SUMMARY: Interleukin (IL)-17A affects the immune system of the lung. Legionella infection can potentially lead to severe pneumonia. The present study aimed to evaluate the role of IL-17A in Legionella pneumonia. Serum IL-17A levels were quantified in both patients with Legionella pneumonia and control subjects; IL-17 was detected in sera from 4 out of 31 patients with Legionella pneumonia but in any controls. There were no differences in peripheral white blood cell counts or other serum biomarkers (C-reactive protein, and lactate dehydrogenase) between IL-17A-positive and IL-17A-negative patients. All IL-17A-positive patients in this cohort survived, whereas 8 of 27 IL-17A-negative patients did not. IL-17A was detected in available bronchoalveolar (BA) fluid samples from 7 patients with Legionella pneumonia within our cohort. However, the IL-17A and IFN-γ concentrations in BA fluids did not correlate with each other. IL-17A might play a significant role in some cases of Legionella pneumonia.

Bacteria of the genus Legionella are important causative agents of epidemic and sporadic pneumonia in humans (1). The most common species is Legionella pneumophila. Legionella pneumonia progresses rapidly and can be fatal when complicated by acute lung injury and/or multiple organ failure (2). Along with Streptococcus pneumoniae, Legionella species are the leading cause of severe community-acquired bacterial pneumonia requiring intensive care unit admission (3). The cell-mediated immune response to Legionella plays a key role in the resolution of Legionella pneumonia (4). Serum levels of interferon gamma (IFN-γ), a Th1 cytokine, are increased in patients with Legionella pneumonia, whereas serum levels of Th2 cytokines such as interleukin (IL)-4 and IL-10 are not increased in such patients (5).

IL-17A, which is produced by Th17 cells and γδ T cells, exhibits unique activity in the pulmonary immune system (6). Recently, several groups examined the clinical roles of IL-17 in patients with community-acquired pneumonia (CAP) (7–9). Remmelts et al. reported low systemic concentrations of IL-17 in CAP cases (7). Paats et al. demonstrated that IL-17A was not detectable in the bronchoalveolar lavage (BAL) fluid and sera from patients with CAP (8), whereas such patients exhibited increased numbers of Th17 cells in the peripheral blood and bronchoalveolar (BA) space of patients with CAP (9). In a murine pneumonia model, IL-17 was found to contribute to immunity against Legionella pneumonia (10). However, the clinical role of IL-17 in human Legionella infections remains unclear. In this study, we attempted to detect IL-17A in the sera and BA fluid samples from patients with L. pneumophila pneumonia.

Thirty-six consecutive patients with Legionella pneumonia diagnosed in our laboratory between 1997 and 2007 were included in this study. Five patients were excluded because of a lack of appropriate samples. Pneumonia diagnosis was based on clinical presentation, chest radiography interpretation, and laboratory data. Legionella pneumonia diagnosis was confirmed by the detection of Legionella via culture, elevation of antibody titers in paired sera, and/or detection of specific Legionella antigen in the urine (11). Blood samples were obtained from each patient for conventional clinical diagnosis. BAL/bronchial washing fluid samples were obtained with written informed consent when required for the diagnosis of Legionella pneumonia. All samples were collected in accordance with the hospital protocol to assist with the establishment of diagnoses. The samples were stored at −80°C until required. Medical chart reviews were used to obtain information regarding the laboratory findings and clinical outcomes. This study was approved by the Institutional Review Board of the University of the Ryukus, Okinawa, Japan. The need for informed consent from each patient for study inclusion was waived because of the retrospective nature of this study, which did not result in the experience of any additional adverse events by any subject.

IL-17A and IFN-γ levels in clinical samples were determined via sandwich ELISA (R & D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer’s instructions. The lowest IL-17A and IFN-γ detection limits were <15 pg/ml and <8 pg/ml, respectively. All statistical comparisons between groups were performed IBM SPSS statistics software, version 21 (SPSS Inc., Chicago, IL, USA).

