Dynamic changes in clinical features and cytokine/chemokine responses in SARS patients treated with interferon alfacon-1 plus corticosteroids

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Introduction

Severe acute respiratory syndrome (SARS), caused by a novel coronavirus, emerged in early 2003 as a major international health crisis. We report on serum cytokine levels, viral load and clinical parameters over the course of the disease in a cohort of nine adult SARS patients treated with steroids and interferon alfacon-1 at North York General Hospital in Toronto, Ontario. Considerable variation among SARS patients with respect to circulating viral load and patterns of SARS-CoV-evoked cytokine responses was recorded. No single cytokine profile was observed in all patients, yet serum concentrations of interferon (IFN)-γ, interleukin (IL)-10, CXCL10, CCL5 and CXCL8 were found to be elevated above normal levels during the course of the disease in all patients. Expression levels for IL-10, IFN-γ and CXCL10 consistently peaked within 4 days of peak viral load. IL-12p70, IL-4 and tumour necrosis factor-α concentrations were consistently highest within 5 days of peak viral load. These results suggest that elevated levels of inflammatory cytokines are sensitive correlates of disease severity, including lung abnormalities and viral load in serum, and may provide a tool for monitoring disease progression in affected individuals.
day 10–15, provide evidence for an immunopathological phase of disease [10].

While serum cytokine levels, viral load and clinical course and management have been reported for some SARS patients, here we report on all these parameters in a single group of patients treated at North York General Hospital during the recent SARS outbreak in Toronto.

We analysed serum specimens, collected serially over the course of disease, from nine confirmed SARS patients in concert with thorough chart reviews, and report on the association between clinical and biochemical parameters, the presence of viral RNA in serum and serum cytokine concentrations in these patients.

Methods

Patient selection and specimen collection

Data were collected from nine patients treated at North York General Hospital, Toronto, between 23 May 2003 and 5 July 2003 who met the WHO case definition for probable SARS [15] and were confirmed positive for SARS-CoV through either the detection of viral RNA using real-time RT-PCR (seven patients) and/or seroconversion for viral antibodies (nine patients).

All nine patients were treated with corticosteroids, with treatment duration ranging from 9–17 days. Patients received 50 mg oral prednisone twice daily or 40 mg intravenous methylprednisilone every 12 h upon detection of an abnormal chest radiograph. From 26 May 2003, patients with progressive disease received pulsed high-dose intravenous methylprednisolone (500 mg once daily) for 3 days, followed by a taper and step-down to oral prednisone (nine patients).

All nine patients were also treated with interferons (IFN) alfacon-1 (for 8–13 days), beginning with a 9 g dose. To address concerns that cessation of IFN treatment might result in disease rebound in patients receiving steroids, patients who received IFN alfacon-1 were given a rapid steroid taper to allow the course of steroids to be completed one day before the last IFN dose was administered. In addition to steroids and IFN alfacon-1, seven patients also received antimicrobials.

Blood specimens were collected prior to administration of the first dose of IFN alfacon-1, with subsequent specimens collected on days 2, 4, 7 and 14 following initiation of treatment for measurements of viral RNA and cytokine and chemokine expression. Sample collection was discontinued upon patient discharge. This study was approved by North York General Hospital’s research ethics board.

RNA extraction

Viral RNA was extracted from 1 ml samples of serum or plasma using the QiAamp UltraSens Virus kit (Qiagen Inc, Mississauga, ON, Canada) according to the manufacturer’s instructions.

RT-PCR — quantification of viral RNA

Quantification of SARS-CoV expression was carried out by real-time PCR using the Roche Diagnostics’ LightCycler instrument (Roche Diagnostics, Laval, QC, Canada) and the RealArt™ HPA-coronavirus LC RT PCR kit (Artus GmbH, Hamburg, Germany).

