Inflammatory Cytokine Profile in Children With Severe Acute Respiratory Syndrome

Pak C. Ng, MD, FRCPC¹; Christopher W.K. Lam, PhD²; Albert M. Li, MRCP²; Chun K. Wong, PhD²; Frankie W.T. Cheng, MRCPCH³; Ting F. Leung, MRCP³; Ellis K.L. Hon, FAAP³; Iris H.S. Chan, PhD³; Chi K. Li, FRCP³; Kitty S.C. Fung, FRCPA⁴; and Tai F. Fok, MD, FRCPC³

ABSTRACT. Objective. To study the inflammatory cytokine profile in children with severe acute respiratory syndrome (SARS) and to investigate whether monoclonal antibody to tumor necrosis factor-α (TNF-α) could be considered for treatment of these patients.

Methods. Plasma inflammatory cytokine concentrations (interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-12p70, and TNF-α) were monitored longitudinally on admission, immediately before corticosteroids, and 1 to 2 days and 7 to 10 days after the drug treatment in a cohort of pediatric patients (n = 8) with virologic confirmed SARS-associated coronavirus infection. None of the patients required mechanical ventilation or intensive care treatment. All children except 1 (patient 3) received corticosteroids.

Results. Plasma IL-1β levels (excluding patient 3) were substantially elevated immediately before (range: 7–721 ng/L) and 7 to 10 days after (range: 7–664 ng/L) corticosteroid treatment. In contrast, the plasma concentrations of other key proinflammatory cytokines, including IL-6 and TNF-α, were not overtly increased in any of the patients throughout the course of illness. In addition, plasma IL-10 concentration was significantly lower 1 to 2 days and 7 to 10 days after corticosteroid treatment, compared with the immediate pretreatment level. Similarly, plasma IL-6 and IL-8 concentrations were significantly decreased 7 to 10 days after the drug treatment.

Conclusions. Pediatric SARS patients have markedly elevated circulating IL-1β levels, which suggests selective activation of the caspase-1–dependent pathway. Other key proinflammatory cytokines, IL-6 and TNF-α, showed only mildly elevated levels at the initial phase of the illness. The current evidence does not support the use of TNF-α monoclonal antibody in this group of children. Pediatrics 2004;113:e7–e14. URL: http://www.pediatrics.org/cgi/content/full/113/1/e7; children, cytokines, SARS.

ABBREVIATIONS. SARS, severe acute respiratory syndrome; SARS-CoV, SARS-associated coronavirus; ARDS, acute respiratory distress syndrome; TNF-α, tumor necrosis factor-α; IL, interleukin; RSV, respiratory syncytial virus.

The severe acute respiratory syndrome (SARS) is a newly discovered infectious disease caused by a novel coronavirus.¹² Hong Kong is one of the most severely affected cities.¹³ The outbreaks of SARS at the Prince of Wales Hospital and in a densely populated housing estate, Amoy Gardens, have affected 1755 local residents and claimed 299 lives (as of July 16, 2003). Similar to the H5N1 “avian flu” influenza infection, patients with SARS-associated coronavirus (SARS-CoV) infection develop primary viral pneumonia and lymphopenia, and severe cases may involve acute respiratory distress syndrome (ARDS).⁵,⁶ The H5N1 influenza virus has been shown to be a potent inducer of proinflammatory cytokines.⁶ In particular, there is substantial upregulation in tumor necrosis factor-α (TNF-α) production.⁷ Whether SARS-CoV may induce a similar inflammatory cytokine pattern and contribute to the unusual severity of human disease is not known. Recently, it was proposed that cytolysis may be associated with viral amplification in the early stage of SARS, which is then followed by an adaptive immunologic response with clearance of virus and severe tissue inflammation.⁸ The use of immunomodulating drugs, such as corticosteroids or monoclonal antibody to TNF-α, may be useful in suppressing the host immune response. Thus, knowing the cytokine pattern could assist the clinicians in understanding the disease mechanism and in designing a treatment strategy most effective for the management of this condition. We measured prospectively a panel of proinflammatory and anti-inflammatory cytokines, using the flow cytometric technique during the acute phase of illness, to investigate whether monoclonal antibody to TNF-α is indicated for treatment of SARS. This report describes the inflammatory cytokine profile in a cohort of children with SARS.

