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Short communication

Comparison of the chemokine profiles in the bronchoalveolar lavage fluid between IgG4-related respiratory disease and sarcoidosis: CC-chemokine ligand 1 might be involved in the pathogenesis of sarcoidosis

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ABSTRACT

Background: We previously reported that the cytokine profiles in the bronchoalveolar lavage fluid (BALF) of IgG4-related respiratory disease (IgG4-RRD) more closely resemble the T-helper (Th) 2 response than sarcoidosis. The present study aimed to assess the chemokines in the BALF of IgG4-RRD and sarcoidosis in order to evaluate any possible associations between these chemokines and other markers.

Methods: We examined 12 chemokines using a MILLIPLEX® MAP Kit (Millipore, Darmstadt, Germany) in the same BALF samples of the same 44 patients (IgG4-RRD, n = 11; sarcoidosis, n = 33) in which we had previously evaluated the cytokines.

Results: The levels of CC-chemokine ligand (CCL)26 in the BALF of IgG4-RRD patients (median 24.5, range 3.1–401.1 pg/mL) were significantly higher than those in the BALF of sarcoidosis patients (median 3.1, range 3.1–155.6 pg/mL, $p < 0.05$). Interestingly, the BALF levels of CCL1 in the sarcoidosis patients (median 13.1, range 0.1–106.9 pg/mL) were significantly higher than those of the IgG4-RRD patients (median 9.8, range 0.1–14.7 pg/mL, $p < 0.05$). Furthermore, the CCL1 levels in the BALF were correlated with the total cell count ($\rho = 0.539$, $p < 0.001$), lymphocyte fraction ($R = 0.406$, $P < 0.05$), lymphocyte count ($R = 0.686$, $P < 0.001$), TNF-α level, ($R = 0.748$, $P < 0.001$), and IL-2 level ($R = 0.757$, $P < 0.001$) in the BALF of sarcoidosis patients.

Conclusions: CCL1 might reflect disease activity and its involvement in the pathogenesis of sarcoidosis might be more closely related to Th1 than to Th2.

1. Introduction

Immunoglobulin G4-related disease (IgG4-RD) is a chronic fibrotic inflammatory disease that presents with multi-organ involvement characterized by the infiltration of IgG4-positive plasma cells and elevated serum levels of IgG4 [1]. On the other hand, sarcoidosis is a systemic disease of unknown cause that is characterized by granulomas in various organs and which involves the lungs and lymphatic system, such as the hilar lymph nodes [2,3]. Both of the diseases develop through lymphatic routes of the lungs, and bilateral hilar lymphadenopathy (BHL) is frequently observed on chest computed tomography (CT). Thus, the images of IgG4-RD often mimic findings of sarcoidosis on chest CT [4,5]. However, the clinical conditions of the two diseases are completely different. While T-helper (Th)1 immune responses are predominant in the organs affected by sarcoidosis [2,3], the autoimmunity of IgG4-RD is attributed to the activation of the Th2 immune response and the Th2-cell responses at the affected sites [1]. We previously reported that the cytokine profiles in the bronchoalveolar lavage fluid (BALF) of IgG4-related respiratory disease (IgG4-RRD) with BHL on chest CT more closely resembled the Th2 response in patients with eosinophilia than it did in patients with sarcoidosis [5].