Cytometric bead array — serum cytokine levels

Serum cytokine levels were assayed using three commercial cytometric bead array kits: Human Th1/Th2 Cytokine CBA II: interleukin (IL)-2, IL-4, IL-6, IL-10, tumour necrosis factor (TNF)-α, IFN-γ; Human Inflammation CBA: CXCL8 (IL-8), IL-1β, IL-6, IL-10, TNF-α, IL-12p70; and Human Chemokine CBA: CXCL10 (IP-10), CCL2 (MCP-1), CXCL9 (MIG), CCL5 (RANTES), CXCL8 (IL-8) (BD Biosciences, Mississauga, ON, Canada). Prepared serum samples (50 μl) were analysed in triplicate using a BD FACS Calibur flow cytometer.

Blood samples were also obtained from nine healthy adult controls with no known exposure to SARS. The mean concentration of each cytokine in the serum of the healthy controls ± the standard error was considered the normal range for that cytokine.

Clinical, laboratory and radiographic parameters

For all data, day 1 was identified as the day of symptom onset, as recorded in the patient’s chart. Serial data for LDH, CK and maximum daily temperature were obtained through chart reviews. These parameters were recorded daily through the initial phase of the disease and on alternating days during the recovery phase, that is, once the patient was determined to be improving clinically.

Serial chest radiographs were obtained for all nine patients. Chest X-ray (CXR) involvement scores were calculated on the basis of the radiologists’ interpretation of the radiographs by three radiologists who were blinded to the identity, diagnosis and treatment protocol of the patients. The mean value of percentage lung involvement assigned by the three radiologists was used as the CXR involvement score for each radiograph [16].

Results

All nine confirmed SARS patients in this study (three men and six women) were healthcare workers. Five Caucasians, three Asians and one black patient were included in the study, their ages ranging from 27–56
years with a median age of 48 years. Comorbid conditions were recorded in three patients. Table 1 provides demographic and treatment information for each study subject.

All patients presented with fever and dyspnea and 89% presented with a dry cough. The median duration from onset of symptoms to hospital admission was 2 days (range 0–4 days). Over the course of their disease, seven patients (78%) required supplemental oxygen, three patients (33%) were admitted to the intensive care unit (ICU) with the length of stay ranging from 24 h (patient 8) to 17 days (patient 6) and one patient (11%) required mechanical ventilation [16]. The initial chest radiograph was found to be abnormal in five patients (56%), with all nine patients developing abnormal radiographic findings over the course of their disease. Clinical data are shown in Figure 1. Radiographic evidence of pulmonary disease was first seen on days 5–7 after symptom onset, with peak involvement on days 10–14, correlating with changes in oxygen saturation and plasma viral titres. Liver involvement, manifested by elevated liver enzymes, was seen in a number of patients, as previously reported [16]. Data indicating the peak values and day of occurrence (from symptom onset) for various clinical and laboratory parameters are presented in Table 2.

The results of real-time RT-PCR analyses of SARS-CoV expression and serum cytokine and chemokine assays are presented in Figures 1, 2 and 3. SARS-CoV RNA was detected in the serum and/or plasma of seven patients (78%) (Figure 1). Peak concentrations of viral RNA were typically identified in the serum or plasma close to day 10 from onset of symptoms (interquartile ranges: days 9–11 in plasma, days 8–11 in serum). This observation is consistent with reports that the ‘peak’ stage of SARS typically occurs around days 8–14 [13], with the viral load in serum beginning to decrease from days 10–15, immediately following seroconversion, which typically occurs around day 10 [10]. A number of other disease parameters also peaked within the same time period (see Table 2). CXR involvement score, oxygen saturation and maximum supplemental oxygen requirement consistently peaked within 4 days following the peak viral load recorded in serum or plasma. These observations are consistent with the peak disease severity occurring around day 10 from symptom onset.

Serum concentrations of IFN-γ, IL-10, CXCL10 (IP-10), CCL5 [regulated on activation, normal T-cell expressed and secreted (RANTES)] and CXCL8 (IL-8) were found to be elevated above normal levels during the course of disease in all patients (Figures 2 and 3). IL-6 (Figure 3) was elevated above normal in eight patients (89%), while IL-4 and TNF-α (Figure 3) were marginally elevated in seven patients (78%). CCL2 (MCP-1; Figure 2) was elevated in five patients (56%). The remaining cytokines were elevated above normal in less than half of the patients studied: CXCL9 (MIG; Figure 2) was elevated in four patients (44%), while both IL-12p70 (Figure 3) and IL-1β (Figure 3) were only elevated in three patients (33%). LDH (Figure 1) was elevated above normal in all nine patients and CK (Figure 1) was at least marginally elevated in five patients.

