Legionnaires' disease is an important cause of epidemic and sporadic pneumonia in humans (1). The most common causative agent, *Legionella pneumophila*, is a facultative intracellular bacterium that attacks mononuclear phagocytes. Diagnosis is still difficult because this organism does not grow on routine bacteriological media, so application of specific techniques, such as PCR, for identification of bacteria is required for accurate diagnosis (7). Marston et al. (13) documented more than 3,000 cases of Legionnaires' disease that were reported to the Centers for Disease Control and Prevention, Atlanta, Ga., from 1980 through 1989. They pointed out that Legionnaires' disease was underreported, most likely because of underdiagnosis. Epidemiological data predict that an estimated 17,000 to 23,000 cases of community-acquired *Legionella* pneumonia occur annually in the United States. Unfortunately, the mortality rate is still high, particularly in immunocompromised hosts (8).

Development of cell-mediated immunity in response to *L. pneumophila* plays a key role in the inhibition of bacterial growth and resolution of legionellosis. Although effector mechanisms of cell-mediated immunity directed against this organism in the lung are not completely understood, in vitro studies indicate that gamma interferon (IFN-γ)-activated macrophages inhibit the intracellular growth of the bacterium (17, 23). Recently, two subsets of CD4⁺ T cells, Th1 and Th2, have been defined on the basis of their cytokine profiles (12, 20). Th1 cells produce interleukin-2 (IL-2) and IFN-γ and are involved in cell-mediated immunity, while Th2 cells produce IL-4, IL-5, IL-6, and IL-10 and are associated with humoral immunity. Furthermore, it has been demonstrated that some of these immunoregulatory cytokines possess cross-regulatory properties. For example, IL-12 not only enhances Th1 clones and their cytokine production but also suppresses Th2 clones and their respective cytokines (24). Despite accumulating evidence indicating a crucial role for Th1/Th2 cytokine balance in animal models of infectious diseases (4, 19), only limited data exist for human bacterial diseases in terms of Th1 and Th2 cytokine profiles. In this study, we examined the immune status of patients with *Legionella* disease by examining Th1 and Th2 cytokines in serial serum samples obtained from patients with *Legionella* pneumonia.

Clinical specimens such as sputum, bronchoalveolar lavage fluid, serum, and urine from suspected cases of *Legionella* pneumonia were sent to our department. We defined a case of *Legionella* disease as radiographically confirmed pneumonia accompanied by at least one of the following: (i) isolation of *Legionella* from respiratory secretions; (ii) a fourfold increase in antibody titer (microagglutination kit; Denka-seiken, Tokyo, Japan); (iii) detection of urinary antigen (*Legionella* urinary antigen enzyme immunoassay; Binax, Portland, Maine), or (iv) detection of *Legionella* DNA by PCR (2). In confirmed cases of *Legionella* pneumonia, information such as basic personal data and data concerning underlying diseases, including previous and/or concurrent infections, medications, symptoms, outcomes, and results of specific laboratory tests, was collected. For determination of serum cytokine levels, 75 samples from 36 cases were stored in aliquots at −80°C until assayed for cytokines. Levels of IL-1β, IL-4, IL-6, IL-10, tumor necrosis factor alpha (TNF-α), IFN-γ, and IL-12 (p40 and p70) in serum were quantified by enzyme-linked immunosorbent assay with a detection limit in the picogram-per-milliliter range (IL-1β and IFN-γ were from Otuka Pharmaceutical; IL-4, IL-6, and IL-10 were from Genzyme; TNF-α was from PerSeptive Diagnostics; IL-12 was from Biokine T Cell Diagnostics, Woburn, Mass.). In preliminary studies, it was confirmed that these cytokines were not detectable in sera of healthy volunteers (n = 5).

Fourteen patients were confirmed as having *Legionella* pneumonia. They were diagnosed by serum antibody (five cases), culture (one case), urinary antigen detection (six cases), and/or PCR (six cases). Among these cases, 11 were diagnosed by a single method while three were diagnosed by multiple methods. Etiologic organisms were *L. pneumophila* (12 cases) and *Legionella bozemanii* (one case), and the pathogen in remain-
ing case was presumed to be *L. pneumophila* or *Legionella dumoffii* pneumonia, as significant increases of antibody titer against both organisms were observed. One patient with *L. pneumophila* pneumonia died, whereas the others survived. Figure 1 shows levels of C-reactive protein (CRP), IL-1β, IL-6, and TNF-α in the sera of 14 patients with *Legionella* pneumonia. All patients had high serum CRP levels in the acute phase. In contrast, in only four cases (one involving IL-1β, three involving IL-6, and none involving TNF-α) were cytokine concentrations >100 pg/ml.