In a study investigating the chemokine ligands and chemokine

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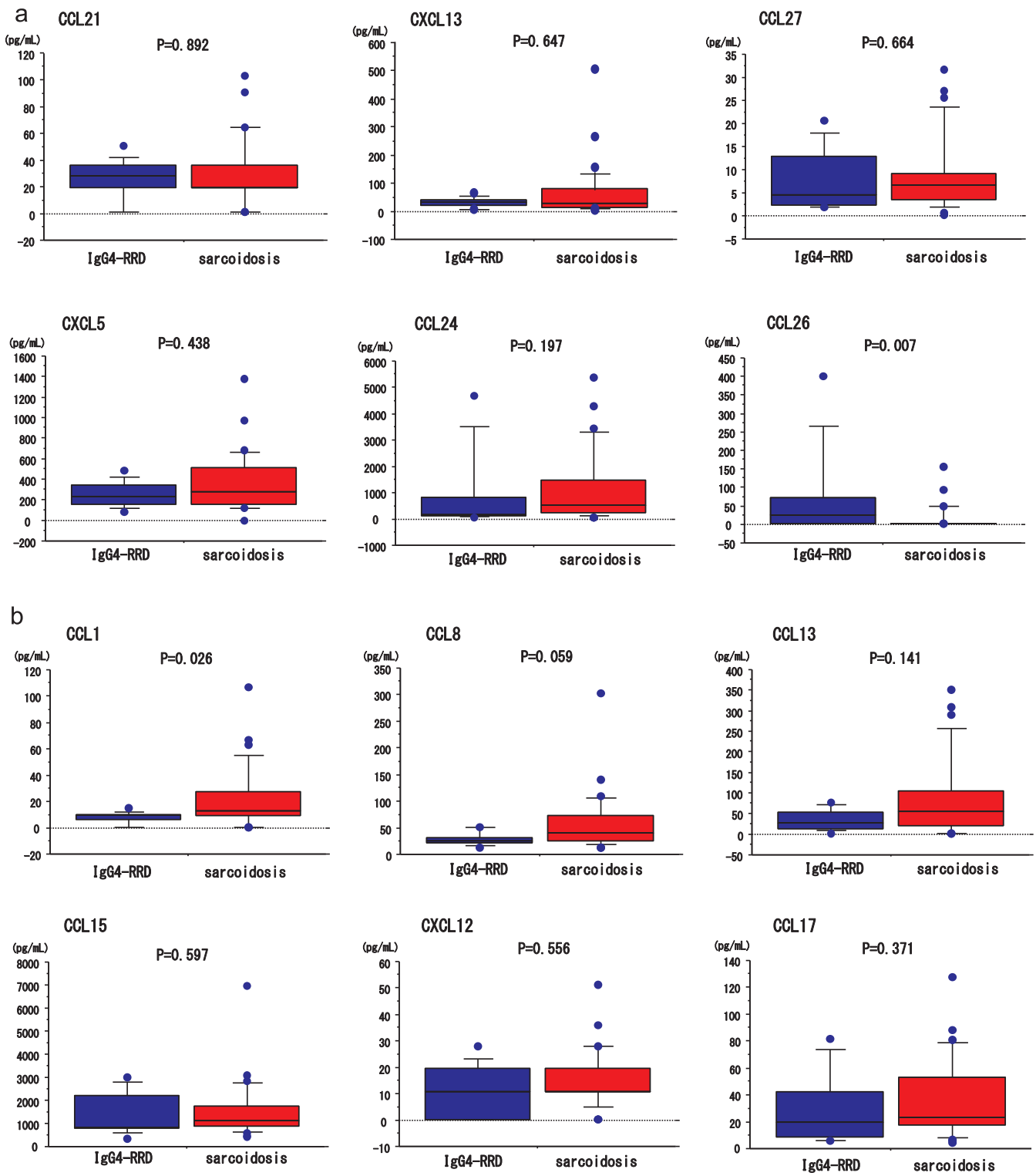

Fig. 1. a, b. The boxplot shows the bronchoalveolar lavage fluid (BALF) levels of 12 chemokines (CC-chemokine ligand [CCL]21, CXC chemokine ligand [CXCL] 13, CCL27, CXCL5, CCL24, CCL26, CCL1, CCL8, CCL13, CCL15, CXCL12, and CCL17) in samples from patients with IgG4-related respiratory disease (IgG4-RRD) (n = 11) and sarcoidosis (n = 33). Significant differences between the two groups were observed in the BALF levels of two of these chemokines. The level of CCL26 in the BALF of IgG4-RRD patients (median 24.5, range 3.1–401.1 pg/mL) was significantly higher than in sarcoidosis patients (median 3.1, range 3.1–155.6 pg/mL, $p < 0.05$). The level of CCL1 in the BALF of sarcoidosis patients (median 13.1, range 0.1–106.9 pg/mL) was significantly higher than in IgG4-RRD patients (median 9.8, range 0.1–14.7 pg/mL, $p < 0.05$). The BALF levels of several cytokines were analysed using the Mann-Whitney U Test.

receptors in patients with IgG4-related sclerosing cholangitis/pancreatitis, Zen et al. reported that CC-chemokine ligand (CCL)1-CC-chemokine receptor (CCR)8 interaction may play a critical role in recruiting inflammatory cells, particularly Th2 lymphocytes and regulatory T cells (Tregs) [6]. However, few reports have explored the chemokine responses in IgG4-RRD and sarcoidosis. The present study investigated the chemokines in the BALF of patients with the two diseases and analysed the differences in the chemokine profiles of patients with the

two diseases in an attempt to shed light on the pathogeneses with regard to the cytokines [5].