While almost all patients experienced an initial fever followed by a spike in LDH and CK concentrations, serum cytokine concentrations varied considerably among patients over the course of disease (Table 3). As this study was implemented acutely during a SARS outbreak, treatment decisions were based on clinical findings, which varied among patients. Patients received different doses of steroids and IFN alfacon-1 depending on the severity and course of their disease. Both steroids and IFN alfacon-1 can affect cytokine production and these treatments probably affected the serum cytokine profiles observed in our patients. Despite these considerations, all patients were found to have elevations of a number of pro-inflammatory cytokines.

Table 1. Patient demographics and treatment protocols

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Comorbidities</th>
<th>Max. steroid dose, mg</th>
<th>Day of max. steroid dose</th>
<th>Max. IFN dose, µg</th>
<th>Day of 1st IFN dose</th>
<th>Mechanical ventilation</th>
<th>ICU</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>F</td>
<td>mitral valve prolapse</td>
<td>500</td>
<td>12</td>
<td>9</td>
<td>11</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>F</td>
<td>—</td>
<td>100</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>F</td>
<td>hypertension</td>
<td>500</td>
<td>14</td>
<td>9</td>
<td>12</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>M</td>
<td>—</td>
<td>500</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>F</td>
<td>—</td>
<td>100</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>F</td>
<td>asthma</td>
<td>500</td>
<td>8</td>
<td>15</td>
<td>8</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>51</td>
<td>M</td>
<td>—</td>
<td>500</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>M</td>
<td>—</td>
<td>500</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>F</td>
<td>—</td>
<td>100</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>Y</td>
<td>N</td>
</tr>
</tbody>
</table>

IFN indicates interferon alfacon-1; 02, supplemental oxygen required; ICU, admitted to intensive care unit; max, maximum.
A) Maximum daily temperature

![Graph showing temperature changes over time](image)

B) Alanine aminotransferase (ALT)

![Graph showing ALT levels over time](image)

C) Aspartate aminotransferase (AST)

![Graph showing AST levels over time](image)

D) Alkaline phosphatase (ALP)

![Graph showing ALP levels over time](image)

Clinical normal ranges are shown for each parameter. Dashes (—) indicate median values. Some of these data have appeared in a different format in [16] (adapted with permission from *Journal of the American Medical Association*, 2003, 290:3222–3228. © 2003, American Medical Association. All rights reserved), but have been included for comparison with the data presented in Figures 2 and 3. IFN, interferon.
Figure 1. continued

E) Lactate dehydrogenase (LDH)

F) Creatine kinase (CK)

