Expression of Lymphocytes and Lymphocyte Subsets in Patients with Severe Acute Respiratory Syndrome

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In a cohort of 38 patients with severe acute respiratory syndrome (SARS), we observed leukopenia in 47% of patients, lymphopenia in 84%, and T lymphopenia in 95%. CD4+ T lymphocyte levels were reduced in 100% of patients, CD8+ T lymphocyte levels were reduced in 87%, B lymphocyte levels were reduced in 76%, and natural killer cell levels were reduced in 55%. Our data suggested that these patients’ immune systems were impaired during the course of SARS. The absolute counts of lymphocyte subsets demonstrated a clinical significance for patients with SARS.

Since November 2002, an outbreak of severe acute respiratory syndrome (SARS) has been taking place throughout the world [1, 2]. The World Health Organization announced that a novel coronavirus is the cause of SARS [3–5]. In the People’s Republic of China, Beijing is the worst-affected city. As of 6 June 2003, there were 2521 cases of SARS and 191 deaths reported in this city [6].

Unfortunately, because SARS is a new type of highly contagious disease in humans, there are no accurate and adequately field-tested laboratory diagnostic methods available yet. The diagnosis of SARS remains based on clinical and epidemiological findings [7–8]. Because the immune response is likely to be a useful indicator in the development of effective methods of diagnosis and treatment of SARS, we analyzed the expression of lymphocytes and lymphocyte subsets by flow cytometry in patients with SARS.

**Patients and methods.** We enrolled a total of 38 patients with SARS (20 male and 18 female; age range, 15–80 years) whose illnesses fit the authoritative definition of SARS [9, 10]. All these patients were initially admitted to Peking Union Medical College Hospital (Beijing) and had not received any ribavirin and corticosteroid therapy. In addition to the 38 patients with SARS, we had previously tested 200 healthy blood donors to establish interlaboratory reference ranges for various parameters. We used these reference values as data for healthy control subjects in this study.

After informed consent was obtained, 2-mL samples of EDTA anti-coagulated peripheral blood were collected from patients with SARS before they received initial treatment. All the samples were prepared and tested within 6 h after being obtained.

First, a complete blood count and a differential count were performed with an automated hematology analyzer (Advia 120; Bayer). Second, 5 aliquots of 100 μL from each blood sample were placed in 5-mL polystyrene tubes. These blood specimens were then incubated in the dark for 20 min at room temperature with 20 μL of each of the following reagents: fluorescein isothiocyanate (FITC)–CD4/phycoerythrin (PE)–CD8/ peridinin chlorophyll protein (PerCP)–CD3 three-color antibody, FITC/CD3/PE/CD16+56 two-color antibody, PE–CD19 antibody, FITC–IgG1/PE–IgG1/PerCP–CD3 isotype control, FITC–IgG1/PE–IgG1 isotype control and PE–IgG1 isotype control (all from Becton Dickinson). To remove contaminating erythrocytes and to fix the cells, 2 mL of lysing solution (Becton Dickinson) was individually added to 5 tubes. After 8 min, the samples were centrifuged (5 min at 300 g) and resuspended in 2 mL of PBS. After the second wash, samples were resuspended in 500 μL of PBS with 0.5% bovine serum albumin. The cell preparations were stored at 4°C in the dark until measurement, which was done within 4 h.

Every sample was measured using the Epics Elite ESP flow cytometer (Beckman Coulter). Before measurement, the optical path was adjusted by testing with the optics calibrator Flow Check (Beckman Coulter). The result of one-half of the value of the coefficient of variation should be <2%. We then tested Cyto-Trol (Beckman Coulter Immunotech), as a control, to confirm that the results were within the target range.

Data acquisition and analysis were performed with the Elite workstation. A count cycle contained 10,000 cells. By means of proper gating and compensation, we could distinguish lymphocytes from other clusters in a forward-versus-side-scatter dot plot and then, by use of fluorescence signals, analyze the expression of CD4+ T cells (CD3+CD4+CD8-), CD8+ T cells...
Table 1. Leukocyte and lymphocyte levels in 38 patients with severe acute respiratory syndrome and in 200 healthy control subjects.

| Variable                      | Mean value ± SD            | Case patients | Control subjects | P     |
|-------------------------------|-----------------------------|---------------|------------------|-------|
| Leukocyte count, cells × 10^9/L | 4.77 ± 1.65                 | 7.00 ± 1.50   | <.001            |
| Lymphocytes, %                | 17.53 ± 10.61               | 32.82 ± 6.13  | <.001            |
| Lymphocyte count, cells × 10^9/L | 0.91 ± 0.44                 | 3.00 ± 1.00   | <.001            |

The initial blood counts showed leukopenia (a total leukocyte count of <4 × 10^9 cells/L) in 18 (47%) of 38 patients with SARS. The difference in this value between patients with SARS and healthy control subjects was statistically significant (P < .001). Leukocytosis was not observed. Lymphopenia (an absolute lymphocyte count of <1.00 × 10^9 cells/L) was noted in most of patients with SARS. Of the 38 patients, 32 patients (84%) showed a decreased lymphocyte count and 31 (82%) had a decreased lymphocyte percentage (a lymphocyte percentage of <20.6%). The mean lymphocyte count of patients with SARS was significantly lower than that of healthy control subjects (P < .001; table 1).