2. Material and methods

The Ethics Committee of Shinshu University School of Medicine approved this study (Approval Number: 3458). We re-evaluated the same 44 untreated patients (IgG4-RRD, n = 11; sarcoidosis, n = 33) who visited our hospital (Shinshu University Hospital) from September 2007 to March 2014 [5]. Written informed consent was obtained from all patients. Eleven consecutive patients with IgG4-RRD showed BHL and bronchial wall thickening on chest CT and underwent a transbronchial lung biopsy and bronchial biopsy per our routine protocol [4,5]. Eleven patients were diagnosed with IgG4-RRD based on the diagnostic criteria following the observation of IgG4-positive cell infiltration in their biopsy specimens [7]. We also assessed 33 consecutive patients with sarcoidosis who were diagnosed based on biopsy-proven evidence of sarcoidosis (stage I–II) [3] and who visited our hospital during the same period.

The levels of 12 chemokines (CCL21, CXC chemokine ligand [CXCL] 13, CCL27, CXCL5, CCL24, CCL26, CCL1, CCL8, CCL13, CCL15, CXCL12, and CCL17) in the BALF were assessed using a MILLIPLEX® MAP Kit (Millipore, Darmstadt, Germany) and Luminex® magnetic beads (Luminex, Austin, TX, USA), as described previously [5]. The chi-squared test was used to compare the sex ratio and smoking status between the two groups, and the Mann-Whitney *U* test was used to compare the laboratory data and the levels of the abovementioned chemokines in the BALF between the two groups. In the sarcoidosis patients, the correlation between the CCL1 levels in BALF and the total cell count; lymphocyte and eosinophil fractions; lymphocyte and eosinophil counts; and the BALF levels of several cytokines (evaluated in our previous study) [5] were analysed using Spearman's correlation coefficient (SPSS Statistics version 22; IBM, Armonk, NY, USA).

3. Results

The 11 patients with IgG4-RRD (male, n = 9; female, n = 2; median age, 62 years [range: 50–78]) had a higher percentage of male patients ($p < 0.01$) and were older ($p < 0.05$) in comparison to the 33 patients with sarcoidosis (male, n = 9; female, n = 24; median age, 53 years [range: 21–77]). No significant differences in the number of smokers or smoking status were noted.

General blood tests were performed in 11 patients with IgG4-RRD and 31 patients with sarcoidosis. The serum IgG (IgG4-RRD, n = 11; sarcoidosis, n = 28), IgG4 (IgG4-RRD, n = 11; sarcoidosis, n = 15), angiotensin-converting enzyme (ACE) (IgG4-RRD, n = 11; sarcoidosis, n = 29), and soluble interleukin-2 receptor (sIL-2R) (IgG4-RRD, n = 11; sarcoidosis, n = 22) levels were evaluated.

The median white blood cell count, peripheral eosinophil fraction, and peripheral eosinophil count of IgG4-RRD patients (6440 [range: 3680–9010]/μL, 5.0% [range: 1.8–25.0%], and 392 [range: 147–1483]/μL, respectively) were significantly higher than those of sarcoidosis patients (5040 [range: 2990–10,160]/μL, $p < 0.05$; 2.9% [range: 0.5–8.2%], $p < 0.001$; and 149 [range: 21–477]/μL, $p < 0.001$, respectively). The median serum total protein and IgG of IgG4-RRD patients (8.4 [range: 7.6–10.1] g/dL and 2889 [range: 1854–5545] mg/dL, respectively) were significantly higher than those of sarcoidosis patients (7.6 [range: 6.6–8.5] g/dL, $p < 0.001$; and 1437 [range: 812–1800] mg/dL, $p < 0.001$, respectively). Conversely, the median serum albumin of sarcoidosis patients (4.3 [range: 3.0–4.8] g/dL) was significantly higher than that of IgG4-RRD patients (3.6 [range: 3.0–4.5] g/dL, $p < 0.005$). The median values of serum blood urea nitrogen (BUN), creatinine, lactate dehydrogenase (LDH), and C-reactive protein (CRP) in both groups were within normal limits, and no significant differences were noted between the two groups. The median serum IgG4 level in IgG4-RRD patients (1610 [range: 323–2970] mg/dL) was significantly higher than that in sarcoidosis patients (21 [range: 3–92] mg/dL, $p < 0.001$). Conversely, the median serum ACE level in sarcoidosis patients (25.6 [range: 13.3–45.3] U/L) was significantly higher than that in IgG4-RRD patients (14.6 [range: 5.3–20.2] U/L, $p < 0.001$). However, the median serum sIL-2R level of IgG4-RRD patients (1225 [range: 439–4695] U/mL) was not significantly higher than that in sarcoidosis patients (902 [range: 407–2423] U/mL, $p = 0.089$).