G) Chest radiograph involvement

H) Serum/plasma viral RNA
Table 2. Clinical features among SARS patients treated with corticosteroids and IFN alfacon-1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median peak value (IQR)</th>
<th>Median day of peak value (IQR)</th>
<th>Median time to resolution from onset of symptoms (IQR)</th>
<th>Median value immediately prior to IFN treatment (IQR)</th>
<th>Median time to resolution from start of IFN treatment (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max temp, °C</td>
<td>39.4 (39–39.7)</td>
<td>4 (3–5)</td>
<td>12 (11–15)</td>
<td>38.3 (38.1–38.8)</td>
<td>4 (2–5)</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>65 (52–169)</td>
<td>11 (8–11)</td>
<td>15 (14–18)</td>
<td>33.5 (29.8–53)</td>
<td>6 (6–7)</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>84 (72–359)</td>
<td>13 (12–15)</td>
<td>N/A* N/A</td>
<td>44.5 (23–104.8)</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>ALP, U/l</td>
<td>96 (72–122)</td>
<td>10 (7–13)</td>
<td>N/A† N/A</td>
<td>69 (57.5–95)</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>CXR</td>
<td>45 (35–50)</td>
<td>11 (9–11)</td>
<td>23 (22–23)</td>
<td>25 (20–35)</td>
<td>12 (11–12)</td>
</tr>
<tr>
<td>LDH, U/l</td>
<td>432 (316–531)</td>
<td>12 (11–13)</td>
<td>20 (19–22)</td>
<td>309 (277.5–410.8)</td>
<td>10 (7–12)</td>
</tr>
<tr>
<td>O₂ sat (%)‡</td>
<td>92 (90–93)</td>
<td>9 (9–10)</td>
<td>N/A‡ N/A</td>
<td>95 (93–95)</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>O₂ req'd, l/min</td>
<td>4 (3–6)</td>
<td>10 (10–11)</td>
<td>17 (17–20)</td>
<td>0 (0–0)</td>
<td>11 (6–11)</td>
</tr>
<tr>
<td>CK(F), U/l</td>
<td>269 (166–555)</td>
<td>8.5 (5–10)</td>
<td>11 (9–12)</td>
<td>97 (49–108)</td>
<td>4 (1–6)</td>
</tr>
<tr>
<td>CK(M), U/l</td>
<td>218 (152–262)</td>
<td>7 (5–12)</td>
<td>12 (7–15)</td>
<td>172 (119–187)</td>
<td>6 (3–8)</td>
</tr>
<tr>
<td>RNA-p, copies/ml</td>
<td>137.5 (93–176)</td>
<td>10.5 (9–11)</td>
<td>14 (12–15)</td>
<td>24.2 (0–41.5)</td>
<td>6 (4–7)</td>
</tr>
<tr>
<td>RNA-s, copies/ml</td>
<td>55.5 (46-65)</td>
<td>10 (8–11)</td>
<td>14 (12–15)</td>
<td>0 (0–41.8)</td>
<td>6 (4–7)</td>
</tr>
</tbody>
</table>

Time to resolution from onset of symptoms indicates the median time (in days) from onset of symptoms until each parameter returned to the normal range. Time to resolution from start of IFN treatment indicates the median time (in days) from the start of interferon alfacon-1 treatment until each parameter returned to the normal range. *No time to resolution is provided for ALT as none of the nine patients resolved to normal during the course of the study. †No time to resolution is provided for ALP, as all patients remained below normal throughout the course of the study. ‡For O₂ sat, ‘peak’ value indicates the lowest value recorded. § No time to resolution calculated, as supplemental oxygen was administered to patients with low O₂ sat. Max temp, maximum daily temperature; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; CXR, chest X-ray involvement score; LDH, lactate dehydrogenase; O₂ sat, oxygen saturation; O₂ req’d, maximum daily supplemental oxygen requirement; CK(F), creatinine kinase (female patients); CK(M), creatinine kinase (male patients); RNA-p, SARS-CoV RNA detected in plasma; RNA-s, SARS-CoV RNA detected in serum.
A) CXCL8 (IL-8)

B) CXCL10 (IL-10)

C) CCL2 (MCP-1)

Asterisk (*) indicates day on which the median value is significantly elevated (P<0.05) in comparison with the median for healthy controls, using the Mann–Whitney test. Dashes (—) indicate median values. IFN, interferon; IL, interleukin.
Generally, serum cytokine concentrations peaked between days 9 and 13, and chemokine concentrations peaked from days 10–15. For specific cytokines, peak serum expression appeared closely associated with peak serum or plasma viral load, with IL-10, IFN-γ and CXCL10 levels peaking within 4 days of peak viral load and IL-12p70, IL-4 and TNF-α concentrations being highest within 5 days of peak viral load. Other cytokines were less consistently associated with elevated viral loads, although in many patients coordinate increases in expression of multiple pro-inflammatory cytokines were apparent.

While no single cytokine profile was observed in all patients within the study, similar patterns of increased cytokine expression were observed among some patients. Patients 1 and 4, for example, both exhibited increases above normal levels of all cytokines and chemokines with the exception of CXCL9. These patients both received a maximum steroid dose of 500 mg and both required supplemental oxygen.

