Cytokine

The utility of serum C-C chemokine ligand 1 in sarcoidosis: A comparison to IgG4-related disease

Masamichi Komatsu, MD[1]; Hiroshi Yamamoto, MD, PhD[1]; Masanori Yasuo, MD, PhD[1]; Atsuhito Ushiki, MD, PhD[1]; Tomoyuki Nakajima PhD[2]; Takeshi Uehara, MD, PhD[2]; Satoshi Kawakami, MD[3]; and Masayuki Hanaoka, MD, PhD[1]

[1]First Department of Internal Medicine, [2]Department of Laboratory Medicine; [3]Department of Radiology; Shinshu University School of Medicine, Matsumoto, Japan

*Corresponding Author: Hiroshi Yamamoto, MD, PhD, First Department of Internal Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, 390-8621, Japan; e-mail yama5252@shinshu-u.ac.jp

E-mail Addresses:

MK: mskomatsu@shinshu-u.ac.jp

HY: yama5252@shinshu-u.ac.jp

MY: yasumasa@shinshu-u.ac.jp

AU: atsuhito@shinshu-u.ac.jp

TN: tnakajima@shinshu-u.ac.jp

TU: tuehara@shinshu-u.ac.jp

SK: kawasato@shinshu-u.ac.jp

MH: masayuki@shinshu-u.ac.jp

Abstract word count: 299 words.

Main text word count: 3353 words.

Abstract

We previously reported higher levels of C-C chemokine ligand (CCL) 1 in the bronchoalveolar lavage (BAL[1]) fluid (BALF) of patients with sarcoidosis than in BALF of patients with immunoglobulin G4 (IgG4)-related disease (IgG4-RD), indicating that CCL1 might act as a marker of disease activity in sarcoidosis. Notably, less invasive sampling sources are desirable, as BAL cannot always be performed due to its inherent risk. In this study, we sought to decipher the correlation between serum levels of CCL1 and clinical characteristics of sarcoidosis. Serum samples were obtained from 44 patients with clinically confirmed sarcoidosis, 14 patients with IgG4-RD, and 14 healthy controls. The clinical and radiological findings were retrospectively evaluated. Serum levels of CCL1 were measured using a sandwich enzyme-linked immunosorbent assay. Serum levels of other 17 cytokines and chemokines were measured using a MILLIPLEX® MAP KIT and Luminex® magnetic beads. Serum levels of CCL1 were significantly higher in patients with sarcoidosis than in patients with IgG4-RD and healthy controls. Serum CCL1 was positively correlated with the degree of hilar lymph node swelling on chest computed tomography and serum levels of soluble interleukin 2 receptor. Positive

[1] Abbreviations
ACE, Angiotensin-converting enzyme; BALF, Bronchoalveolar Lavage Fluid; CT, Computed tomography; FVC, Forced vital capacity; IP, Inducible protein; TARC, Thymus-and activation-regulated chemokine; TNF, Tumor necrosis factor

correlations were also observed between serum CCL1 and total cell counts, lymphocyte counts in BALF, and serum T helper 1 mediators such as IP-10 and TNF-α in patients with sarcoidosis. Serum CCL1 levels were significantly elevated in sarcoidosis and correlated with clinical parameters of the disease. In addition, serum and BALF levels of CCL1 were positively correlated in a statistically significant manner. Although further research in this field is necessary, CCL1 might have the potential to be a reliable serological marker of disease activity in sarcoidosis.

Key words:

chemokine, cytokine; IgG4-related disease; sarcoidosis; C-C chemokine ligand 1

1. Introduction

Sarcoidosis is a systemic disease of unknown etiology characterized by granulomas in various organs, including the lungs and lymphatic system [1, 2]. Granulomatous inflammation in sarcoidosis is considered a T helper (Th) 1-immune response mediated by lymphocytes, macrophages, and Th1 cytokines [3, 4].

Immunoglobulin G (IgG)4-related disease (IgG4-RD) is a chronic fibrotic inflammatory disease with multi-organ involvement characterized by infiltration of IgG4-positive plasma cells and elevated serum levels of IgG4 [5]. Both sarcoidosis and IgG4-RD develop through pulmonary lymphatics and frequently present with hilar lymphadenopathy on chest computed tomography (CT) [6].

Although sarcoidosis and IgG4-RD often have similar imaging features, the pathology of IgG4-RD is attributed to the activation of Th2 immune response at the affected sites [5]. We previously reported the cytokine and chemokine profiles of bronchoalveolar lavage (BAL) fluid (BALF) in patients with sarcoidosis and IgG4-RD, and we demonstrated that the lung lesion of IgG4-RD was more characteristic of the Th2 response than that of sarcoidosis [7, 8].

C-C chemokine ligand (CCL) 1 belongs to the C-C chemokine family. It is an inflammatory mediator and Th2 cell attractant and stimulates the migration of human

monocytes [9-11]. Several studies have suggested the involvement of CCL1 in allergic diseases such as bronchial asthma and atopic dermatitis [12, 13]. Recently, we found that CCL1 levels were significantly higher in the BALF of sarcoidosis patients than in that of patients with IgG4-RD [8]. As CCL1 levels have been significantly correlated with the total cell counts and lymphocyte counts in the BALF of patients with sarcoidosis, we presumed that CCL1 could reflect disease activity in sarcoidosis. Although the utility of BALF in sarcoidosis is recognized [14], BAL is not always performed, as it is a relatively invasive bronchoscopic procedure. In addition, hypoxemia is reportedly associated with BALF [15]. Therefore, less-invasive sampling strategies are desirable for diagnostic purpose.

In this study, we evaluated the serum levels of CCL1 in three study groups (sarcoidosis, IgG4-RD, and healthy controls) and assessed the correlations between serum CCL1 levels, sarcoidosis characteristics, and the serum profile of several cytokines and chemokines to reveal the clinical utility of serum CCL1 in sarcoidosis.

2. Methods

2.1. Patients and diagnostic criteria

This study recruited 44 consecutive patients with clinically confirmed sarcoidosis who

visited Shinshu University Hospital between April 2010 and September 2018. The diagnosis of sarcoidosis was based on the criteria laid by the 'World Association of Sarcoidosis and Other Granulomatous Disorders' consensus statement [1, 2]. All patients had histologically proven non-caseating granulomas in at least one organ and presented a chronic phenotype.

Fourteen patients with IgG4-RD and an equal number of healthy controls with no history of allergic disease were also included in the study. The patients with IgG4-RD were diagnosed based on the comprehensive diagnosis criteria [16] with pulmonary involvement. None of the patients were treated with corticosteroids or other immunosuppressive drugs during sample collection. Informed consent was obtained from all the participants, and the study design was approved by the Ethics Committee of Shinshu University School of Medicine (Matsumoto, Japan) (Approval Number: 4002).

2.2. Study design

2.2.1. Data collection

The clinical, radiological, and pathological data were retrieved from the medical records and analyzed retrospectively. To assess the pulmonary parenchymal, hilar, and mediastinal lymph node enlargement, we used the high-resolution CT (HRCT), and HRCT score as previously reported [17]. Four parameters were evaluated

(bronchovascular bundle, intra-parenchymal nodules, septal and non-septal lines, and parenchymal consolidation) on a four-grade scale to analyze the parenchymal involvement in the afflicted area. Lymph node swelling and pleural thickening were also assessed on a four-grade scale. The HRCT score was obtained by adding up the six individual scores. The HRCT score was assessed by a trained radiologist (S.K.) without clinical information.

2.3. CCL1, cytokines, and chemokines

Serum levels of CCL1 were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (R&D Systems Inc., Minneapolis, MN, USA). Further, serum levels of 17 cytokines and chemokines (interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, eotaxin, interferon (IFN)-γ, interferon-inducible protein (IP)-10, monocyte-chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, and tumor necrosis factor (TNF)-α) were analyzed using MILLIPLEX® MAP KIT (Millipore, Darmstadt, Germany) and Luminex® magnetic beads (Luminex, Austin, TX, USA), as described previously [18].