Only two chemokines in the BALF were significantly different between the two groups (Fig. 1a, b). The level of CCL26 in the BALF of IgG4-RRD patients (median 24.5, range 3.1–401.1 pg/mL) was significantly higher than that in the BALF of sarcoidosis patients (median 3.1, range 3.1–155.6 pg/mL, $p < 0.05$) (Fig. 1a). Conversely, the level of CCL1 in the BALF of sarcoidosis patients (median 13.1, range 0.1–106.9 pg/mL) was significantly higher than that in the fluid of IgG4-RRD patients (median 9.8, range 0.1–14.7 pg/mL, $p < 0.05$) (Fig. 1b).

As we previously reported, the BALF concentrations of TNF-α, IL-2 and IL-6 in sarcoidosis patients (mean ± SE 2.47 ± 0.39, 0.16 ± 0.01 and 2.69 ± 0.59 pg/mL) were significantly higher than those of IgG4-RRD patients (0.66 ± 0.07 [$p < 0.01$], 0.08 ± 0.01 [$p < 0.05$] and 0.89 ± 0.17 pg/mL [$p < 0.05$], respectively) (5). In contrast, the concentrations of IL-5 and IL-13 in IgG4-RRD patients (0.18 ± 0.02 and 0.24 ± 0.06 pg/mL) were significantly higher than those in sarcoidosis patients (0.17 ± 0.04 and 0.09 ± 0.02 pg/mL, both $p < 0.05$), and IL-4 showed the same tendency. There were no significant differences in the concentrations of IFN-γ or other cytokines between the two groups (5).

The correlation analyses showed that the CCL1 level in the BALF was significantly positively correlated with the total cell count ($\rho = 0.539$, $p < 0.001$), lymphocyte fraction ($R = 0.406$, $P < 0.05$), lymphocyte count ($R = 0.686$, $P < 0.001$), TNF-α, ($R = 0.748$, $P < 0.001$), and IL-2 ($R = 0.757$, $P < 0.001$) in the BALF (Fig. 2). However, there was no statistically significant correlation between the CCL1 level in the BALF and the eosinophil fraction ($R = -0.04$, $P = 0.81$), eosinophil count ($R = -0.03$, $P = 0.84$), IFN-γ ($R = 0.30$, $P = 0.08$) (Fig. 1b), IL-4 ($\rho = -0.12$, $p = 0.49$), IL-5 ($R = 0.05$, $P = 0.74$), IL-6 ($R = 0.23$, $P = 0.18$), IL-8 ($R = 0.14$, $P = 0.43$), or IL-13 ($R = -0.06$, $P = 0.72$).

4. Discussion

Few reports have described the cytokine and chemokine profiles in the BALF of patients with IgG4-RRD. To our knowledge, this is the first report showing that the CCL26 values in the BALF of IgG4-RRD patients were significantly higher than those in sarcoidosis patients. The CCL26 chemokine was reported to be highly expressed by bronchial epithelial cells on treatment with IL-13 in Th2-dominant eosinophilic asthma [8]. We previously reported that the eosinophil fractions and the IL-13 values in BALF from IgG4-RRD patients were significantly higher than those in BALF from sarcoidosis patients [5]. These results might explain why the BALF levels of CCL26 in patients with Th2-weighted IgG4-RRD were significantly higher than those in sarcoidosis patients. That the levels of CCL1 in the BALF in sarcoidosis patients were significantly higher than those in IgG4-RRD patients was a noteworthy finding.