METHODS

Patients

During the SARS outbreak between March 13 and May 17, 2003, 8 children with virologic confirmed SARS-CoV infection were admitted to the pediatric unit of the Prince of Wales Hospital. Their clinical characteristics are summarized in Table 1.

Drug Regimen

All febrile children with suspected SARS were initially given a broad-spectrum antibiotic, cefotaxime, and an antimicrobial, erythromycin or clarithromycin, for treatment of atypical pneumonia. Oral ribavirin (40–60 mg/kg/d) was also started when the child had a definitive contact history of SARS or had high fever.
### TABLE 1. Clinical Features and Treatment Outcomes Among Children With SARS

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
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| Sex (M/F) | F | M | M | M | M | F | F |

<table>
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<th>Clinical features</th>
<th>Age (y)</th>
<th>Patient 1</th>
<th>Patient 2</th>
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<th>Patient 4</th>
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<th>Patient 6</th>
<th>Patient 7</th>
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<td>Parents</td>
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<td>Right upper zone consolidation</td>
<td>Right upper zone consolidation</td>
<td>Left middle zone consolidation</td>
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<td>Chest radiograph</td>
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<td>Right and left lower zone air space consolidation</td>
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<td>Pos (stool)</td>
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<td>Duration of fever (d)</td>
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<td>6 (day 1–6)</td>
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<td>10 (day 1–10)</td>
<td>4 (day 1–4)</td>
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</tbody>
</table>

CT indicates computed tomography; RT-PCR, reverse-transcriptase polymerase chain reaction; Neg, negative test; Pos, positive test; N/A, not applicable.
(>38.5°C) and abnormal radiographic changes suggestive of pneumonia on admission. When the patient remained febrile 48 to 72 hours after commencement of antibiotics and antiviral treatment, oral prednisolone (0.5–1 mg/kg/d) was added. Intravenous ribavirin (20 mg/kg/d) and pulse methylprednisolone (10 mg/kg/d, for 2–3 days) were reserved for those who had persistent fever, continuing clinical deterioration, or progressive consolidation on chest radiographs or computed tomography scan of the thorax. Thereafter, a dose-tapering course of oral prednisolone was prescribed in the following 2 weeks. This pediatric treatment regimen was extrapolated from the adult experience, and the dose and timing of commencement of these medications were constantly under review during the outbreak.

**Laboratory Investigations**

In view of the difficulty in diagnosing SARS at the initial stage of clinical presentation, serial blood tests for monitoring lymphocyte counts, biochemical enzymes, and inflammatory cytokines were performed in all children with probable SARS. Blood samples for cytokine measurement were obtained 1) on admission, 2) immediately (ie, within 24 hours) before commencement of corticosteroids, 3) 1 to 2 days after the drug treatment, and 4) 7 to 10 days after the drug treatment. All specimens were collected during the routine blood sampling procedure to minimize the disturbance to the patients. On each occasion, 0.6 mL of venous blood was collected in a chilled container. The blood samples were immediately immersed in ice and transported to the laboratory for processing.

Plasma was separated by centrifugation (1900 × g for 5 minutes) at 4°C and stored in 200-μL aliquots at −80°C until analysis. A panel of proinflammatory and anti-inflammatory cytokines— including interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70, and TNF-α—were simultaneously quantified by the Human Inflammatory Cytokine Cytometric Bead Array Kit (BD Pharmingen, San Diego, CA) using flow cytometry (FACSCalibur system, Becton Dickinson Corp, San Jose, CA). The assay sensitivities for IL-1β, IL-6, IL-8, IL-10, IL-12p70, and TNF-α were 7.2, 2.5, 3.6, 3.3, 1.9, and 3.7 ng/L, respectively. In cytometric bead array, 6 bead populations with distinct fluorescence intensities were coated with specific antibodies for capturing different cytokines in plasma. The cytokine-captured beads were then mixed with phycoerythrin-conjugated detection antibodies to form sandwich complexes. After incubation, washing, and acquisition of sample data, the result was generated in a graphic format using the BD cytometric bead array analysis software. The coefficients of variation for all cytokine assays were <10%.