In sarcoidosis patients, CCL17 and CCL18 serum levels were measured using a sandwich ELISA kit (R&D Systems Inc., Minneapolis, MN, USA). In addition, the levels

of CCL1, CCL17, and CCL18 in the BALF of sarcoidosis patients were measured using a sandwich ELISA kit.

2.4. Statistical analysis

Data distribution of the variables in three or more groups was first assessed by using the Kolmogorov-Smirnov test; those showing a normal distribution were analyzed using one-way analysis of variance with Tukey-Kramer's *posthoc* test, while those with non-normal distribution were transformed with using the Johnson system [19]. The transformed data with a normal distribution were analyzed using a one-way analysis of variance with Tukey-Kramer's *posthoc* test, while those with a non-normal distribution were analyzed using the Kruskal-Wallis test followed by Dunn's *posthoc* test. Data were statistically analyzed using the Mann-Whitney U-test for comparing two groups. Correlations between the groups were examined with Spearman correlation coefficient. Statistical analyses were performed using StatFlex® Version 7.0 (Artech, Osaka, Japan), and the Johnson system was performed using JMP® Version 14.1 (SAS, Cary, NC, USA). The statistical significance was established at p values of < 0.05.

3. Results

3.1. Patient characteristics

The characteristics of the patients with sarcoidosis and IgG4-RD and healthy controls are summarized in Table 1. The patients with IgG4-RD (females, n =5, males, n =9; median age, 68.5 years [range: 54-87]) were older than those with sarcoidosis (females, n =17, males, n =27; median age, 49 years [range: 23-75 years]) ($p < 0.001$) and healthy controls (females, n =0, males, n =14; median age, 34.5 years [range: 25-49 years]) ($p < 0.001$). The sarcoidosis group had a higher percentage of female patients ($p < 0.001$) than did healthy control groups.

The patients with sarcoidosis were divided into five groups based on the chest radiographic findings (Stage 0; n=5, Stage I; n=19, Stage II; n=19, Stage III; n=1, Stage IV; n=0). The median HRCT score of sarcoidosis patients was 4 (range: 1-13). A total of 27 out of 44 sarcoidosis patients had extrapulmonary lesions, while 12 out of 14 IgG4-RD patients showed extrapulmonary involvement.

Table 2 showed the laboratory findings and pulmonary function tests of the patients with sarcoidosis and IgG4-RD. Our results showed that the serum levels of calcium, albumin, and angiotensin-converting enzyme (ACE) were significantly higher in patients with sarcoidosis than in those with IgG4-RD. On the other hand, the serum levels of IgG and IgG4 were significantly higher in patients with IgG4-RD than in those with sarcoidosis. In addition, the peripheral blood samples from the patients with IgG4-RD showed

significantly higher counts of lymphocytes and eosinophils than those from the sarcoidosis patients. For the pulmonary function test, the forced expiratory volume in one second (FEV_1) / forced vital capacity (FVC) ratio was significantly lower in patients with IgG4-RD than in those with sarcoidosis. Bronchoalveolar lavage was performed on 19 out of 44 sarcoidosis patients, and 3 out of 14 IgG4-RD patients, and the total number of cells and CD4/CD8 ratio were analyzed in BALF, but we observed no significant difference between the two groups.

Table 1. Clinical characteristics of the study population.

	Sarcoidosis	IgG4-RD	Healthy controls	*p* value [a]
Number	44	14	14	
Sex, female/male	17 / 27 [c]	5 / 9	0 / 14	**< 0.001**
Age (yr), median (range)	49.0 (23, 75)	68.5 (54, 87) [b, d]	34.5 (25, 49)	**< 0.001**
smoking status				
smoker/ex-smoker/non-smoker	10 / 13 / 21	0 / 9 / 5	0 / 6 / 8	NS
Radiographic stage, no.		ND	ND	
0	5			
I	19			
II	19			
III, IV	1			
HRCT socre, median (range)	4 (1, 13)	ND	ND	
Extrapulmonary disease, yes/no	27 / 17	12 / 2		NS

Data are presented as median (range), or number.

[a] Data were analyzed using the Kruskal-Wallis test.

[b, c, d] Data were analyzed using the Dunn's test. [b] $p < 0.05$; sarcoidosis vs IgG4-RD. [c] $p < 0.05$; sarcoidosis vs healthy controls. [d] $p < 0.05$; IgG4-RD vs healthy controls.

Abbreviations. HRCT: high-resolution computed tomography; IgG4-RD: immunoglobulin G4-related disease; ND: not done; NS: not significant

Table 2. Laboratory findings and pulmonary function test.

	Sarcoidosis	n	IgG4-RD	n	*p* value
Laboratory findings					
ACE (U/mL)	20.3 (2.5, 41.2)	14	16.8 (9.3, 19.6)	9	**0.029**
sIL-2R (U/mL)	448 (302, 4516)	42	550.5 (224, 1710)	14	0.268
serum Calcium (mg/dL)	9.4 (8.3, 10.1)	41	8.8 (8.1, 9.9)	14	**0.001**
Total protein (g/dL)	7.3 (6.2, 8.7)	44	7.4 (6.7, 10.0)	14	0.212
albumin (g/dL)	4.30 (3.3, 5.0)	44	4.0 (2.6, 4.3)	14	**0.005**
urea nitrogen (mg/dL)	14.35 (1.7, 29.3)	44	12.5 (1.5, 24.8)	14	0.059
creatinine (mg/dL)	0.70 (0.44, 2.17)	44	0.78 (0.54, 1.05)	14	0.256
LDH (U/L)	181 (124, 225)	44	174 (141, 255)	13	0.506
CRP (mg/dL)	0.07 (0.00, 3.73)	43	0.10 (0.00, 0.77)	14	0.334
IgG (mg/dL)	1383 (938, 2226)	35	1680 (1096, 6186)	14	0.005
IgG4 (mg/dL)	45 (14, 182)	11	241 (6, 2330)	14	**< 0.001**
KL-6 (U/mL)	311 (149, 1921)	16	273 (239, 628)	4	0.741
White blood cell (cell/μL)	4815 (2690, 9200)	44	5100 (2850, 7410)	14	0.203
Lymphocyte count (cell/μL)	1061 (478, 2566)	44	1774 (976, 2119)	14	**0.008**
Eosinophil count (cell/μL)	121 (0, 1542)	44	226 (31, 634)	14	**0.008**
Pulmonary functional tests					
VC (L)	2.75 (1.50, 4.50)	18	3.03 (2.01, 3.84)	9	0.877
predicted VC (%)	89.1 (55.1, 115.6)	18	91.8 (77.0, 117.8)	9	0.425
FVC (L)	3.00 (1.45, 4.97)	38	3.04 (1.97, 5.04)	12	0.838
predicted FVC (%)	94.5 (55.3, 123.5)	38	96.0 (74.9, 127.6)	12	0.727
FEV_1 (L)	2.40 (1.25, 4.38)	38	1.99 (1.43, 3.55)	12	0.061
FEV_1/FVC ratio (%)	83.1(45.1, 98.0)	38	70.9 (37.8, 79.2)	12	**< 0.001**
predicted DLco (%)	77.6 (28.6, 102.5)	14	91.1 (62.7, 108.0)	9	0.244
BALF findings					
Total cell count (10^5/mL)	1.96 (0.84, 5.08)	19	1.71 (0.60, 1.90)	3	0.196
Macrophage (%)	62.6 (24.3, 95.6)	19	71.3 (54.8, 87.5)	3	0.738
Lymphocyte (%)	26.2 (3.9, 74.0)	19	27.8 (8.6, 36.7)	3	0.599
Eosinophil (%)	0.5 (0.0, 5.0)	19	2.7 (0.7, 6.7)	3	0.068
CD4/8 ratio (%)	3.69 (1.46, 7.49)	19	1.79 (1.50, 2.60)	3	0.104

Data are presented as median (range).