Several studies have suggested the potential involvement of CCL1-CCR 8 interaction in allergic diseases [9–11]. The serum CCL1 levels in atopic dermatitis patients were reported to be significantly higher than those in healthy subjects [9]. The BALF CCL1 concentrations in asthmatic patients were significantly increased in comparison to normal subjects [10,11]. The expression of CCL1 and CCR8 was confirmed in the bronchial epithelium and infiltrating lymphocytes, respectively [11]. Moreover, IL-4 and IL-13 stimulated primary bronchial airway epithelial cells to release CCL1, which may play a role in lymphocyte recruitment in bronchial asthma [11]. As the IgG4-RRD is a Th2-dominant condition [5], we expected that the CCL1 concentrations in

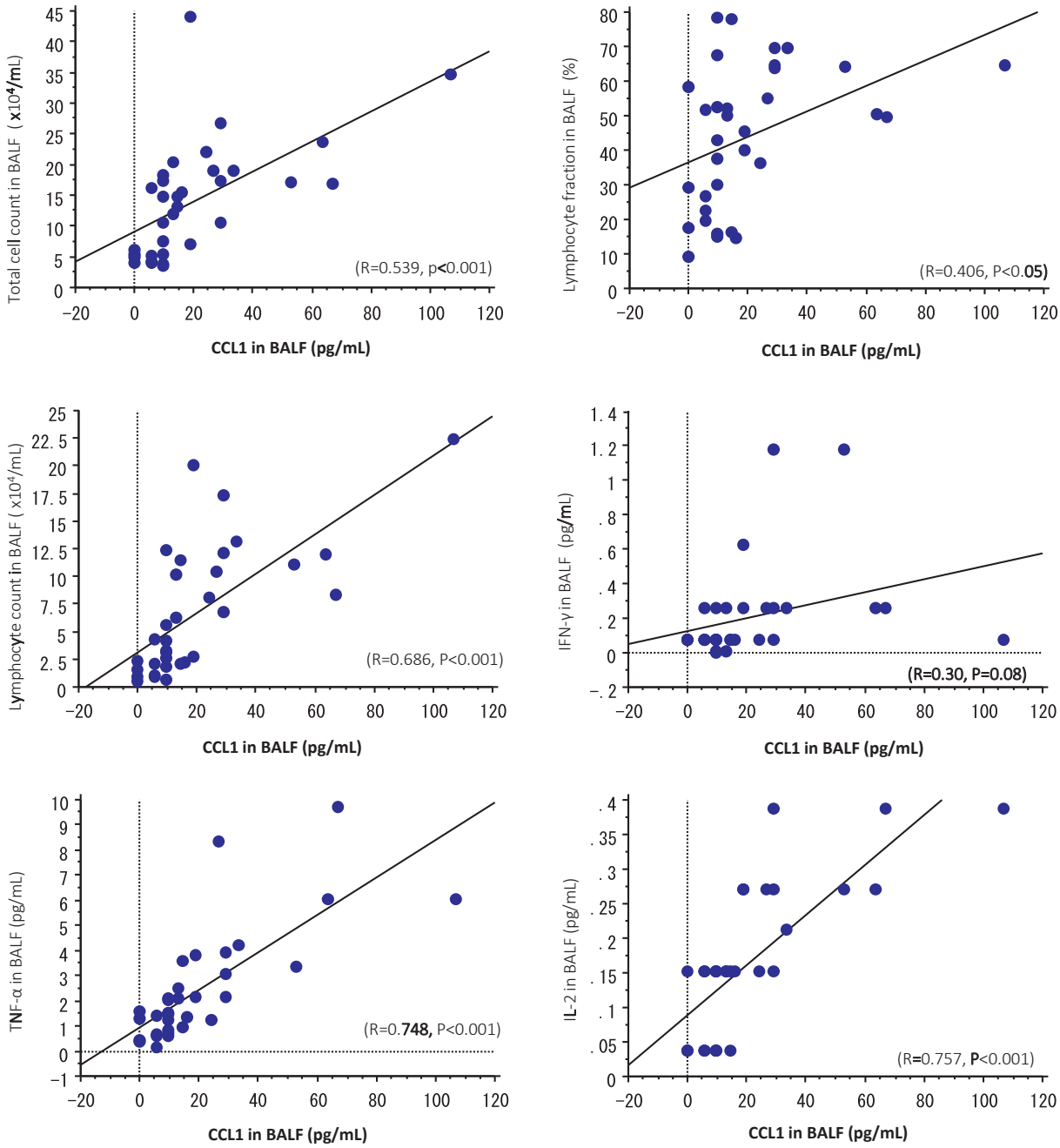

Fig. 2. In sarcoidosis patients (n = 33), the CCL1 levels in the BALF were positively correlated with the total cell counts (R = 0.539, p < 0.001), lymphocyte fraction (R = 0.406, P < 0.05), lymphocyte counts (R = 0.686, P < 0.001), TNF-α level, (R = 0.748, P < 0.001), and IL-2 level (R = 0.757, P < 0.001). There was no statistically significant correlation between the CCL1 levels in the BALF and IFN-γ (R = 0.30, P = 0.08). The correlation between the CCL1 levels in the BALF and the total cell counts; lymphocyte fractions; lymphocyte and eosinophil counts; and several cytokine levels in BALF were analysed using Spearman's correlation coefficient.

the BALF of IgG4-RRD patients would likely be higher in comparison to sarcoidosis patients. However, the present results showed that the CCL1 concentrations in the BALF of sarcoidosis patients were significantly higher than those in IgG4-RRD patients. Furthermore, the BALF CCL1 levels were not positively correlated with the BALF levels of IL-4 and IL-13 in sarcoidosis patients. Thus, we thought that the current results could not be explained by the Th2 response alone.

CCL1 is known to be produced by peripheral blood mononuclear cells, monocytes, activated T lymphocytes, and endothelial cells [11]. Moreover, CCL1 is produced by both Th1 and Th2 cells in response to T cell receptor triggering [12]. Generally, the Th1-polarized immune response plays an important role in the onset and development of granulomas [13]. A previous study demonstrated that granuloma formation is divided into 4 stages: initial, accumulation, effector phase, and resolution/fibrosis progression. In the last stage, the immune response is shifted toward a Th2 response [14]. The present results showed that the CCL1 levels in the BALF were significantly correlated with the total cell