The average age of the group of 31 patients with L. pneumophila pneumonia was 61.2 ± 10.9 years, which included 27 men and 4 women. Control subjects were
randomly selected from patients who visited our hospital for health check-ups and did not present with relevant respiratory and infectious diseases. The average age of the group of 17 control subjects was 64.1 ± 11.8 years, which included 10 men and 7 women. Most samples were collected during the acute phase of infection (average: 10.7 ± 9.1 days; median: 8 days; Table 1). IL-17A was detected in the sera from 4 of 31 patients with Legionella pneumonia but was not detected in the sera of any control subjects. Serum IL-17A concentrations in the 4 positive patients were 30.7, 36.3, 48.3, and 590.7 pg/ml. For additional analysis, we categorized patients as either IL-17A-positive or IL-17A-negative patients; there were no significant differences with respect to age, sex, and clinical biomarkers (white blood cell counts, C-reactive protein, and lactate dehydrogenase) between these 2 groups. The days during the disease course when serum samples taken also did not differ between these 2 groups (Table 1). The ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2 ratio) appeared to be better in IL-17A-positive patients than in IL-17A-negative patients. All 4 IL-17A-positive patients survived, whereas 8 out of 27 IL-17A-negative patients succumbed to infection (Table 1).

Next, we determined cytokine levels in the available BAL fluid samples from 7 out of the 31 patients with Legionella pneumonia. IL-17A was detected in the 7 BAL fluid samples at concentrations ranging from 1.34 to 53.4 pg/ml (Fig. 1A). The IL-17A levels in the 2 available BAL fluid samples from IL-17A-negative patients were 53.4 and 8.8 pg/ml (Fig. 1A). In most cases, the IL-17A levels were higher in BAL fluids than in sera (Fig. 1A). A comparison of the IL-17A and IFN-γ concentrations (12) in BAL fluids found no significant correlation (Fig. 1B).

IL-17 is a cytokine that induces neutrophil-mediated inflammation and its role in protective immunity against bacterial infection has been reported (6,7). Our study detected IL-17A in sera from several patients with Legionella pneumonia. The presence of IL-17A in sera might be associated with a favorable outcome. In addition, IL-17A was detected in BAL fluids from Legionella pneumonia patients. The present findings suggest a clinical role for IL-17A in Legionella pneumonia, similar to the role reported in CAP associated with other bacterial pathogens (7–9). In addition, we demonstrated that the serum IL-17A levels did not correlate with the serum IFN-γ or BAL fluid IL-17 levels in patients with Legionella pneumonia. This finding suggests that IL-17A and IFN-γ are regulated differently during pneumonia. Innate immune cells and Th17 cells might therefore be responsible for intra-pulmonary IL-17A production, and further studies are warranted.

This study has several limitations. First, the study was retrospective and only evaluated samples collected at a single time point during the acute disease phase. Accordingly, the significant increase in IL-17A levels during infection might have been understated. Second, BA fluid samples were not available for the control subjects and therefore, the normal IL-17A concentration in the BA fluid is unknown. Even with these limitations, the present data demonstrate significant changes in the IL-17A levels in patients with Legionella pneumonia.

Table 1. Comparison of IL-17A positive and negative cases of Legionella pneumonia

|                          | Serum IL-17A positive (4 patients) | Serum IL-17A negative (27 patients) | P value |
|--------------------------|-----------------------------------|------------------------------------|---------|
| Age (years)              | 63.3 ± 16.4                       | 60.9 ± 10.2                        | 0.69 (1) |
| Sex (male: female)       | 4:0                               | 23:4                               | 0.56 (2) |
| Co-morbidity (number of patients) | No (3)                              | None (12)                          | 0.33 (2) |
|                          | Yes (1)                           | Yes (15)                           |         |
| Disease days when the sample was taken (median) | 6.8 ± 4.0 (range: 1–10) | 12.0 ± 10.2 (range: 2–42) | 0.10 (2) |
| White blood cells (×/μl) | 8,900 ± 2,483                     | 12,663 ± 8,309                     | 0.38 (1) |
| C-reactive protein (mg/dl)| 16.1 ± 11.8                       | 26.0 ± 12.7                        | 0.16 (1) |
| Lactate dehydrogenase (IU/l) | 647 ± 441                           | 891 ± 804                          | 0.56 (3) |
| PaO2/FiO2                | 312.7 ± 31.1                      | 212.3 ± 98.0                       | 0.055 (3) |
| Non-survivors (%)        | 0 (0)                             | 8 (29.6)                           | 0.28 (2) |

Serum IL-17A concentrations in “IL-17A-positive patients” were between 30.7 and 590.7 pg/ml. Serum IL-17A was not detected in IL-17A-negative patients.

1): Student t test.
2): Fischer’s exact test.
3): one case had renal disease and steroid treatment.
In conclusion, the present study suggests that IL-17A might play a role in certain clinical cases of *Legionella* pneumonia. Further studies are warranted to elucidate the clinical role of IL-17A in pulmonary infections due to *Legionella* and other pathogens.

**Acknowledgment** We thank Gretchen Parrott, MPH for reviewing this manuscript.

**Conflict of interest** None to declare.

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