Abbreviations. ACE: angiotensin-converting enzyme; BALF: bronchoalveolar lavage

fluid; CRP: C-reactive protein; DLco: diffusing capacity of the lung for carbon monoxide; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; Ig: immunoglobulin; IgG4: immunoglobulin G4; IgG4-RD: immunoglobulin G4-related disease; KL-6; Krebs von Lungen-6; LDH: lactate dehydrogenase; sIL-2R: soluble interleukin 2 receptor; VC: vital capacity.

3.2. Serum levels of CCL1

The serum levels of CCL1 were measured for all the study participants and were significantly higher in patients with sarcoidosis (median: 55.5, range: 0.0-721.5 pg/mL) than in healthy controls (median: 0.0, range: 0.0-6.0 pg/mL, $p < 0.001$) and in patients with IgG4-RD (median: 7.1, range: 0.0-325.1 pg/mL, $p < 0.001$) (Figure 1).

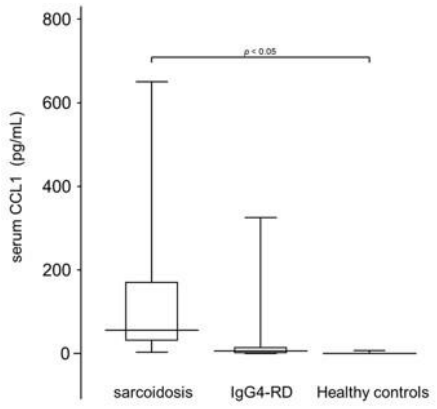

Figure 1. Serum levels of CCL1 of patients with sarcoidosis, IgG4-RD, and healthy controls. Horizontal lines, boxes, and error bars represent the median, the 25th, and 75th

percentiles, and 10th and 90th percentiles, respectively.

Abbreviations. CCL: C-C chemokine ligand; IgG4-RD: immunoglobulin G4-related disease

3.3. Serum CCL1 and Radiographic findings in sarcoidosis patients

We assessed the relationship between serum CCL1 levels and radiographic findings in patients with sarcoidosis. Data for serum CCL1 levels were transformed using the Johnson system and followed a normal distribution. The patients were divided into five stages based on the chest radiographic findings, as explained under patient characteristics. The serum levels of CCL1 in patients with stage II sarcoidosis were higher than those in patients with stage 0 and stage I (Figure 2A).

We assessed the relationship between serum levels of CCL1 and the size of hilar and mediastinal lymph nodes (Figure 2B). In patients with sarcoidosis, the size of hilar and mediastinal lymph nodes was graded as per the classification by Drent et al.[17] on a four-grade scale (score 0- none; n =2, 1- minor; n= 20, 2- moderate; n= 12, 3- pronounced; n=10). We found that the serum levels of CCL1 were significantly higher in patients with pronounced lymph nodes (score 3) than in those with none (score 0) and minor lymph nodes (score 1) ($p < 0.05$). Moreover, the serum levels of CCL1 in patients

with moderate lymph nodes (score 2) were significantly higher than those in patients with a score of 0 ($p < 0.05$). The serum levels of CCL1 in patients with minor lymph nodes (score 1) were significantly higher than those in patients with a score of 0. We found no correlation between serum CCL1 levels and parenchymal involvement or pleural thickening.

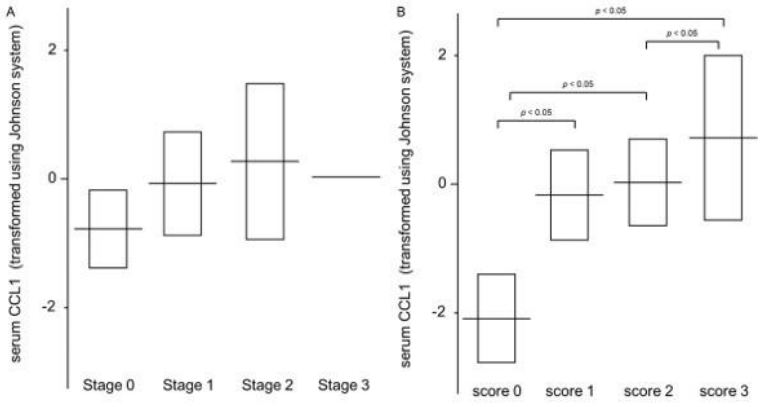

Figure 2. Serum levels of CCL1 of patients with sarcoidosis according to radiographic findings. The Y-axis shows the Serum CCL1 transformed using the Johnson system. A) Serum CCL1 by radiographic Stage. B) Serum CCL1 by hilar and mediastinal lymph nodes swelling. Lymph node swelling was graded on a four-grade scale (score 0- none, 1- minor, 2- moderate, 3- pronounced).

Horizontal lines and boxes represent the mean, and the standard deviation, respectively.

Abbreviations. CCL: C-C chemokine ligand

3.4. Serum CCL1 and Laboratory and BALF findings in sarcoidosis patients

We investigated the relationship between serum CCL1 levels and the BALF findings in patients with sarcoidosis. The serum levels of CCL1 correlated positively with the serum levels of soluble interleukin 2 receptor (sIL-2R) (r = 0.661, $p < 0.001$) (Figure 3), but not with serum ACE levels (r = 0.194, $p = 0.205$). Positive relationships were also observed between serum CCL1 levels and the total cell counts (r = 0.573, $p = 0.010$) and lymphocyte counts (r = 0.715, $p < 0.001$) in BALF of sarcoidosis patients (Figure 3).

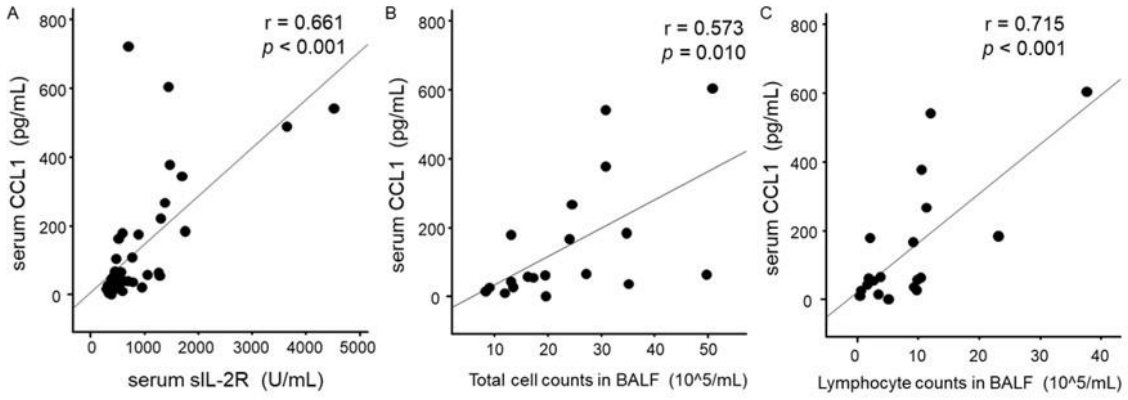

Figure 3. Correlation between serum CCL1 and A) serum levels of sIL-2R (r = 0.661, $p < 0.001$), B) the total cell counts (r = 0.573, $p = 0.010$), and C) lymphocyte counts (r=0.715, $p < 0.001$) in BALF of sarcoidosis patients.

Abbreviations. BALF: bronchoalveolar lavage fluid; CCL: C-C chemokine ligand; sIL-2R: soluble interleukin 2 receptor.

3.5. Serum levels of cytokines and chemokines in patients with sarcoidosis, IgG4-RD, and healthy controls

Serum levels of 17 cytokines and chemokines analyzed using MILLIPLEX® MAP KIT, and Luminex® magnetic beads were shown in Table 3. Eight cytokines and chemokines, namely IL-5, IL-8, IL-13, eotaxin, IFN-γ, IP-10, MCP-1, and TNF-α, differed significantly among three groups using the Kruskal-Wallis test and Dunn's test.

Four cytokines and chemokines, namely IL-5, IFN-γ, IP-10, and MCP-1, differed significantly between the two groups of sarcoidosis and IgG4-RD. The serum levels of IFN-γ and IP-10 were significantly higher in patients with sarcoidosis than in IgG4-RD. On the other hand, the serum levels of IL-5 and MCP-1 were significantly higher in patients with IgG4-RD than in patients with sarcoidosis.