The results of lymphocyte immunophenotyping demonstrated that the absolute counts of all lymphocyte subsets declined in patients with SARS (table 2). A sharply decreased T cell count was observed in most patients: 36 (95%) of the 38 patients had T cell absolute counts lower than the mean count for the healthy control subjects. Decreased B cell counts were observed in 29 patients (76%). A moderate reduction in the NK cell count was noted in 21 patients (55%). Among the T cell subsets, CD4+ T cell counts were found to have decreased in all patients; the lowest value was 0.04 × 10^9 cells/L. A decrease in the CD8+ T cell count was observed in 33 patients (87%); the lowest value was only 0.07 × 10^9 cells/L. There were significant differences between patients with SARS and healthy control subjects for these indices (P < .01).

As for the percentages of lymphocyte subsets, the comparative percentage of T cells decreased in 22 patients (58%). In contrast, the B cell and NK cell percentages were normal or elevated in 37 patients (97.4%) and in 36 patients (94.7%), respectively. Only 1 patient (2.6%) was noted to have a relative decrease in the B cell count, and 2 patients (5.3%) were noted to have a decreased NK cell count. With respect to the subsets of T cells, the percentage of CD4+ T cells relatively decreased in 31 patients (82%). The percentage of CD8+ T cells was also reduced in 13 patients (34%). Seventeen patients (44%) had a decreased ratio of CD4+ T cells to CD8+ T cells. Compared with healthy individuals, the T cell and B cell percentages were significant different (P < .001). The data are summarized in table 3.

Discussion. Levels of lymphocytes and lymphocyte subsets are of great importance to keep the immune system functional. Usually viral infection, immunodeficiency diseases, and other infectious diseases lead to abnormal changes in the levels of lymphocyte subsets [11–13]. At present, SARS is spreading over the world as a new clinical entity. Although a novel coronavirus has been identified as the cause of SARS, we know little, as yet, about the mechanisms by which SARS impacts the human immune system.

In our study, we found that lymphopenia—in particular, T lymphopenia—was common among patients with SARS, which suggests that the patient’s immune system is impaired during the course of early infection with the SARS virus. In addition to reductions in T lymphocyte level, reductions of B lymphocyte and NK cell levels are observed in patients with SARS. Because of the depletion of lymphocytes, leukopenia or a low-normal leukocyte count is noted in some patients. All these findings are quite different from those associated with pneumonia caused by common respiratory viruses, which usually is associated with a normal or elevated lymphocyte count [14].

Our results with respect to the levels of T cell subsets support the hypothesis that, in patients with SARS, the percentage of CD4+ T cells is much lower than that of CD8+ T cells. This implies that CD4+ T cells are more severely damaged by the SARS virus than are CD8+ T cells. However, 87% of the patients with SARS also show decreases in of the CD8+ T cell level, in contrast to the findings for patients with HIV infection. In general, the adaptive immune response to viral infections occurs by means of the cytotoxic T lymphocyte (CTL) response.
Table 3. Percentages of lymphocyte subsets in 38 patients with severe acute respiratory syndrome and in 200 healthy control subjects.

| Lymphocyte subset | Percentage, mean ± SD | Case patients | Control subjects | P  |
|-------------------|-----------------------|---------------|------------------|----|
| T cells           | 61.15 ± 13.22         | 73.22 ± 6.05  | <.001            |
| CD4+ T cells      | 27.90 ± 7.49          | 38.52 ± 4.49  | <.001            |
| CD8+ T cells      | 28.27 ± 9.01          | 33.81 ± 7.13  | <.001            |
| B cells           | 16.56 ± 4.55          | 12.25 ± 3.65  | <.001            |
| Natural killer cells | 17.90 ± 7.51        | 17.33 ± 4.52  | >.05             |
| CD4+/CD8+ cells   | 1.06 ± 0.40           | 1.47 ± 0.53   | <.001            |

CTLs are generated in response to an invading pathogen, and they specifically recognize and kill virus-infected cells and/or release inhibitory antiviral soluble factors [12, 13]. Therefore, CD8+ T cell counts should be sharply increased in patients with SARS. The mechanism of reduction of CD8+ T cell counts in patients with SARS needs further investigation.

The human immune system shows a different response to the SARS coronavirus than it does to HIV and other viruses. Our study shows that the absolute counts of CD4+ T cells and CD8+ T cells have clinical significance in patients with SARS and that surveillance of lymphocytes and lymphocyte subsets is helpful in the diagnosis and treatment of SARS. Study of lymphocytes and lymphocyte subsets is important in order to reveal the mechanism of SARS infection. Further study of lymphocyte levels in health care workers who were exposed to SARS is ongoing in our laboratory.

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