In addition, microbiologic investigations were performed to determine the potential bacterial and viral pathogens associated with community-acquired pneumonia. Throat swab, sputum samples, and blood samples were taken for routine bacterial cultures. Throat swab in infants and throat gargle samples from older children were also obtained for 1) antigen detection of influenza A and B; respiratory syncytial virus (RSV); adenovirus; and parainfluenza 1, 2, and 3; 2) the commercial indirect immunofluorescence assay; 4) virus isolation using different culture cell lines to recover common respiratory viruses and SARS-CoV; and 3) reverse-transcriptase–polymerase chain reaction for detecting influenza A and B, RSV, enteroviruses, and SARS-CoV. Tracheal aspirate sample was not available for virologic or cytokine analysis, as none of the patients required intubation or mechanical ventilation. Paired acute and convalescent serum samples were tested for Chlamydia pneumoniae, C psittaci, Mycoplasma pneumoniae, and SARS-CoV antibodies. It was estimated that reverse-transcriptase–polymerase chain reaction for detecting influenza A and B, RSV, enteroviruses, and SARS-CoV was 10%.

**Ethical Approval**

The study was approved by the research ethics committee of the Chinese University of Hong Kong. Informed consent was obtained from the parents or guardians for all patients.

**RESULTS**

The clinical features; laboratory, radiology, and virology findings; and treatment outcomes are summarized in Table 1. All patients had significant increase in their convalescent viral titers specific for SARS-CoV. All except 1 child (patient 3) received oral prednisolone. In most cases (except patients 2 and 6), subsidence of fever and improvement in chest radiographic appearances occurred within 48 hours of corticosteroid treatment (Table 1). Patient 6 also received intravenous ribavirin because of persistent high fever and severe pneumonia. Patients 2 and 6 required pulse methylprednisolone for treatment of progressive lung condition, and both patients responded with substantial clearance of chest radiograph 72 hours after treatment. None of the children required mechanical ventilation, and all patients survived.

Four children (patients 4, 5, 6, and 8) did not have blood taken for cytokines on admission. The longitudinal profile of the inflammatory cytokines are illustrated in Fig 1. Plasma IL-1β concentrations (excluding patient 3) were substantially elevated both immediately before (range: 7–721 ng/L) and 7 to 10 days after corticosteroid treatment (range: 7–664 ng/L; Fig 1A). In contrast, the plasma levels of other key inflammatory cytokines, including IL-6 (range: 2.5–99 ng/L) and TNF-α (range: 0–18 ng/L), were not overtly increased in any patient throughout the course of illness (Fig 1B–F).

The cytokine results before and after corticosteroid treatment are summarized in Table 2. Plasma IL-10 concentrations were significantly lower 1 to 2 days and 7 to 10 days after corticosteroid treatment, compared with the immediate pretreatment level (P = .028, for both time points). Similarly, both plasma IL-6 and IL-8 concentrations were significantly decreased 7 to 10 days after the drug treatment (P = .018 and P = .043, respectively). The overall trend of these cytokines suggests elevated levels at the initial phase of the disease (ie, the first 2 time points), which was then followed by a decline in plasma concentration with time (Fig 1B–D, Table 2). The reductions in plasma cytokine levels 7 to 10 days after corticosteroid therapy paralleled improvements in the clinical conditions. Plasma TNF-α concentrations, however, were not significantly different before and after corticosteroid treatment (P = .08, for both time points).

Patient 6 was the sickest child in our cohort. His cytokine profile indicated that he had the highest proinflammatory cytokine levels—IL-1β (721 ng/L), IL-6 (88 ng/L), IL-8 (26.7 ng/L), and TNF-α (18 ng/L)—immediately before corticosteroid treatment. The decline in plasma cytokine concentrations was also much slower compared with other patients (Fig 1).

