and then gradually decreased, with no significant differences between IC and non-IC patients (**Fig. 4B**). Concentrations of IL-7 and IL-15 were similar to

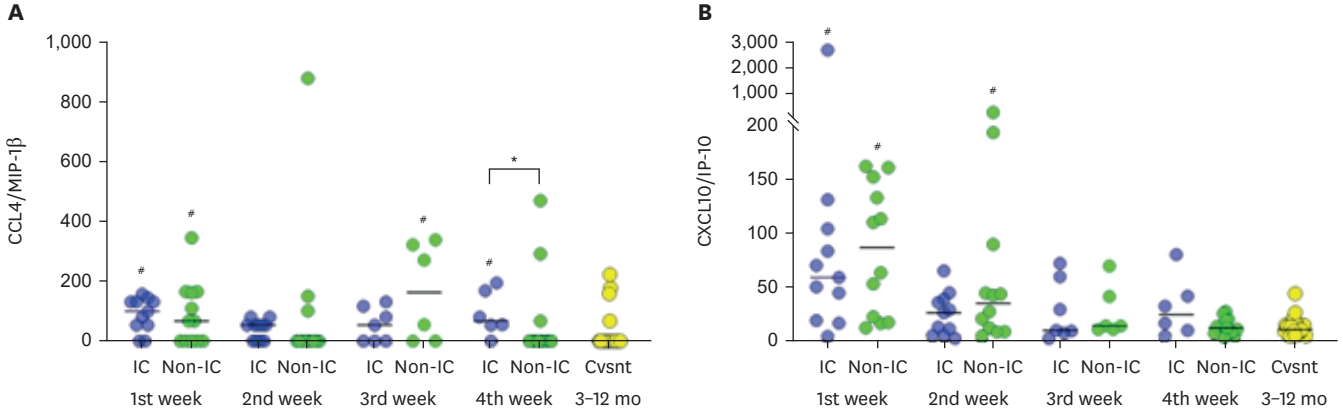

Fig. 4. Comparison of cytokine responses of IC pediatric patients, non-IC adult patients and in convalescent sera. Concentrations of (**A**) CCL4/MIP-1β, (**B**) CXCL10/IP-10, (**C**) IL-1Ra, (**D**) IL-6, (**E**) IFN-γ, (**F**) IL-18, (**G**) TNF-α, and (**H**) IL-17A for the first to fourth weeks and third to twelfth months after the onset of illness, compared with those of non-IC adult comparators. Each point is also compared with the concentrations of the same cytokines in convalescent non-IC adult patients.
IC = immunocompromised, Cvsnt = convalescent, IL = interleukin, MIP = macrophage inflammatory protein, IP = interferon-gamma inducible protein, IFN = interferon, TNF = tumor necrosis factor.
$^{*}P \le 0.05$; $^{**}P \le 0.01$; $^{***}P \le 0.001$; and $^{****}P \le 0.0001$. $^{\#}P \le 0.05$, in comparison with the convalescent sera.