The serum levels of five cytokines and chemokines, namely IL-8, IL-13, eotaxin, IP-10, and TNF-α were significantly higher in patients with sarcoidosis than in those with healthy controls. The serum levels of IL-5 and eotaxin were significantly higher in patients with IgG-RD than in patients with healthy controls.

In short, serum levels of IP-10 were significantly higher in patients with sarcoidosis than in the other two groups. The serum level of IL-5 in patients with IgG4-RD was

significantly higher than in the other two groups.

Table 3. Serum cytokines and chemokines in patients with sarcoidosis, IgG4-RD, and healthy controls.

	Sarcoidosis	n	IgG4-RD	n	Healthy controls	n	*p* value [a]
CCL1	55.46 (0.00, 721.50) [b, c]	44	6.23 (0.00, 325.14)	14	0.00 (0.00, 6.60)	14	**< 0.001**
IL-1β	0.00 (0.00, 80.45)	39	0.26 (0.00, 2.54)	11	0.00 (0.00, 34.95)	14	0.601
IL-2	0.00 (0.00, 12.11)	39	0.00 (0.00, 1.76)	11	0.00 (0.00, 22.49)	14	0.511
IL-4	0.00 (0.00, 38.13)	39	0.00 (0.00, 0.00)	11	0.00 (0.00, 94.37)	14	0.261
IL-5	0.00 (0.00, 1.29)	39	0.76 (0.00, 15.96) [b, d]	11	0.00 (0.0, 2.11)	14	**0.006**
IL-6	0.00 (0.00, 13.50)	39	0.00 (0.00, 4.44)	11	0.00 (0.00, 0.00)	14	0.108
IL-8	8.09 (2.86, 979.63) [c]	39	7.37 (2.67, 37.29)	11	5.48 (2.01, 18.22)	14	**0.044**
IL-10	0.00 (0.00, 14.13)	39	0.00 (0.00, 34.04)	11	0.00 (0.00, 25.78)	14	0.510
IL-12 (p40)	0.00 (0.00, 31.73)	39	0.00 (0.00, 0.00)	11	0.00 (0.00, 326.84)	14	0.557
IL-12 (p70)	0.00 (0.00, 29.89)	39	0.00 (0.00, 1.81)	11	0.00 (0.00, 47.08)	14	0.426
IL-13	4.52 (0.00, 44.10) [c]	39	0.00 (0.00, 17.06)	11	0.00 (0.00, 6.10)	14	**< 0.001**
Eotaxin	94.56 (47.86, 179.20) [c]	39	99.14 (65.76, 139.27) [d]	11	54.89 (22.05, 139.12)	14	**< 0.001**
IFN-γ	5.84 (0.00, 378.69) [b]	39	0.37 (0.00, 5.16)	11	1.57 (0.00, 52.58)	14	**< 0.001**
IP-10	1074.03 (112.34, 3544.59) [b, c]	39	459.95 (312.50, 1303.31)	11	217.90 (94.63, 466.69)	14	**< 0.001**
MCP-1	412.53 (209.86, 2317.33)	39	611.09 (369.57, 780.01) [b]	11	508.73 (311.86, 780.50)	14	**0.016**
MIP-1α	0.00 (0.00, 45.92)	39	0.00 (0.00, 0.00)	11	0.00 (0.00, 15.33)	14	**0.024**
MIP-1β	67.22 (17.37, 166.90)	39	62.80 (44.38, 82.94)	11	61.38 (34.17, 99.07)	14	0.948
TNF-α	23.52 (5.48, 89.53) [c]	39	20.50 (1.96, 28.36)	11	10.96 (7.18, 73.88)	14	**< 0.001**

Data are presented as median (range).

[a] Data were analyzed using the Kruskal-Wallis test.

[b, c] Data were analyzed using Dunn's test. [b] $p < 0.05$; sarcoidosis vs IgG4-RD. [c] $p < 0.05$; IgG4-RD vs Healthy controls.

CCL: C-C chemokine ligand; IFN: interferon; IgG4-RD: immunoglobulin G4-related disease; IL: interleukin; IP: Interferon-inducible protein; MCP: monocyte-chemotactic protein; MIP: macrophage inflammatory protein; TNF: tumor necrosis factor.

3.6. Relationship between serum levels of CCL-1 and the other cytokines and chemokines profile in sarcoidosis patients

The relationship between serum levels of CCL1 and the other cytokines and chemokines profile in patients with sarcoidosis was also investigated. We found that the serum levels of CCL1 in patients with sarcoidosis showed a significant positive correlation with the serum levels of IP-10 (r = 0.545, $p < 0.001$) and TNF-α (r = 0.517, $p < 0.001$) (Figure 4).

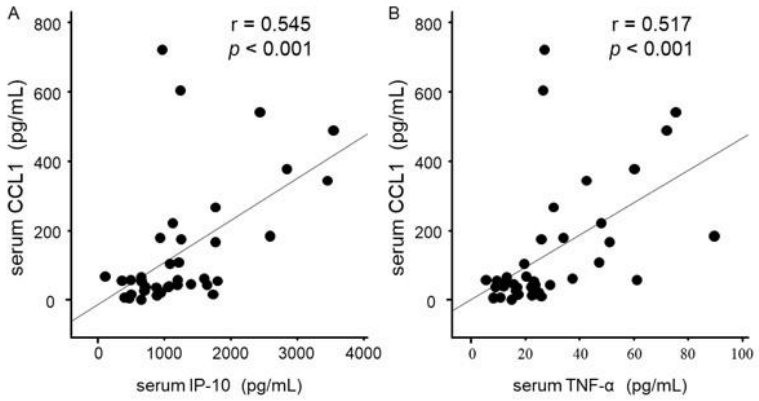

Figure 4. Correlation between serum levels of CCL1 and A) serum levels of IP-10 (r = 0.545, $p < 0.001$), and B) TNF-α of sarcoidosis patients (r = 0.517, $p < 0.001$)).

Abbreviations. CCL: C-C chemokine ligand; IP: interferon-inducible protein; TNF: tumor necrosis factor

3.7. Relationship between serum and BALF levels of chemokines in patients with

sarcoidosis

The median BALF levels of CCL1 in sarcoidosis was 0.62, range 0.00-8.64 pg/mL, n = 19. Serum and BALF CCL1 levels positively correlated (r = 0.491, p = 0.033) (Figure 5).

In addition, the levels and correlation of serum and BALF chemokine levels were as follows. CCL17: serum level, median 736.50, range 59.12-4105.78 pg/mL, n = 36, BALF level, median 0.00, range 0.00-3.41, n =18, with correlation between serum and BALF levels, r = 0.451, p = 0.105; CCL18: serum level, median 364.52, range 147.14-1352.58 pg/mL n = 39, BALF level, median 58.28, range 0.11-376.48, n = 19, with a correlation between serum and BALF levels, r = 0.426, p = 0.088..

No statistically significant linear relationships were observed between serum CCL1, CCL17, and CCL18 levels (Figure 6.).

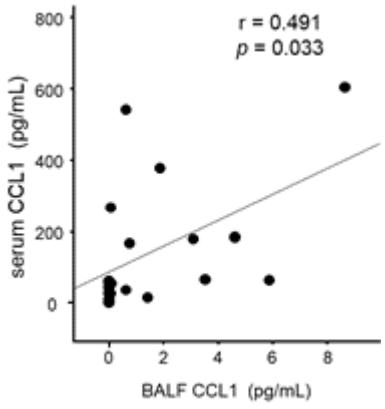

Figure 5. Correlation between serum and BALF levels of CCL1 (r = 0.491, p = 0.033).