In patients 2 and 9, IL-12p70, IL-1β and TNF-α were not elevated. These patients had similar peak CXR involvement scores (25–35) and received the same maximum steroid dose of 100 mg, although patient 9 required supplemental oxygen and patient 2 did not.

Patient 6 exhibited a unique profile of cytokine elevation, with all cytokines except CCL2 elevated above normal. This patient also showed sustained elevations in IL-6, IL-12p70, IL-1β, CCL5 and CXCL8 following a decrease in viral load. Patient 6 received a maximal steroid dose of 500 mg, required mechanical ventilation and was admitted to the ICU for the longest duration of any patient in the study. Interestingly, this patient also suffered from asthma, which may account for the particularly severe course of disease and sustained elevation of pro-inflammatory cytokines.
Figure 3. Comparison of cytokine concentrations in serum from nine SARS patients treated with IFN alfacon-1

A) IL-6

B) IL-1β

C) IL-4

D) IL-10

Asterisk (*) indicates day on which the median value is significantly elevated (P<0.05) in comparison to the median for healthy controls, using the Mann–Whitney test. Dashes (—) indicate median values. IFN, interferon; IL, interleukin; TNF, tumour necrosis factor.
**Discussion**

The role of pro-inflammatory cytokines in various respiratory viral infections as well as acute respiratory distress syndrome (ARDS) has been well documented [17–22]. For example, chemokines associated with the recruitment of inflammatory cells, including CCL2 and CCL3 (MIP-1α), are elevated in children with respiratory syncytial virus infections [20]. Elevations in IL-1β, TNF-α, IFN-γ and IL-12 levels have been reported in mice experimentally infected with subspecies B1 human adenovirus [23]. In addition, respiratory syncytial virus has been shown to induce both in vitro and in vivo production of chemokines and cytokines such as CCL2, CCL5, CXCL10, CXCL9, TNF-α, IL-6, IFN-γ, IL-4, IL-10 and KC (an IL-8 homologue) [24,25]. Elevated expression of IL-6, TNF-α and CXCL8, but not IL-1β, have been reported in volunteers experimentally infected with influenza H1N1 [21]. In these patients, it was noted that IL-6
Table 3. Changes in cytokine profile in SARS patients before and after treatment with IFN alfacon-1