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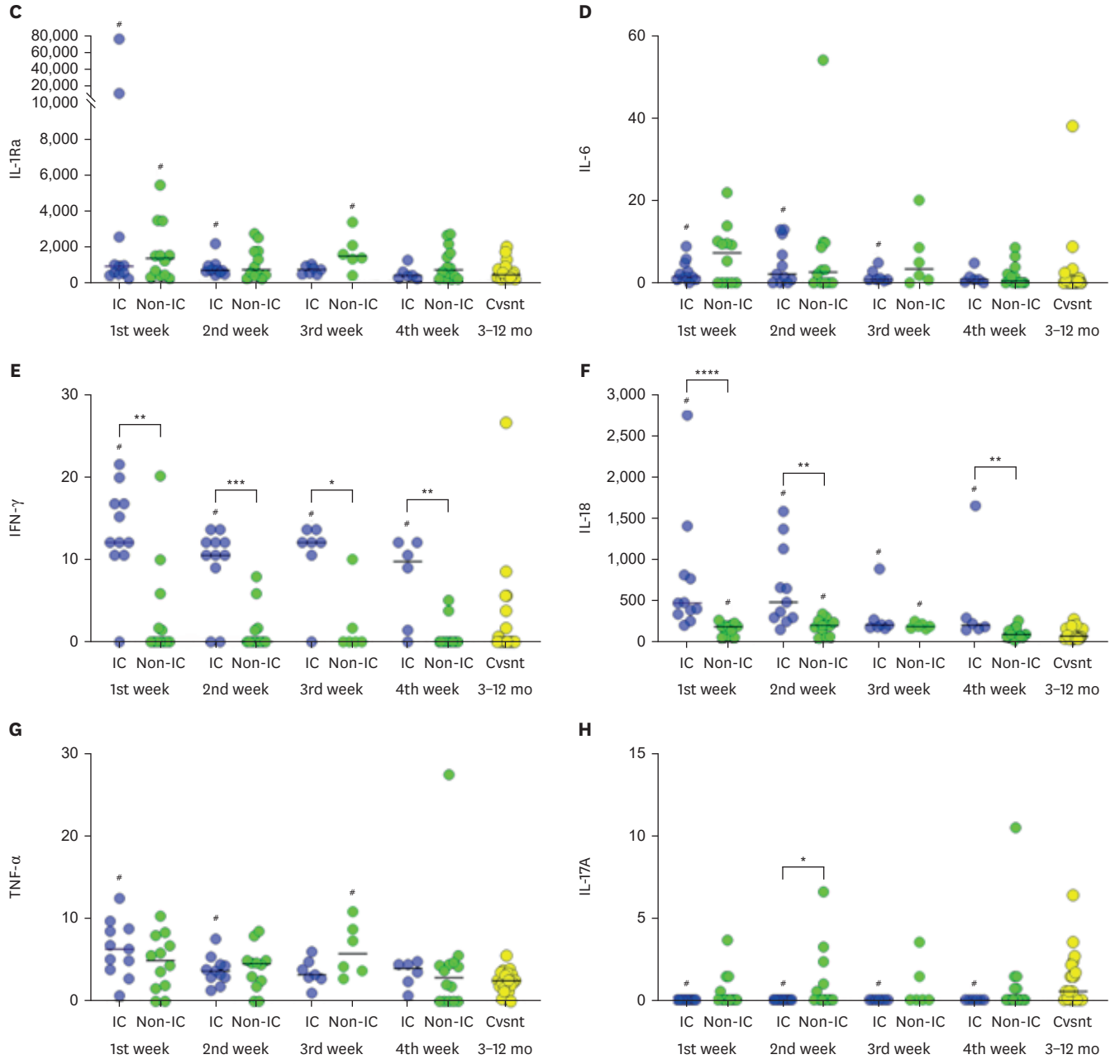

Fig. 4. (Continued) Comparison of cytokine responses of IC pediatric patients, non-IC adult patients and in convalescent sera. Concentrations of (**A**) CCL4/MIP-1β, (**B**) CXCL10/IP-10, (**C**) IL-1Ra, (**D**) IL-6, (**E**) IFN-γ, (**F**) IL-18, (**G**) TNF-α, and (**H**) IL-17A for the first to fourth weeks and third to twelfth months after the onset of illness, compared with those of non-IC adult comparators. Each point is also compared with the concentrations of the same cytokines in convalescent non-IC adult patients.
IC = immunocompromised, Cvsnt = convalescent, IL = interleukin, MIP = macrophage inflammatory protein, IP = interferon-gamma inducible protein, IFN = interferon, TNF = tumor necrosis factor.
$^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; and $^{****}P \leq 0.0001$. $^{\#}P \leq 0.05$, in comparison with the convalescent sera.

those in convalescent sera in both IC and non-IC patients (**Supplementary Fig. 2C and D**), while GM-CSF and IL-3 concentrations were mostly undetectable in both IC and non-IC patients (**Supplementary Fig. 2E and F**), and IL-8 did not show a significant elevation (**Supplementary Fig. 2G**).