count and lymphocyte count in sarcoidosis, which might indicate the pathological activity of sarcoidosis. Furthermore, in sarcoidosis patients, the BALF CCL1 levels were significantly correlated with the BALF levels of Th1 cytokines, such as TNF-α and IL-2, rather than Th2 cytokines. Although the detailed mechanisms are still unknown, CCL1 might play an important role in sarcoidosis that is more closely related to Th1 than to Th2.

In a chronic lung inflammation model using the intratracheal administration of Bacille de Calmette et Guérin (BCG), a significant increase in granuloma formation was seen in CCL1-transgenic mice in comparison to control mice [15]. We found no reports describing the relationship between CCL1 and sarcoidosis; however, CCL1 may be involved in the granuloma formation of sarcoidosis.

This study is associated with several limitations. First, we compared IgG4-RRD patients to sarcoidosis patients and did not evaluate healthy control subjects. Second, we only assessed the BALF without evaluating the peripheral blood in this study. Third, this was a small-scale, retrospective, single-institution study. Thus, we cannot further analyse any of the relationships in which CCL26 is involved. According to these limitations, we cannot draw any firm conclusions. However, despite these limitations, to our knowledge, this is the first report describing the association between CCL1 in the BALF and sarcoidosis. Furthermore, CCL1 might have potential application as a biomarker in sarcoidosis.

5. Conclusions

The CCL1 values in the BALF of sarcoidosis patients were significantly higher than those in IgG4-RRD patients. CCL1 might reflect disease activity and may be involved in the pathogenesis of sarcoidosis in a manner that is more closely related to Th1 than to Th2.

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Conflict of interest

The authors have no conflict of interest to declare.

CRediT authorship contribution statement

Hiroshi Yamamoto: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Masanori Yasuo:** Conceptualization, Investigation, Resources, Writing - review & editing, Supervision. **Masamichi Komatsu:** Writing - original draft, Visualization. **Atsuhito Ushiki:** Writing - review & editing. **Hideaki Hamano:** Supervision. **Atsushi Hori:** Investigation, Resources. **Tomoyuki Nakajima:** Investigation, Resources. **Takeshi Uehara:** Supervision. **Yasunari Fujinaga:** Supervision. **Shoko Matsui:** Supervision, Funding acquisition. **Masayuki Hanaoka:** Supervision, Project administration.

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