**Statistical Analysis**

Wilcoxon signed rank test was used to assess the difference in plasma cytokine concentrations immediately before and 1 to 2 days or 7 to 10 days after commencement of corticosteroids. All statistical tests were performed by SPSS for Windows (Release 10; SPSS Inc, Chicago, IL). The level of significance was set at 5% in all comparisons.
Fig 1. A, Change in plasma IL-1β concentrations in 4 different time points after the onset of illness—on admission, immediately before corticosteroid treatment, and 1 to 2 days and 7 to 10 days after treatment—in 8 children with mild and moderate SARS. All patients (except patient 3) received corticosteroid treatment. B, Change in plasma IL-6 concentrations in 4 different time points after the onset of illness—on admission, immediately before corticosteroid treatment, and 1 to 2 days and 7 to 10 days after treatment—in 8 children with mild and moderate SARS. All patients (except patient 3) received corticosteroid treatment.
Fig 1. C, Change in plasma IL-8 concentrations in 4 different time points after the onset of illness—on admission, immediately before corticosteroid treatment, and 1 to 2 days and 7 to 10 days after treatment—in 8 children with mild and moderate SARS. All patients (except patient 3) received corticosteroid treatment. D, Change in plasma IL-10 concentrations in 4 different time points after the onset of illness—on admission, immediately before corticosteroid treatment, and 1 to 2 days and 7 to 10 days after treatment—in 8 children with mild and moderate SARS. All patients (except patient 3) received corticosteroid treatment.
Fig 1. E, Change in plasma IL-12p70 concentrations in 4 different time points after the onset of illness—on admission, immediately before corticosteroid treatment, and 1 to 2 days and 7 to 10 days after treatment—in 8 children with mild and moderate SARS. All patients (except patient 3) received corticosteroid treatment. F, Change in plasma TNF-α concentrations in 4 different time points after the onset of illness—on admission, immediately before corticosteroid treatment, and 1 to 2 days and 7 to 10 days after treatment—in 8 children with mild and moderate SARS. All patients (except patient 3) received corticosteroid treatment.
SARS has not been reported. Our results revealed a pathway in SARS-CoV infection. Nonetheless, the selective activation of the caspase-1 mediated production is that there has been a marked increase in TNF-α and other proinflammatory cytokines, including IL-6 and interferon-γ, have been documented in human influenza A H5N1 infection. To date, the cytokine pattern in children with SARS has not been reported. Our results revealed a predominant upregulation of IL-1β but not TNF-α or IL-6 after SARS-CoV infection in children. In experimental respiratory infection of pig lungs, Van Reeth et al14 showed that IL-1 but not TNF-α or interferon-α was increased after porcine reproductive-respiratory syndrome virus infection, whereas all 3 proinflammatory cytokines were upregulated in response to swine influenza virus. Similarly, IL-1β but not TNF-α was secreted from macrophages infected with a neurotropic strain of coronavirus in irradiated mice.15 A unique feature of IL-1β production is that this pyrogenic cytokine is synthesized as an inactive precursor (pro-IL-1β), and virus-induced activation of caspase-1 (IL-1β-converting enzyme) is required for converting this precursor protein into its biologically active form (IL-1β).16 Pirhonen et al16 found that influenza A and Sendai virus could infect human macrophages, and induced the activation of caspase-1-dependent pathway resulting in enhancement of IL-1β production. Hence, one plausible explanation of our observation is that there has been a selective activation of the caspase-1-dependent pathway in SARS-CoV infection. Nonetheless, the exact mechanism that leads to SARS-CoV-induced immunomodulation remains to be elucidated. Our results also suggest that other key circulating proinflammatory and anti-inflammatory cytokines—TNF-α, IL-6, and IL-10—on admission and immediately before corticosteroid treatment were not overtly elevated as observed in H5N1 infection. Monoclonal antibody to TNF, therefore, is unlikely to be useful in this group of children, and the proinflammatory and antiviral action of normal TNF-α response may even be adversely attenuated by this treatment. However, we must point out that the circulating TNF-α levels may not necessarily reflect the levels produced locally in the lungs, although a substantial increase in regional TNF-α level is likely to overspill into the circulation. Hence, more research into pulmonary cytokine production and in severe cases is required for clinicians to understand better the immunologic aspect of this new disease.