Abbreviations. CCL: C-C chemokine ligand; BALF: bronchoalveolar lavage fluid

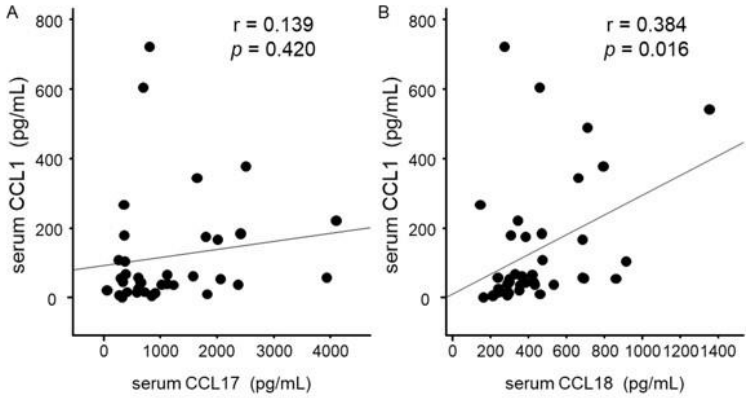

Figure 6. Correlation between serum CCL1 and A) serum CCL17 (r = 0.139, p = 0.420), and B) serum CCL18 (r = 0.384, p = 0.016).

Abbreviations. CCL: C-C chemokine ligand

4. Discussion

Our study demonstrated that patients with sarcoidosis exhibit high serum CCL1 levels than those with IgG4-RD and healthy controls. The serum levels of CCL1 were positively correlated with serum sIL-2R and the degree of hilar lymph nodes swelling on chest CT. Significant positive correlations were also observed between serum levels of CCL1 and total cell counts, and lymphocyte counts in BALF from patients with sarcoidosis. In addition, there was a positive relationship between serum and BALF CCL1 levels.

We have previously reported the cytokine and chemokine profiles in the BALF from

patients with sarcoidosis and IgG4-RD [7, 8]. The levels of Th1 cytokines, such as IL-2, IL-6, and TNF-α in BALF, were significantly higher in patients with sarcoidosis than in those with IgG4-RD. On the other hand, the levels of Th2 cytokines and chemokines, such as IL-5, IL-13, and CCL26, were significantly higher in BALF from IgG4-RD patients.

Interestingly, the higher levels of CCL1 in the BALF from patients with sarcoidosis than in that from those with IgG4-RD was a significant finding. Furthermore, the levels of BALF CCL1 showed a significant positive correlation with the total cell counts, lymphocyte fraction, lymphocyte counts, TNF-α, and IL-2 in the BALF of patients with sarcoidosis. Based on our previous findings, we concluded that CCL1 levels in BALF from patients with sarcoidosis might reflect the close association of disease activity in sarcoidosis with Th1 compared with Th2. However, we did not evaluate parameters other than BALF, nor did we compare the results from patients with sarcoidosis with those from healthy controls [8].

In this study, we demonstrated that serum levels of CCL1 were significantly higher in patients with sarcoidosis than the other two groups. To our knowledge, this is the first report showing the high expression of serum CCL1 in sarcoidosis patients. We found a positive relationship between serum levels of CCL1 and sIL-2R, a recently evaluated

marker for disease activity of sarcoidosis [20, 21]. A positive correlation was also observed between the serum levels of CCL1 and lymph node swelling on HRCT. The levels of serum CCL1 were significantly positively correlated with total cell counts, lymphocyte fraction, and lymphocyte counts in BALF from sarcoidosis patients. These results were consistent with our previous report [8]. Collectively, these results indicate that serum levels of CCL1 might have the potential for predicting disease activity in sarcoidosis.

In addition, serum and BALF CCL1 levels positively correlated. In sarcoidosis, several alternative procedures are recommended for histological diagnosis. Bronchoscopic methods, such as endobronchial biopsy, transbronchial lung biopsy, and transbronchial needle aspiration, are frequently performed. BALF is recommended as an additional procedure for sarcoidosis diagnosis, as BAL is relatively invasive. In addition, BALF is reportedly associated with the risk of hypoxemia; therefore, it could not be performed in all patients. The positive correlation between serum and BALF levels of CCL1 might represent a novel, minimally-invasive sarcoidosis marker.

CCL1 belongs to the C-C chemokine family and is produced by activated monocytes/macrophages, T lymphocytes (Th1, Th2, and cytotoxic T cells), and endothelial cells [9-11]. C-C chemokine receptor (CCR) 8, which is present on

lymphocytes and monocytes, is the receptor for CCL1 and CCL18 [22-24]. Several studies have suggested the possible involvement of CCL1-CCR8 interaction in allergic diseases [12, 13]. For instance, serum levels of CCL1 were higher in patients with atopic dermatitis than in healthy controls [12]. Similarly, BALF CCL1 concentrations were elevated in patients with bronchial asthma [13]. It has been suggested that CCL1 may play a key role in regulating the immune system and defending against infection [25]. CCL1 was also reported to be a potential biomarker in lung adenocarcinoma [26]; however, the roles of CCL1 in the lungs are still unclear.

We analyzed CCL18, which can bind to CCR8, similar to CCL1. CCL18 is highly expressed in the lung, and it might contribute to the development of various interstitial lung diseases [27, 28]. Although it has been reported that CCL18 levels were higher in sarcoidosis patients than in healthy controls [28], no positive correlation between CCL1 and CCL18 levels was identified in this study. The lack of correlation might be explained by the fact that CCL1 might not be directly associated with Th2 lymphocytes as the Th2-associate chemokine, CCL18 [29].

It is presumed that sarcoidosis results from chronic immune response to unknown antigens, possibly of pathogenic origin. *Propionibacterium acnes* [30] and *M. tuberculosis* [31] are considered the possible causes of sarcoidosis. Kishi et al. showed

that the intratracheal administration of *Bacille de Calmette et Guérin* in the chronic lung inflammation model significantly increased granuloma formation in CCL1- transgenic mice compared with the control mice [32]. This study demonstrated a novel *in vivo* role for CCL1 in regulating the adaptive immune response. Chiu et al. reported that CCL1 expression was upregulated in granuloma formation in an experimental animal model of granuloma by *Mycobacterium bovis* [33]. These reports might indicate that CCL1 could regulate granuloma formation in sarcoidosis.

In our study, the levels of serum Th1 cytokines and chemokines, such as IFN-γ and IP-10, were significantly higher in patients with sarcoidosis than in IgG4-RD patients. These results follow our previous BALF report [7].

IP-10, a ligand for CXC chemokine receptor 3, is reportedly expressed on Th1 cells [34, 35], and its expression is strongly induced by IFN-γ [36]. In patients with sarcoidosis, IP-10 has been reported to contribute to granuloma formation by facilitating migration and activation of Th1 cells [36]. These findings strongly indicate that IP-10 may play an important role in the dysregulated Th1 response associated with sarcoidosis. In our study, a significantly positive relationship was observed between serum levels of CCL1 and IP-10. Hence, CCL1 might have a close association with Th1 immune response in sarcoidosis compared with that of Th2.

The role of Th2 cytokines has not been ruled out in granuloma formation associated with sarcoidosis [37]. Several reports have shown that serum levels of CCL17, also known as thymus-and activation-regulated chemokine (TARC) and IL-13, are elevated in patients with sarcoidosis [38-40]. We also observed significantly higher levels of IL-13 and eotaxin in patients with sarcoidosis than in healthy controls. Furthermore, we analyzed serum CCL17 in patients with sarcoidosis. CCL17 acts on the chemokine receptor CCR4, which is expressed on Th2 lymphocytes [41]. CCL17 is a chemokine specifically involved in the trafficking of Th2 cells. In this study, no statistically significant correlation between serum CCL1 and CCL17 levels was identified. Although the detailed mechanisms are still unknown, we presumed that CCL1 might have a more important role as a regulator of Th1 rather than Th2 response in sarcoidosis.

The serum levels of IL-13 were not significantly higher in patients with sarcoidosis than in those with IgG4-RD, inconsistent with our previous BALF study [8]. These findings might suggest that the activation of the Th2 response in IgG4-RD could be more of a local rather than a systemic phenomenon.