Figure 2 shows the concentrations in serum of IL-4, IL-10, IFN-γ, and IL-12. IL-4 and IL-10 were detected in only one case each. The patient showing the highest level of IL-10 in serum also had detectable levels of IL-1β and IL-6, as shown in Fig. 1. This patient died 48 days after the onset of pneumonia because of complications involving interstitial pneumonitis of unknown etiology. The concentrations of IFN-γ and IL-12 in the sera of these patients were clearly different from those of other cytokines; significant increases in the levels of IFN-γ (range, 414 to 1,028 pg/ml) and IL-12 (range, 161 to 1,006 pg/ml) in serum were observed in the acute phases of 6 and 11 cases, respectively. Although serum IFN-γ levels diminished thereafter, seven cases sustained high levels or showed even further increases of IL-12 levels in the 20 days after the onset of pneumonia. With one exception, these patients showed no signs of exacerbation of the pneumonia or other complications during the observation period.

We also examined serum levels of IL-12 in an additional 22 cases of pneumonia clinically suspected to be but not diagnosed as *Legionella* pneumonia. Those cases fell into two distinct groups; 16 cases showed significant increases of levels of IL-12 in serum, as in the confirmed cases of *Legionella* pneumonia (range, 230 to 1,049 pg/ml), whereas IL-12 was not detected in the remaining six cases (data not shown). Considering the fact that diagnosis of *Legionella* disease is still difficult and more than 10,000 cases may be overlooked annually in the United States, it is likely that *Legionella* pneumonia is involved in these cases.

The major finding of our study is the relative predominance of cellular immune responses in patients with *Legionella* pneumonia, as evidenced by the significant increase of levels of Th1 cytokines (IFN-γ and IL-12) in serum. In addition, our data
demonstrate for the first time the potential of IL-12 as a critical mediator of host immunity against legionellosis.

The balance of Th1/Th2 cytokine responses is believed to play an important role in orchestrating the immune response against invading microbes (12, 20). Of special importance are cytokines (IFN-γ and IL-12) and the Th2 cytokines (IL-4 and IL-10). Several experimental models of infectious diseases, such as those caused by Leishmania spp. (4, 19), Toxoplasma gondii (6), Mycobacterium spp. (3), Listeria monocytogenes (5), and Candida albicans (21), shed light on the critical role of the Th1/Th2 balance in innate and adaptive immune responses to infections. However, in the clinical setting only a few diseases, such as pleuritis caused by Mycobacterium tuberculosis (11), leprosy (22), and leishmaniasis (14), have been analyzed in terms of their immune status and Th1/Th2 balance.

Newton et al. (18) showed that suppression of Th1 activity by marijuana significantly sensitized mice to a lethal challenge of L. pneumophila. Kitsukawa et al. (9) detected mRNA encoding IFN-γ but not IL-4 in the supernatant of human peripheral blood mononuclear leukocytes cultured with L. pneumophila. Our results confirm these early findings and further demonstrate the crucial role of Th1-polarized immune responses in patients with Legionella pneumonia.

IL-12, a recently described cytokine, appears to possess the characteristics necessary to link the innate and cognate cellular immune systems (24). The ability of IL-12 to induce production of IFN-γ and other phagocytic cell-activating cytokines is particularly important during acute bacterial infections. In addition, IL-12 induces the differentiation of Th1 cells from uncommitted T cells, thus initiating cell-mediated immunity, which generally protects against intracellular parasites in the chronic stages of infections. Interestingly, in the present study, we observed sustained high levels of IL-12 in the sera of patients with Legionella pneumonia even in the convalescent phase, although signs of continuous colonization of the lungs or exacerbation of pneumonia were not observed. In this regard, Naot et al. (16) and Morley et al. (15) reported possible reactivation of Legionella pneumonia in immunocompromised patients. In addition, Kohler et al. (10) reported that 10 of 23 patients with Legionella pneumonia excreted antigen in their urine for 42 days or longer despite full recovery from Legionnaires' disease and an absence of clinical disease during this phase. We also observed continued urinary antigen excretion for more than two weeks postrecovery in four of our patients (data not shown). These data strongly suggest the continuous presence in these patients of bacteria or bacterial components or products, which may be associated with IL-12 production.

In the present study, we could not compare serum cytokines in pneumonia cases of different etiologies: we have only presented cytokine profiles of Legionella disease as a preliminary report. Whether these cytokine characteristics are specific to Legionella disease, in addition to whether blood monocytes from patients with Legionella pneumonia would preferentially synthesize Th1-type cytokines in response to Legionella antigens, remains to be determined in future studies. It would also be of great interest to determine if IL-12 could be used as a diagnostic indicator for certain infectious diseases which preferentially stimulate cell-mediated immunity. These issues would have important implications for our understanding of the pathological and immunological status of patients with legionellosis.

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