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Among cytokines related to inflammatory responses, serum IL-1Ra and IL-6 concentrations increased in the 1st and 2nd week after the onset of illness and gradually decreased thereafter (**Fig. 4C and D**). The IL-1Ra and IL-6 concentrations in IC patients were significantly greater than in convalescent sera but not significantly different from non-IC comparators. Notably, IFN-γ concentrations were consistently higher in IC patients throughout the follow-up period compared with both non-IC patients and convalescent sera (**Fig. 4E**), while IL-1β levels remained undetectable (**Supplementary Fig. 3A**). Since cellular immune responses were not analyzed, the source of IFN-γ (T cells or natural killer cells) remains unidentified. Among Th_1 cytokines, IL-18 levels were significantly elevated in IC patients throughout the follow-up period compared with convalescent sera (**Fig. 4F**) and were also greater than in non-IC comparators in the 1st, 2nd, and 4th weeks. In addition, TNF-α concentrations increased in the 1st week after the onset of illness and decreased gradually thereafter, with no significant difference between IC and non-IC patients (**Fig. 4G**). None of IL-2, IL-12p70, and TNF-β showed any notable elevation (**Supplementary Fig. 3B-D**). Among Th_2 cytokines, serum IL-4 and IL-5 levels increased in the 1st week and then decreased (**Supplementary Fig. 3E and F**). However, IL-17E/IL-25 levels were mostly undetectable (**Supplementary Fig. 3G**). Regarding cytokines related to Th_{17} responses, IL-17A concentrations were significantly lower in IC patients compared to convalescent sera (**Fig. 4H**) and IL-23 concentrations increased in IC patients only in the 1st week after the onset of illness (**Supplementary Fig. 4A**). Among cytokines reflecting Treg responses, IL-10 concentrations were generally undetectable in IC patients, while a significant elevation was detected in non-IC patients in the 1st week after the symptom onset (**Supplementary Fig. 4B**). TGF-β1 concentrations were lower than those in convalescent sera in the 1st and 2nd week after the onset of illness (**Supplementary Fig. 4C**). In summary, multiplex cytokine analyses in IC pediatric patients indicated monocyte/macrophage activation and recruitment of the Th_1 response. Proinflammatory cytokines were activated briefly, while Th_{17} signals were suppressed. The continuous activation of IFN-γ and IL-18 signals distinguished IC pediatric patients from non-IC adult comparators.

DISCUSSION

During the COVID-19 pandemic, there has been extensive study of the clinical, microbiological, and immunological aspects of the disease. However, unexplored areas remain, with IC pediatric patients being one such area. In this analysis, we comprehensively investigated the pathophysiology of COVID-19 in IC children, focusing on virological and sero-immunological aspects. We found that the IC pediatric patients exhibited mild symptoms with low viral loads. Neutralizing antibody production was limited, but cytokine analysis suggested IFN-γ- and IL-18-mediated immune response without excessive activation of inflammatory cascades.

The virologic analysis included RT-PCR assays and viral cultures using saliva samples, given the low accessibility of respiratory specimens in young children. In our study, the overall viral load and culture viability of saliva swab specimens were low in the IC pediatric patients. A significantly greater viral load in initial NP swab specimens implies that the diagnosis of COVID-19 was not delayed, while saliva specimen would not be an optimal alternative to NP swab. Nevertheless, individual plotting of viral load kinetics illustrated a rapid reduction of viral shedding through saliva. Culture positivity was not associated with worsening of clinical symptoms. Although previous studies demonstrated the usefulness of saliva specimens for RT-PCR and viral culture in adult patients,[18-22] studies in children have reported inconsistent

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results.[35-38] Studies using adults' saliva samples found a similar collection process (e.g., 2 mL of self-collected saliva). However, challenges in children's self-collection hinder a standardized protocol. Studies used either saliva swabs or self-collected saliva, with varying swabbing techniques and sample volumes.[35-38] This may have contributed to the inconsistent results.

Interpretating antibody responses in IC children is complicated by the significant hindrance of proper antibody production caused by IC conditions.[28,39] Therefore, having an appropriate comparator from non-IC hosts is important. In a study of non-IC adult patients, seroconversion rates of neutralizing antibodies were observed in 80.0% of asymptomatic and 93.9% of mildly symptomatic patients, with PRNT titers peaking in the 3rd week and gradually declining over three months.[16,30] Therefore, having non-IC adults as a comparator group may be still helpful.

These findings suggested that the infecting-strain-specific antibody response in IC pediatric patients is definitively weak. Furthermore, the poor correlation between Sab and PRNT titers and the low cross-reactivity between PRNT titers against omicron sublineages imply decreased production of neutralizing antibodies. Previous reports have indicated that mature B cell responses, resulting from repeated antigenic stimulation, lead to increased cross-reactivity by targeting more conserved regions of the receptor-binding domain.[3,40,41] The presence of pre-existing neutralizing antibodies in IC patients suggested previous subclinical exposure to SARS-CoV-2, but the antibody response was still limited. In previous reports, a poor neutralizing antibody response was strongly associated with persistent viral shedding and/or delayed recovery.[10,12,42,43] The rapid recovery of IC children in out cohort, despite a limited antibody response, may suggest the potential role of innate and cellular immunity along with low viral affinity associated with reduced ACE2 expression.[44-46]