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>Normal range</th>
<th>Immediately prior to interferon treatment</th>
<th>Day 2 after interferon treatment</th>
<th>Percent change, %</th>
<th>Day 4 after interferon treatment</th>
<th>Percent change, %</th>
<th>Day 7 after interferon treatment</th>
<th>Percent change, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>1–4</td>
<td>4.1 (10.8–68.9)</td>
<td>20.7 (13.7–94.5)</td>
<td>15.1</td>
<td>17.9 (6.1–42.4)</td>
<td>30</td>
<td>9.2 (14.5–25.4)</td>
<td>31.7</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0–1</td>
<td>41.6 (28.5–86.8)</td>
<td>19.5 (13.1–40.6)*</td>
<td>53.1</td>
<td>17.8 (12.6–31.5)</td>
<td>35.2</td>
<td>20.6 (13.2–27.928)</td>
<td>30.5</td>
</tr>
<tr>
<td>IL-10</td>
<td>3–5</td>
<td>9.9 (6.6–14)</td>
<td>11.1 (5.4–13.5)</td>
<td>12.1</td>
<td>7.2 (6.15–10.9)</td>
<td>31</td>
<td>7.9 (5.9–8.7)</td>
<td>32.4</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>13–34</td>
<td>5.3 (3.4–65.5)</td>
<td>6.3 (5–47.4)</td>
<td>19.9</td>
<td>4.8 (1–25.3)</td>
<td>34.6</td>
<td>6.7 (4.6–34.55)</td>
<td>35.1</td>
</tr>
<tr>
<td>IL-1β</td>
<td>21–67</td>
<td>20 (0–361.6)</td>
<td>24.2 (0–257.3)</td>
<td>21</td>
<td>0 (0–112.0)*</td>
<td>100</td>
<td>28.5 (0–196.4)</td>
<td>42.5</td>
</tr>
<tr>
<td>IL-4</td>
<td>1–4</td>
<td>3.8 (0–4.2)</td>
<td>2.6 (0–4.25)</td>
<td>31.6</td>
<td>3.5 (0.25–4.9)</td>
<td>7.9</td>
<td>3.1 (0–4.7)</td>
<td>26.4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1–2</td>
<td>1.9 (1.6–2)</td>
<td>1.8 (1.4–2.5)</td>
<td>5.3</td>
<td>1.6 (0.8–1.9)</td>
<td>15.8</td>
<td>2 (1.8–2)</td>
<td>5.3</td>
</tr>
<tr>
<td>MCP-1</td>
<td>145–192</td>
<td>89.6 (64.6–131.2)</td>
<td>100.1 (86.4–130.7)</td>
<td>11.7</td>
<td>89.9 (69.6–118.4)</td>
<td>0.3</td>
<td>154.7 (93.7–308.4)</td>
<td>72.7</td>
</tr>
<tr>
<td>IP-10</td>
<td>370–477</td>
<td>1475 (941.4–2200.5)</td>
<td>919 (657.9–1996.2)*</td>
<td>37.7</td>
<td>705.9 (423.9–1391.9)</td>
<td>52.1</td>
<td>919 (705.9–1017.1)*</td>
<td>37.7</td>
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<td>MIG</td>
<td>252–304</td>
<td>282.9 (129.3–351.9)</td>
<td>125.2 (83–369.2)</td>
<td>55.7</td>
<td>136.4 (64.4–187.2)</td>
<td>51.8</td>
<td>169.2 (67.2–222.9)</td>
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<tr>
<td>IL-8</td>
<td>12–18</td>
<td>21.3 (19.3–31.7)</td>
<td>36.4 (30.5–87.3)*</td>
<td>70.9</td>
<td>39.3 (23.8–83.6)</td>
<td>84.5</td>
<td>55.8 (40.5–202.8)</td>
<td>162</td>
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</tbody>
</table>

Results are median (interquartile range). *P<0.05 for Wilcoxon signed ranks test comparing median value on treatment day with the median value pre-treatment. IFN, interferon; IL, interleukin; TNF, tumour necrosis factor.

levels peaked early in disease (day 2), while TNF-α peaked on days 3–4 and CXCL8 peaked later in the course of disease. Notably, it has been reported that lower initial levels of both IL-6 and TNF-α were associated with better responses to steroid treatment in ARDS [17].

The hypothesis that an immunopathological mechanism may underly the lung damage associated with SARS [9,10] is consistent with our findings of elevated pro-inflammatory cytokine levels that persisted beyond the decline in viral load in the majority of patients. To minimize lung injury due to inflammatory processes, our patients received corticosteroids, a treatment which may have affected peak cytokine expression levels. Indeed, one study investigating changes in cytokine profile following corticosteroid treatment of children with SARS reported significant decreases in IL-6, IL-1 and CXCL8 and IL-1a treated with corticosteroids compared with untreated patients in a cohort followed in Beijing [27].

In addition to their antiviral properties, IFNs exert anti-inflammatory activity, specifically inducing the antagonists soluble TNF receptor and IL-1 receptor antagonist [28]. Thus, the use of IFN alfacon-1 in our patient cohort may also have limited the peak expression levels for the different pro-inflammatory cytokines. An analysis of serum cytokine and chemokine levels before and after IFN alfacon-1 treatment indicated that IL-6, IL-1β, IFN-γ and CXCL10 were all significantly reduced ranging from day 2 to day 7 following the initiation of treatment. Interestingly, a recent study reports that steroid treatment alone resulted in a reduction in CCL2, CXCL10, and CXCL8 from 5–8 days following treatment [29].