More important, previous studies on cytokines and ARDS suggested that nonsurvivors who received intensive care treatment had 1) significantly higher circulating TNF-α, IL-6, and IL-8 levels on admission; 2) persistent elevation of proinflammatory cytokine levels over time; and that 3) such levels remained elevated despite corticosteroid treatment, compared with survivors.13,17 Again, our findings indicate a decreasing trend in most key inflammatory cytokines with time and a significant decrease in plasma concentrations of IL-6, IL-8, and IL-10 after 7 to 10 days of corticosteroid treatment (Fig 1). The reduction in plasma cytokine levels corresponded closely with improvement in the clinical condition and resolution of inflammatory shadows on chest radiographs. It has been suggested that effective containment of host inflammatory response, as a result of either spontaneous improvement or suppression by anti-inflammatory drugs such as corticosteroids, was associated with a reduction in circulating inflammatory cytokine levels. The reduction in cytokine levels also correlated with improvement in general clinical condition, pulmonary function, and survival.13,17 In view of rapid deterioration in lung function in many adult patients during the acute phase of SARS-CoV infection,3,8 it would be deemed unethical to withhold corticosteroid treatment in any patients who had persistent fever or progressive radiographic changes at the onset of the outbreak.8 Hence, all except 1 patient in this cohort were treated with corticosteroids. Consequently, we are unable to conclude with confidence that the reduction in circulating cytokine levels was associated with corticosteroid usage or just the natural recovery process of illness independent of any drug treatment. The absence of overtly elevated plasma IL-6 and TNF-α levels, together with a progressive reduction in concentrations of other cytokines, IL-8 and IL-10, did predict a favorable outcome for our patients. Such findings concur with our previous clinical observation that children tend to have milder disease and a

### DISCUSSION

This study was designed to investigate prospectively the longitudinal cytokine profile of children with SARS. Similar to H5N1 “avian flu” pneumonia, SARS-CoV–induced atypical pneumonia is also associated with pulmonary epithelial cell proliferation, infiltration of alveolar macrophages, early hyaline membrane formation, hemophagocytosis, and other histopathologic features suggestive of ARDS.6,12 The presence of hemophagocytosis supports the notion that cytokine dysregulation may be, at least partly, responsible for an exaggerated and persistent inflammatory response causing diffuse alveolar damage and alveolar fibroproliferation.6,12,13 Marked increase in TNF-α and other proinflammatory cytokines, including IL-6 and interferon-γ, have been documented in human influenza A H5N1 infection.6,7 To date, the cytokine pattern in children with SARS has not been reported. Our results revealed a predominant upregulation of IL-1β but not TNF-α or IL-6 after SARS-CoV infection in children. In experimental respiratory infection of pig lungs, Van Reeth et al14 showed that IL-1 but not TNF-α or interferon-α was increased after porcine reproductive-respiratory syndrome virus infection, whereas all 3 proinflammatory cytokines were upregulated in response to swine influenza virus. Similarly, IL-1β but not TNF-α was secreted from macrophages infected with a neurotropic strain of coronavirus in irradiated mice.15 A unique feature of IL-1β production is that this pyrogenic cytokine is synthesized as an inactive precursor (pro-IL-1β), and virus-induced activation of caspase-1 (IL-1β-converting enzyme) is required for converting this precursor protein into its biologically active form (IL-1β).16 Pirhonen et al16 found that influenza A and Sendai virus could infect human macrophages, and induced the activation of caspase-1-dependent pathway resulting in enhancement of IL-1β production. Hence, one plausible explanation of our observation is that there has been a selective activation of the caspase-1-dependent pathway in SARS-CoV infection. Nonetheless, the exact mechanism that leads to SARS-CoV-induced immunomodulation remains to be elucidated. Our results also suggest that other key circulating proinflammatory and anti-inflammatory cytokines—TNF-α, IL-6, and IL-10—on admission and immediately before corticosteroid treatment were not overtly elevated as observed in H5N1 infection. Monoclonal antibody to TNF, therefore, is unlikely to be useful in this group of children, and the proinflammatory and antiviral action of normal TNF-α response may even be adversely attenuated by this treatment. However, we must point out that the circulating TNF-α levels may not necessarily reflect the levels produced locally in the lungs, although a substantial increase in regional TNF-α level is likely to overspill into the circulation. Hence, more research into pulmonary cytokine production and in severe cases is required for clinicians to understand better the immunologic aspect of this new disease.

