Serum Cytokine Responses in Primary Pneumonic Plague Patients

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The serum levels of interleukin-2 (IL-2), gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-4, IL-6, and IL-10 of pneumonic plague patients were determined by enzyme-linked immunosorbent assay. IL-6 was the only elevated cytokine in the patients, and its level increased with a clear time course, indicating that IL-6 might be a prognostic marker for predicting the progression of plague.

Most human plague cases clinically represent three forms, bubonic, septicemic, or pneumonic, depending on the route of infection (18). Pneumonic plague is the most threatened form of the disease because of its high mortality without timely and effective treatment (21).

On July 29, 2009, deadly primary pneumonic plague (PPP) hit Xinghai County, Qinghai Province, China, with 12 confirmed cases and 3 deaths (22). This outbreak has been investigated by clinical, epidemiological, bacteriological, and immunological methods. Within 3 days after the onset of symptoms, all patients were treated with streptomycin, ceftriaxone sodium, and ciprofloxacin for 15 days except one, who was treated for 26 days.

Although pneumonic plagues have been reported in the United States, India, Uganda, Zambia, Ecuador, and Madagascar (3–6, 10, 15, 19, 23–24), there has been no report on inflammatory cytokines in response to Yersinia pestis in pneumonic plague patients until now because they were all retrospective investigations. We investigated the dynamics of serum cytokines in nine pneumonic plague patients to reveal the relationship between cytokine production and the disease course.

Thirty-six serum samples from nine patients were collected on days 8, 11, 16, and 18 after the onset of clinical symptoms, and 80 control serum samples were obtained from healthy herdsmen. The symptoms of the patients were obviously alleviated after 1 week of antibiotic treatment, indicating the end of the acute phase of PPP for them, which suggested that the sample from day 8 was at the later acute phase and the sample from day 11 at the early convalescent stage. The serum levels of interleukin-2 (IL-2), gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-4, IL-6, and IL-10 were measured by a high-sensitivity enzyme-linked immunosorbent assay (ELISA: Bender MedSystems) according to the manufacturer’s instructions. The difference in cytokine levels between pneumonic plague patients and healthy controls was revealed by analysis of variance (ANOVA) with SARS 8.0 software, and cytokine concentrations at various time points were compared by the Student t test. A probability value of <0.05 was considered significant.

Among the six cytokines measured by ELISA, the elevated levels of IL-6 were observed in the pneumonic patients at all time points (P < 0.05). Moreover, IL-6 production declined in the convalescent phase in patients. There were no differences in concentrations of IL-2, IFN-γ, TNF-α, IL-4, or IL-10 between patients and healthy controls (P > 0.05) (Fig. 1).

In some patients with community-acquired pneumonia (CAP) caused by Mycoplasma pneumoniae, Chlamydia pneumoniae, Streptococcus pneumoniae, or a virus (16) or patients with severe acute respiratory syndrome (SARS) pneumonia (9, 25), cytokine responses were investigated (7). Y. pestis causes a severe pneumonia in both humans and animals when given by the inhalation route. Comparison of serum cytokine responses among patients with CAP, SARS pneumonia, and plague pneumonia revealed significant differences in serum cytokine profiles. It seemed that IL-6 was the only elevated cytokine in patients with pneumonia caused by Y. pestis or S. pneumoniae. Unfortunately, only two cytokines, IL-1β and IL-6, were detected in patients with CAP caused by S. pneumoniae (13), and we could compare only IL-6 changes in patients with pneumonia caused by Y. pestis or S. pneumoniae. Interestingly, serum concentrations of IL-6 were significantly elevated in all patients infected with M. pneumoniae, C. pneumoniae, or influenza A virus (11), S. pneumoniae (13), SARS virus (9, 20, 25), or Y. pestis. Our results further provided evidence for the notion that elevated IL-6 concentrations correlate with the degree of severity of inflammation rather than the specific etiologic agent of pneumonia (16). Therefore, IL-6 might be a prognostic marker for predicting the progression of pneumonic plague.

Interleukin-6 is a multifunctional proinflammatory cytokine (8) which can be produced by both blood leukocytes and endothelial cells within the injured lung tissue. Therefore, elevated serum IL-6 production levels might originate from injured pulmonary bronchial and alveolar epithelial cells (25). Likewise, elevated IL-6 levels might be released...
from endothelial cells within the injured lung tissue of pneumonic plague patients. It has been found that IL-10 and TNF-α levels were elevated in SARS patients (9, 20, 25), and elevated TNF-α and IFN-γ levels were observed in patients with pneumonia caused by *M. pneumoniae*, *C. pneumoniae*, or influenza A virus (11). In the animal experiment, 72 h after infection with aerosolized *Y. pestis*, TNF-α production was significantly increased in mice (2) and rats (1) 72 h after infection with aerosolized *Y. pestis*, indicating that there might be different cytokine profiles in humans and rodents. However, in an intranasally infected mouse model of pneumonic plague, genes encoding a variety of cytokines are upregulated in the early phase (at 12 h postinfection) of infection, but they are downregulated in the middle phase (at 24 h postinfestation), indicating inhibition of the host defense system during the development of plague (14). Unfortunately, due to the sudden outbreak, serum samples from these patients were not collected in the earlier stages. Therefore, the cytokine concentrations in Qinhai plague patients and infected animals with aerosolized *Y. pestis* in the early acute phase of PPP were not available for comparison. Since no associations between cytokine concentrations and patients’ sex or history of antibiotics have been documented for CAP patients (16), we could not determine whether the measured levels of some cytokines had waned due to treatment with antibiotics or differences in patients’ age or sex in this preliminary investigation. IL-10 production was undetectable in plague patients, which is consistent with the results for sera of mice infected with *Y. pestis* by the intranasal route (12, 14).

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**FIG. 1.** Determination of IL-2, IFN-γ, TNF-α, IL-4, IL-6, and IL-10 levels in the sera of nine pneumonic plague patients by ELISA. The x axis shows the days of measurement after the onset of symptoms and a control group (C). The y axis shows the concentrations of six cytokines (pg/ml). An asterisk indicates that the *P* value is <0.05 compared to the control group.

### REFERENCES