Increases in CXCL8 identified through nasal lavage did not always correlate with systemic increases in CXCL8 in a study of experimental human influenza A virus infection [21]. Thus, while we observed increases in systemic CXCL8 in all SARS patients, expression of this chemokine may have been elevated to an even greater extent in lung tissue. Notably, increases in CXCL8 production can be evoked in alveolar macrophages by mechanical ventilation alone [3]. Furthermore, stress of lung cells in vitro is associated with an increase in CXCL8 [30]. The magnitude of CXCL8 production in the one mechanically-ventilated patient in our study (patient 6) was, however, no different than that detected in the other patients, possibly reflecting the treatment of patient 6 with the higher 15 µg dose of IFN alfacon-1 and high-dose intravenous methylprednisone.

Taken together, the elevated expression of some pro-inflammatory cytokines observed in our study are consistent with some of the findings reported for other SARS patient cohorts [13]. The Beijing Group of the National Research Project for SARS reported that IL-6 concentration peaked during the recovery phase (beyond 15 days from symptom onset). In this study, CXCL8 expression was reported to be elevated during peak disease and remission (beyond 8 days) and TNF-α elevated throughout the course of disease [13].

A significant increase in IFN-γ and the pro-inflammatory cytokines IL-1β, IL-6 and IL-12 has been reported for at least 2 weeks after disease onset in SARS patients treated with corticosteroids [29]. In the same study, an increase in the anti-inflammatory cytokine IL-10, occurring approximately 1 week after...
symptom onset in some patients and lagging behind the elevation in pro-inflammatory cytokines, was also observed. These findings are somewhat consistent with our results, although IL-1β and IL-12 were not generally elevated in our patient cohort. These latter differences between the respective study observations may be related to differences in treatment protocols (our patients received IFN alfacon-1), in methodologies for cytokine evaluation and in differences between the study populations – our patients were predominantly Caucasian, also including three Asian and one black patient, while others have reported on an entirely Asian study group [29]. While normal values reported by others [29] are generally similar to those observed in our study, CCL2 levels were higher in our control group, a finding which accounts for the lack of significant CCL2 elevation observed in our SARS patients. Early increases in CXCL8 have been observed in other patient cohorts, followed by a decline that occurred from 5–8 days after the onset of corticosteroid treatment [29]. CXCL8 was found to be elevated later in the course of disease in our study and increased over time for some patients, with the median value peaking on day 18 after symptom onset. There was no single signature pattern of cytokine or chemokine expression in our study cohort. Nevertheless, IFN-γ, IL-10, CXCL8, CXCL10 and CCL5 were elevated above normal for all patients during their course of disease. When detected, IL-6 and TNF-α levels peaked earlier in disease than CXCL8 levels.

In agreement with our data, it has been reported that serum TNF-α levels did not change and IL-6 levels were increased in a cohort of adult SARS patients in Beijing, when compared with a group of healthy controls [27]. In contrast to our finding that CXCL8 was significantly elevated above normal levels on a number of days following disease onset, ranging from day 10–18, the results from the Beijing cohort indicate a decrease in serum CXCL8 levels [27]. Furthermore, they report that serum IL-10 levels were significantly increased in convalescent SARS patients (that is, in samples collected from 35–42 days following the onset of symptoms), but were not significantly differently changed during the acute phase of disease in SARS or severe SARS patients [27]. In our patient cohort, we observed significantly elevated IL-10 levels on a number of days ranging from day 8–10 following the onset of symptoms.

In a recent study, long-term persistence (19–29 days) of viral RNA in the serum and sputum of SARS CoV-specific IgG seroconverters was reported [31]. We did not identify viral RNA in the serum or plasma in this study cohort beyond day 12. We attribute the more rapid clearance of viral RNA from serum and plasma, and lower peak levels of proinflammatory cytokines compared with other SARS patient cohorts, to IFN alfacon-1 plus corticosteroid treatment, which we believe led to improvement in clinical disease and more rapid resolution of disease-associated radiographic lung abnormalities, improved oxygen saturation and decreased requirement for supplemental oxygen, as previously reported [16]. When considered altogether, the dynamic changes in cytokine and chemokine expression levels observed during the course of disease are reflective of pathogenesis and may have some utility as prognostic indicators. However, the data do not suggest a therapeutic strategy for SARS patients that would include anticytokine therapy.

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