Our study had a few limitations. First, this study was carried out at a single institution, had a small sample size, and data were analyzed retrospectively. Second, since most patients in this study were Japanese, racial influence on the clinical course of

sarcoidosis cannot be ruled out. Finally, measurement of the serum levels of CCL1 was performed only at the time of diagnosis. Therefore, it is necessary to validate the outcomes of this study in a larger population with a diverse clinical course, prospectively. Despite these limitations, to our knowledge, this is the first report describing the significant correlation between serum CCL1 and several clinical findings in sarcoidosis patients.

5. Conclusion

The serum levels of CCL1 could reflect disease activity and may be involved in the pathogenesis of sarcoidosis; therefore, it is more closely related to Th1- than to Th2-associated responses. Though further research in this field is necessary, CCL1 might have the potential to be a less-invasive, reliable serological marker of sarcoidosis.

CRediT authorship contribution statement

MK contributed to conceptualization, methodology, validation, formal analysis, data collection and curation, visualization, and wrote the first draft. HY contributed to conceptualization, resources, data curation, supervision, and wrote, reviewed, and edited the manuscript. MY contributed to the research, supervision, data curation, and wrote,

reviewed, and edited the manuscript. AU wrote, reviewed, and edited the manuscript. TN contributed to the research and resources. TU contributed to the research, resources, and supervision. SK contributed to data curation and supervision. MH contributed to supervision and project administration.

Declaration of Competing Interest: The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment: We thank Atsushi Hori and Hitomi Imamura for technical assistance with the measurement of cytokines and chemokines. We are grateful to Masayoshi Koinuma for constructive advice on statistics. We would like to thank Editage for English language editing.

Funding: This work was supported by the Research Program of Intractable Disease, the Ministry of Health, Labor, and Welfare of Japan.

References

[1] Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999, Am J Respir Crit Care Med 160(2) (1999) 736-55.

[2] D.N. Mitchell, J.G. Scadding, Sarcoidosis, Am Rev Respir Dis 110(6) (1974) 774-802.

[3] P. Pinkston, P.B. Bitterman, R.G. Crystal, Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis, N Engl J Med 308(14) (1983) 793-800.

[4] B.W. Robinson, T.L. McLemore, R.G. Crystal, Gamma interferon is spontaneously released by alveolar macrophages and lung T lymphocytes in patients with pulmonary sarcoidosis, J Clin Invest 75(5) (1985) 1488-95.

[5] J.H. Stone, Y. Zen, V. Deshpande, IgG4-related disease, N Engl J Med 366(6) (2012) 539-51.

[6] H. Yamamoto, M. Yasuo, M. Ito, T. Yokoyama, H. Hamano, T. Uehara, S. Kawakami, K. Kubo, Clinical features of central airway involvement in autoimmune pancreatitis, Eur Respir J 38(5) (2011) 1233-6.

[7] H. Yamamoto, M. Yasuo, T. Ichiyama, A. Ushiki, M. Hanaoka, H. Hamano, T. Uehara, S. Kawakami, S. Matsui, K. Kubo, Cytokine profiles in the BAL fluid of IgG4-related respiratory disease compared with sarcoidosis, ERJ Open Res 1(2) (2015).

[8] H. Yamamoto, M. Yasuo, M. Komatsu, A. Ushiki, H. Hamano, A. Hori, T. Nakajima, T. Uehara, Y. Fujinaga, S. Matsui, M. Hanaoka, 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, Cytokine 120 (2019) 125-129.

[9] M.D. Miller, M.S. Krangel, The human cytokine I-309 is a monocyte chemoattractant, Proc Natl Acad Sci U S A 89(7) (1992) 2950-4.

[10] D. D'Ambrosio, A. Iellem, R. Bonecchi, D. Mazzeo, S. Sozzani, A. Mantovani, F. Sinigaglia, Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells, J Immunol 161(10) (1998) 5111-5.

[11] A. Zingoni, H. Soto, J.A. Hedrick, A. Stoppacciaro, C.T. Storlazzi, F. Sinigaglia, D. D'Ambrosio, A. O'Garra, D. Robinson, M. Rocchi, A. Santoni, A. Zlotnik, M. Napolitano, The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells, J Immunol 161(2) (1998) 547-51.

[12] H.O. Kim, S.I. Cho, B.Y. Chung, H.K. Ahn, C.W. Park, C.H. Lee, Expression of CCL1 and CCL18 in atopic dermatitis and psoriasis, Clin Exp Dermatol 37(5) (2012) 521-6.

[13] R. Montes-Vizuet, A. Vega-Miranda, E. Valencia-Maqueda, M.C. Negrete-Garcia, J.R. Velasquez, L.M. Teran, CC chemokine ligand 1 is released into the airways of atopic asthmatics, Eur Respir J 28(1) (2006) 59-67.

[14] C. Pedro, N. Melo, E.B.H. Novais, A. Magalhaes, G. Fernandes, N. Martins, A. Morais, P. Caetano Mota, Role of Bronchoscopic Techniques in the Diagnosis of Thoracic Sarcoidosis, J Clin Med 8(9) (2019).

[15] W.F. Fang, Y.C. Chen, Y.H. Chung, W.T. Woon, C.C. Tseng, H.W. Chang, M.C. Lin, Predictors of oxygen desaturation in patients undergoing diagnostic bronchoscopy, Chang Gung Med J 29(3) (2006) 306-12.

[16] H. Umehara, K. Okazaki, Y. Masaki, M. Kawano, M. Yamamoto, T. Saeki, S. Matsui, T. Yoshino, S. Nakamura, S. Kawa, H. Hamano, T. Kamisawa, T. Shimosegawa, A. Shimatsu, T. Ito, K. Notohara, T. Sumida, Y. Tanaka, T. Mimori, T. Chiba, M. Mishima, T. Hibi, H. Tsubouchi, K. Inui, H. Ohara, Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011, Mod Rheumatol 22(1) (2012) 21-30.

[17] M. Drent, J. De Vries, M. Lenters, R.J. Lamers, S. Rothkranz-Kos, E.F. Wouters, M.P. van Dieijen-Visser, J.A. Verschakelen, Sarcoidosis: assessment of disease severity using HRCT, Eur Radiol 13(11) (2003) 2462-71.

[18] G. Moncunill, J.J. Aponte, A.J. Nhabomba, C. Dobano, Performance of multiplex commercial kits to quantify cytokine and chemokine responses in culture supernatants from Plasmodium falciparum stimulations, PLoS One 8(1) (2013) e52587.

[19] J.F. Slifker, S.S. Shapiro, The Johnson System: Selection and Parameter Estimation,

Technometrics 22(2) (1980) 239-246.

[20] S. Uysal, F. Meriç Yilmaz, N. Boğdaycioğlu, S. Mungan Öztürk, F. Ak, Increased serum levels of some inflammatory markers in patients with multiple sclerosis, Minerva Med 105(3) (2014) 229-35.

[21] C. Thi Hong Nguyen, N. Kambe, I. Kishimoto, I. Ueda-Hayakawa, H. Okamoto, Serum soluble interleukin-2 receptor level is more sensitive than angiotensin-converting enzyme or lysozyme for diagnosis of sarcoidosis and may be a marker of multiple organ involvement, J Dermatol 44(7) (2017) 789-797.

[22] R.S. Selvan, L.J. Zhou, M.S. Krangel, Regulation of I-309 gene expression in human monocytes by endogenous interleukin-1, Eur J Immunol 27(3) (1997) 687-94.

[23] N.S. Haque, X. Zhang, D.L. French, J. Li, M. Poon, J.T. Fallon, B.R. Gabel, M.B. Taubman, M. Koschinsky, P.C. Harpel, CC chemokine I-309 is the principal monocyte chemoattractant induced by apolipoprotein(a) in human vascular endothelial cells, Circulation 102(7) (2000) 786-92.

[24] S.A. Islam, M.F. Ling, J. Leung, W.G. Shreffler, A.D. Luster, Identification of human CCR8 as a CCL18 receptor, J Exp Med (2013) 1889-98.