The serial analysis of 25 cytokines strengthened our understanding of immune responses in IC pediatric patients. Serum CCL4/MIP-1β, TNF-α, and CXCL10/IP10 concentrations were comparable with those of non-IC adult patients, indicating an appropriate innate/adaptive cellular immune response. Measured CCL2/MCP-1 concentrations were within the range of those in convalescent serum samples, and are typically elevated in severe COVID-19.[47] Transient elevations of IL-1Ra and IL-6 concentrations and suppressed Th_{17} signals appear to reflect recovery without excessive activation of inflammatory cascades. Increased serum concentrations of IL-1Ra and IL-6 are well-known signatures of severe COVID-19, which can be suppressed by immune modulator treatment.[47-49] Continuously elevated concentrations of IFN-γ, a major cytokine responsive to viral infection, and IL-18, a monocyte-derived IFN-γ-inducing factor,[50] appeared to be distinguishing features of IC pediatric patients in the present analysis. Greater IFN-γ and IL-18 responses in children than in adults have been reported in previous studies,[51,52] and our data indicate that anti-viral responses mediated by IFN-γ were also enhanced in IC pediatric patients. These findings suggest that although antibody production is impaired, the antiviral activity mediated by innate and adaptive immunity appears to be preserved.[45]

This study had several limitations. First of all, we used specimens from non-IC adults as comparators instead of healthy children. Second, the analyses were conducted using a small number and heterogeneous IC pediatric patients. Third, as viral load measurements and cultures were performed solely on saliva, direct comparisons with NP swab samples were not feasible. Fourth, the immunologic analyses were largely based on serum with limited cell-based experiments. Lastly, the multiplex cytokine assay did not include type I and III

interferons, key regulators of early viral replication in COVID-19 patients. Despite these limitations, our investigation of COVID-19 in IC pediatric patients enhances understanding of this newly emerged respiratory virus that is now becoming endemic.

Previous reports on COVID-19 in IC children, including those with cancer or IEI, generally exhibit mild symptoms and few critical cases,[13,53-55] which was also observed in our study. However, there have been reports of severe COVID-19 cases in pediatric cancer or some IEI patients,[55-57] making it inappropriate to extrapolate that all IC children will have mild disease. Therefore, it is essential to follow recommendations for vaccination and treatment of COVID-19 in IC pediatric patients.

IC children experienced mild COVID-19 symptoms with low viral loads and recovered without complications. Neutralizing antibody production was detected but waned rapidly. Cytokine analysis suggested enhanced IFN-γ- and IL-18-mediated immune response without excessive activation of inflammatory cascades.

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SUPPLEMENTARY MATERIALS

Supplementary Material 1
Detailed description of the methods

Supplementary Table 1
Baseline characteristics and laboratory findings of 11 IC pediatric patients

Supplementary Fig. 1
Titer correlations between Sab titers and the results of PRNT against Omicron sublineages. Titer correlations between Sab titers and PRNT ND_{50} against (**A**) BA.1, (**B**) BA.2, (**C**) BA.5, and (**D**) BA.2.75 as evaluated using a linear regression model.

Supplementary Fig. 2
Comparison of chemokines and cell-proliferation-related cytokine responses in IC pediatric patients, non-IC adult patients, and convalescent sera. Concentrations of (**A**) CCL2/MCP-1 (**B**) CCL3/MIP-1α (**C**) IL-7 (**D**) IL-15 (**E**) GM-CSF (**F**) IL-3 (**G**) IL-8 over the first to fourth weeks and third to twelfth months after the onset of illness compared with those of non-IC adult comparators. Each point is also compared with the concentrations in convalescent sera of non-IC adult patients.

Supplementary Fig. 3
Comparison of cytokines related to inflammatory, Th_1 and Th_2 responses in IC pediatric patients, non-IC adult patients, and convalescent sera. Concentrations of (**A**) IL-1β (**B**) IL-2 (**C**) IL-12p70 (**D**) TNF-β (**E**) IL-4 (**F**) IL-4 (**G**) IL-17E/IL-25 over the first to fourth weeks and third to twelfth months after the onset of illness, compared with those of non-IC adult

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comparators. Each point is also compared with the concentrations in convalescent sera of non-IC adult patients.

Supplementary Fig. 4

Comparison of cytokines related to Th_{17} and Treg responses of IC pediatric patients, non-IC adult patients, and convalescent sera. Concentrations of (**A**) IL-23 (**B**) IL-10 (**C**) TGF-β1 over the first to fourth weeks and third to twelfth months after the onset of illness, compared with those of non-IC adult comparators. Each point is also compared with the concentrations in convalescent sera from non-IC adult patients.

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