More important, previous studies on cytokines and ARDS suggested that nonsurvivors who received intensive care treatment had 1) significantly higher circulating TNF-α, IL-6, and IL-8 levels on admission; 2) persistent elevation of proinflammatory cytokine levels over time; and that 3) such levels remained elevated despite corticosteroid treatment, compared with survivors.13,17 Again, our findings indicate a decreasing trend in most key inflammatory cytokines with time and a significant decrease in plasma concentrations of IL-6, IL-8, and IL-10 after 7 to 10 days of corticosteroid treatment (Fig 1). The reduction in plasma cytokine levels corresponded closely with improvement in the clinical condition and resolution of inflammatory shadows on chest radiographs. It has been suggested that effective containment of host inflammatory response, as a result of either spontaneous improvement or suppression by anti-inflammatory drugs such as corticosteroids, was associated with a reduction in circulating inflammatory cytokine levels. The reduction in cytokine levels also correlated with improvement in general clinical condition, pulmonary function, and survival.13,17 In view of rapid deterioration in lung function in many adult patients during the acute phase of SARS-CoV infection,3,8 it would be deemed unethical to withhold corticosteroid treatment in any patients who had persistent fever or progressive radiographic changes at the onset of the outbreak.8 Hence, all except 1 patient in this cohort were treated with corticosteroids. Consequently, we are unable to conclude with confidence that the reduction in circulating cytokine levels was associated with corticosteroid usage or just the natural recovery process of illness independent of any drug treatment. The absence of overtly elevated plasma IL-6 and TNF-α levels, together with a progressive reduction in concentrations of other cytokines, IL-8 and IL-10, did predict a favorable outcome for our patients. Such findings concur with our previous clinical observation that children tend to have milder disease and a

### TABLE 2. Changes in Cytokine Profile of Children With SARS (Excluding Patient 3) Before and After Corticosteroid Treatment

<table>
<thead>
<tr>
<th>Plasma Cytokine Concentrations (ng/L)</th>
<th>Immediately Before Corticosteroid Treatment (n = 7)</th>
<th>1–2 Days After Corticosteroid Treatment (n = 7)</th>
<th>7–10 Days After Corticosteroid Treatment (n = 7)</th>
<th>Normal Reference Range19–23</th>
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<tr>
<td>IL-1β</td>
<td>127 (105–400)</td>
<td>130 (58–390)</td>
<td>100 (39–433)</td>
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<td>IL-6</td>
<td>29 (24–56)</td>
<td>20 (3–74)</td>
<td>3 (3–27)*</td>
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<tr>
<td>IL-8</td>
<td>6.4 (3.6–24.4)</td>
<td>7.5 (3.6–26.7)</td>
<td>3.6 (3.3–11.8)*</td>
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<td>IL-10</td>
<td>9.2 (6.9–15.9)</td>
<td>5.3 (4.9–6.5)*</td>
<td>3.3 (2.0–3.3)*</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>12.1 (6.2–125)</td>
<td>13.0 (5.5–121)</td>
<td>10.5 (5.9–126)</td>
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<tr>
<td>TNF-α</td>
<td>3.7 (3.7–7.2)</td>
<td>3.7 (2.1–5.8)</td>
<td>3.7 (2.4–7.4)</td>
<td>&lt;3.5</td>
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Results are median (interquartile range).

* P < .05
less aggressive clinical course than adult SARS patients.\textsuperscript{4} The cytokine results, therefore, cast doubt on the liberal use of corticosteroids in pediatric SARS patients, as the host immunologic response did not seem to be as severe as initially anticipated. However, it is important to note that our patients had relatively mild symptoms, and none required mechanical ventilation or intensive care. Thus, the results may not be generalizable to cases with severe disease. Nonetheless, it is reassuring that there was no fatality among the pediatric patients in Hong Kong. It is interesting that corticosteroids were not used in Canada, and all children recovered with supportive treatment.\textsuperscript{19} Corticosteroids should probably be reserved for patients with severe disease, in particular, those who require oxygen supplementation and mechanical ventilatory support.

CONCLUSIONS

Pediatric SARS patients had markedly elevated plasma IL-1β levels, which suggested selective caspase-1–dependent pathway activation in infected macrophages. In contrast to H5N1 influenza infection,\textsuperscript{6,7} other key proinflammatory cytokines, including IL-6 and TNF-α, were only mildly elevated at the initial phase of the illness. Most of these cytokine levels fell with time and coincided with improvement in clinical conditions and radiographic appearances. Thus, the current evidence does not support the use of monoclonal antibody to TNF-α for treatment of children with SARS. A randomized, controlled study would be useful to determine the effectiveness and adverse effects of corticosteroids in pediatric patients with severe SARS-CoV infection.

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