[25] N. Takabatake, Y. Shibata, S. Abe, T. Wada, J. Machiya, A. Igarashi, Y. Tokairin, G. Ji, H. Sato, M. Sata, Y. Takeishi, M. Emi, M. Muramatsu, I. Kubota, A single nucleotide polymorphism in the CCL1 gene predicts acute exacerbations in chronic obstructive pulmonary disease, Am J Respir Crit

Care Med 174(8) (2006) 875-85.

[26] M.D. Pastor, A. Nogal, S. Molina-Pinelo, Á. Quintanal-Villalonga, R. Meléndez, I. Ferrer, B. Romero-Romero, M.J. De Miguel, J.L. López-Campos, J. Corral, R. García-Carboner, A. Carnero, L. Paz-Ares, IL-11 and CCL-1: Novel Protein Diagnostic Biomarkers of Lung Adenocarcinoma in Bronchoalveolar Lavage Fluid (BALF), J Thorac Oncol 11(12) (2016) 2183-2192.

[27] A. Prasse, C. Probst, E. Bargagli, G. Zissel, G.B. Toews, K.R. Flaherty, M. Olschewski, P. Rottoli, J. Muller-Quernheim, Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis, Am J Respir Crit Care Med 179(8) (2009) 717-23.

[28] M. Cai, F. Bonella, X. He, S.U. Sixt, R. Sarria, J. Guzman, U. Costabel, CCL18 in serum, BAL fluid and alveolar macrophage culture supernatant in interstitial lung diseases, Respir Med 107(9) (2013) 1444-52.

[29] V. Kodelja, C. Muller, O. Politz, N. Hakij, C.E. Orfanos, S. Goerdt, Alternative macrophage activation-associated CC-chemokine-1, a novel structural homologue of macrophage inflammatory protein-1 alpha with a Th2-associated expression pattern, J Immunol 160(3) (1998) 1411-8.

[30] J.Y. Homma, C. Abe, H. Chosa, K. Ueda, J. Saegusa, M. Nakayama, H. Homma, M. Washizaki, H. Okano, Bacteriological investigation on biopsy specimens from patients with sarcoidosis, Jpn J Exp Med 48(3) (1978) 251-5.

[31] D.R. Moller, Potential etiologic agents in sarcoidosis, Proc Am Thorac Soc 4(5) (2007) 465-8.

[32] H. Kishi, M. Sato, Y. Shibata, K. Sato, S. Inoue, S. Abe, T. Kimura, M. Nishiwaki, K. Yamauchi, T. Nemoto, A. Igarashi, Y. Tokairin, O. Nakajima, I. Kubota, Role of chemokine C-C motif ligand-1 in acute and chronic pulmonary inflammations, Springerplus 5(1) (2016) 1241.

[33] B.C. Chiu, C.M. Freeman, V.R. Stolberg, E. Komuniecki, P.M. Lincoln, S.L. Kunkel, S.W. Chensue, Cytokine-chemokine networks in experimental mycobacterial and schistosomal pulmonary granuloma formation, Am J Respir Cell Mol Biol 29(1) (2003) 106-16.

[34] M. Loetscher, B. Gerber, P. Loetscher, S.A. Jones, L. Piali, I. Clark-Lewis, M. Baggiolini, B. Moser, Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes, J Exp Med 184(3) (1996) 963-9.

[35] Y. Weng, S.J. Siciliano, K.E. Waldburger, A. Sirotina-Meisher, M.J. Staruch, B.L. Daugherty, S.L. Gould, M.S. Springer, J.A. DeMartino, Binding and functional properties of recombinant and endogenous CXCR3 chemokine receptors, J Biol Chem 273(29) (1998) 18288-91.

[36] S. Narumi, T.A. Hamilton, Inducible expression of murine IP-10 mRNA varies with the state of macrophage inflammatory activity, J Immunol 146(9) (1991) 3038-44.

[37] M.C. Iannuzzi, J.R. Fontana, Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics, Jama 305(4) (2011) 391-9.

[38] C.T.H. Nguyen, N. Kambe, I. Ueda-Hayakawa, I. Kishimoto, N.T.M. Ly, K. Mizuno, H. Okamoto, TARC expression in the circulation and cutaneous granulomas correlates with disease

severity and indicates Th2-mediated progression in patients with sarcoidosis, Allergol Int 67(4) (2018) 487-495.

[39] S. Nureki, E. Miyazaki, M. Ando, T. Ueno, T. Fukami, T. Kumamoto, K. Sugisaki, T. Tsuda, Circulating levels of both Th1 and Th2 chemokines are elevated in patients with sarcoidosis, Respir Med 102(2) (2008) 239-47.

[40] H.P. Hauber, D. Gholami, A. Meyer, A. Pforte, Increased interleukin-13 expression in patients with sarcoidosis, Thorax 58(6) (2003) 519-24.

[41] C.A. Anderson, P. Patel, J.M. Viney, R.M. Phillips, R. Solari, J.E. Pease, A degradatory fate for CCR4 suggests a primary role in Th2 inflammation, J Leukoc Biol (2020) 455-66.

Tables

Table 1. Clinical characteristics of the study population.

	Sarcoidosis	IgG4-RD	Healthy controls	*p* value [a]
Number	44	14	14	
Sex, female/male	17 / 27 [c]	5 / 9	0 / 14	**< 0.001**
Age (yr), median (range)	49.0 (23, 75)	68.5 (54, 87) [b, d]	34.5 (25, 49)	**< 0.001**
smoking status				
smoker/ex-smoker/non-smoker	10 / 13 / 21	0 / 9 / 5	0 / 6 / 8	NS
Radiographic stage, no.		ND	ND	
0	5			
I	19			
II	19			
III, IV	1			
HRCT socre, median (range)	4 (1, 13)	ND	ND	
Extrapulmonary disease, yes/no	27 / 17	12 / 2		NS

Data are presented as median (range), or number.

[a] Data were analyzed using the Kruskal-Wallis test.

[b, c, d] Data were analyzed using the Dunn's test. [b] $p < 0.05$; sarcoidosis vs IgG4-RD. [c] $p < 0.05$; sarcoidosis vs healthy controls. [d] $p < 0.05$; IgG4-RD vs healthy controls.

Abbreviations. HRCT: high-resolution computed tomography; IgG4-RD: immunoglobulin G4-related disease; ND: not done; NS: not significant

Table 2. Laboratory findings and pulmonary function test.

	Sarcoidosis	n	IgG4-RD	n	*p* value
Laboratory findings					
ACE (U/mL)	20.3 (2.5, 41.2)	14	16.8 (9.3, 19.6)	9	**0.029**
sIL-2R (U/mL)	448 (302, 4516)	42	550.5 (224, 1710)	14	0.268
serum Calcium (mg/dL)	9.4 (8.3, 10.1)	41	8.8 (8.1, 9.9)	14	**0.001**
Total protein (g/dL)	7.3 (6.2, 8.7)	44	7.4 (6.7, 10.0)	14	0.212
albumin (g/dL)	4.30 (3.3, 5.0)	44	4.0 (2.6, 4.3)	14	**0.005**
urea nitrogen (mg/dL)	14.35 (1.7, 29.3)	44	12.5 (1.5, 24.8)	14	0.059
creatinine (mg/dL)	0.70 (0.44, 2.17)	44	0.78 (0.54, 1.05)	14	0.256
LDH (U/L)	181 (124, 225)	44	174 (141, 255)	13	0.506
CRP (mg/dL)	0.07 (0.00, 3.73)	43	0.10 (0.00, 0.77)	14	0.334
IgG (mg/dL)	1383 (938, 2226)	35	1680 (1096, 6186)	14	0.005
IgG4 (mg/dL)	45 (14, 182)	11	241 (6, 2330)	14	**< 0.001**
KL-6 (U/mL)	311 (149, 1921)	16	273 (239, 628)	4	0.741
White blood cell (cell/μL)	4815 (2690, 9200)	44	5100 (2850, 7410)	14	0.203
Lymphocyte count (cell/μL)	1061 (478, 2566)	44	1774 (976, 2119)	14	**0.008**
Eosinophil count (cell/μL)	121 (0, 1542)	44	226 (31, 634)	14	**0.008**
Pulmonary functional tests					
VC (L)	2.75 (1.50, 4.50)	18	3.03 (2.01, 3.84)	9	0.877
predicted VC (%)	89.1 (55.1, 115.6)	18	91.8 (77.0, 117.8)	9	0.425
FVC (L)	3.00 (1.45, 4.97)	38	3.04 (1.97, 5.04)	12	0.838
predicted FVC (%)	94.5 (55.3, 123.5)	38	96.0 (74.9, 127.6)	12	0.727
FEV_1 (L)	2.40 (1.25, 4.38)	38	1.99 (1.43, 3.55)	12	0.061
FEV_1/FVC ratio (%)	83.1(45.1, 98.0)	38	70.9 (37.8, 79.2)	12	**< 0.001**
predicted DLco (%)	77.6 (28.6, 102.5)	14	91.1 (62.7, 108.0)	9	0.244
BALF findings					
Total cell count (10^5/mL)	1.96 (0.84, 5.08)	19	1.71 (0.60, 1.90)	3	0.196
Macrophage (%)	62.6 (24.3, 95.6)	19	71.3 (54.8, 87.5)	3	0.738
Lymphocyte (%)	26.2 (3.9, 74.0)	19	27.8 (8.6, 36.7)	3	0.599
Eosinophil (%)	0.5 (0.0, 5.0)	19	2.7 (0.7, 6.7)	3	0.068
CD4/8 ratio (%)	3.69 (1.46, 7.49)	19	1.79 (1.50, 2.60)	3	0.104

Data are presented as median (range).

Abbreviations. ACE: angiotensin-converting enzyme; BALF: bronchoalveolar lavage fluid; CRP: C-reactive protein; DLco: diffusing capacity of the lung for carbon monoxide; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; Ig: immunoglobulin; IgG4: immunoglobulin G4; IgG4-RD: immunoglobulin G4-related

disease; KL-6; Krebs von Lungen-6; LDH: lactate dehydrogenase; sIL-2R: soluble interleukin 2 receptor; VC: vital capacity.

Table 3. Serum cytokines and chemokines in patients with sarcoidosis, IgG4-RD, and healthy controls.

	Sarcoidosis	n	IgG4-RD	n	Healthy controls	n	*p* value [a]
CCL1	55.46 (0.00, 721.50) [b, c]	44	6.23 (0.00, 325.14)	14	0.00 (0.00, 6.60)	14	**< 0.001**
IL-1β	0.00 (0.00, 80.45)	39	0.26 (0.00, 2.54)	11	0.00 (0.00, 34.95)	14	0.601
IL-2	0.00 (0.00, 12.11)	39	0.00 (0.00, 1.76)	11	0.00 (0.00, 22.49)	14	0.511
IL-4	0.00 (0.00, 38.13)	39	0.00 (0.00, 0.00)	11	0.00 (0.00, 94.37)	14	0.261
IL-5	0.00 (0.00, 1.29)	39	0.76 (0.00, 15.96) [b, d]	11	0.00 (0.0, 2.11)	14	**0.006**
IL-6	0.00 (0.00, 13.50)	39	0.00 (0.00, 4.44)	11	0.00 (0.00, 0.00)	14	0.108
IL-8	8.09 (2.86, 979.63) [c]	39	7.37 (2.67, 37.29)	11	5.48 (2.01, 18.22)	14	**0.044**
IL-10	0.00 (0.00, 14.13)	39	0.00 (0.00, 34.04)	11	0.00 (0.00, 25.78)	14	0.510
IL-12 (p40)	0.00 (0.00, 31.73)	39	0.00 (0.00, 0.00)	11	0.00 (0.00, 326.84)	14	0.557
IL-12 (p70)	0.00 (0.00, 29.89)	39	0.00 (0.00, 1.81)	11	0.00 (0.00, 47.08)	14	0.426
IL-13	4.52 (0.00, 44.10) [c]	39	0.00 (0.00, 17.06)	11	0.00 (0.00, 6.10)	14	**< 0.001**
Eotaxin	94.56 (47.86, 179.20) [c]	39	99.14 (65.76, 139.27) [d]	11	54.89 (22.05, 139.12)	14	**< 0.001**
IFN-γ	5.84 (0.00, 378.69) [b]	39	0.37 (0.00, 5.16)	11	1.57 (0.00, 52.58)	14	**< 0.001**
IP-10	1074.03 (112.34, 3544.59) [b, c]	39	459.95 (312.50, 1303.31)	11	217.90 (94.63, 466.69)	14	**< 0.001**
MCP-1	412.53 (209.86, 2317.33)	39	611.09 (369.57, 780.01) [b]	11	508.73 (311.86, 780.50)	14	**0.016**
MIP-1α	0.00 (0.00, 45.92)	39	0.00 (0.00, 0.00)	11	0.00 (0.00, 15.33)	14	**0.024**
MIP-1β	67.22 (17.37, 166.90)	39	62.80 (44.38, 82.94)	11	61.38 (34.17, 99.07)	14	0.948
TNF-α	23.52 (5.48, 89.53) [c]	39	20.50 (1.96, 28.36)	11	10.96 (7.18, 73.88)	14	**< 0.001**

Data are presented as median (range).

[a] Data were analyzed using the Kruskal-Wallis test.

[b, c] Data were analyzed using Dunn's test. [b] $p < 0.05$; sarcoidosis vs IgG4-RD. [c] $p < 0.05$; IgG4-RD vs Healthy controls.

CCL: C-C chemokine ligand; IFN: interferon; IgG4-RD: immunoglobulin G4-related disease; IL: interleukin; IP: Interferon-inducible protein; MCP: monocyte-chemotactic protein; MIP: macrophage inflammatory protein; TNF: tumor necrosis factor.

Figure legends

Figure 1. Serum levels of CCL1 of patients with sarcoidosis, IgG4-RD, and healthy controls. Horizontal lines, boxes, and error bars represent the median, the 25th, and 75th percentiles, and 10th and 90th percentiles, respectively.

Abbreviations. CCL: C-C chemokine ligand; IgG4-RD: immunoglobulin G4-related disease

Figure 2. Serum levels of CCL1 of patients with sarcoidosis according to radiographic findings. The Y-axis shows the Serum CCL1 transformed using the Johnson system. A) Serum CCL1 by radiographic Stage. B) Serum CCL1 by hilar and mediastinal lymph nodes swelling. Lymph node swelling was graded on a four-grade scale (score 0- none, 1- minor, 2- moderate, 3- pronounced).

Horizontal lines and boxes represent the mean, and the standard deviation, respectively.

Abbreviations. CCL: C-C chemokine ligand

Figure 3. Correlation between serum CCL1 and A) serum levels of sIL-2R ($r = 0.661$, $p < 0.001$), B) the total cell counts ($r = 0.573$, $p = 0.010$), and C) lymphocyte counts ($r=0.715$, $p < 0.001$) in BALF of sarcoidosis patients.

Abbreviations. BALF: bronchoalveolar lavage fluid; CCL: C-C chemokine ligand; sIL-2R: soluble interleukin 2 receptor.

Figure 4. Correlation between serum levels of CCL1 and A) serum levels of IP-10 (r = 0.545, $p < 0.001$), and B) TNF-α of sarcoidosis patients (r = 0.517, $p < 0.001$)).

Abbreviations. CCL: C-C chemokine ligand; IP: interferon-inducible protein; TNF: tumor necrosis factor

Figure 5. Correlation between serum and BALF levels of CCL1 (r = 0.491, $p = 0.033$).

Abbreviations. CCL: C-C chemokine ligand; BALF: bronchoalveolar lavage fluid

Figure 6. Correlation between serum CCL1 and A) serum CCL17 (r = 0.139, $p = 0.420$), and B) serum CCL18 (r = 0.384, $p = 0.016$).

Abbreviations. CCL: C-C chemokine ligand

Additional information

DOI: 10.1016/j.cyto.2020.155123.