Clinical Characteristics and Cytokine Profiles of Organizing Pneumonia in Patients with Rheumatoid Arthritis Treated with or without Biologics

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ABSTRACT. ***Objective.*** It has been reported that organizing pneumonia (OP) develops when patients with rheumatoid arthritis (RA) are treated with biologic disease-modifying antirheumatic drugs (bDMARD). However, the clinical characteristics and pathophysiology of OP in RA remain unknown in patients treated with bDMARD. We investigated the clinical characteristics and cytokine profiles of patients with RA-OP treated with bDMARD or conventional synthetic DMARD (csDMARD).

Methods. Twenty-four patients with RA who had developed OP were enrolled. These patients included 12 treated with bDMARD (bDMARD-OP subset) and 12 treated with csDMARD (csDMARD-OP subset). We compared the clinical characteristics and cytokine profiles between the patients with OP (OP subset, n = 24) and non-OP patients (non-OP subset, n = 29).

Results. There was no significant difference in clinical characteristics between the OP subset and the non-OP subset. Four patients developed OP within 2 months of bDMARD administration. In the other 8 patients, OP developed more than 1 year after the initiation of bDMARD. OP improved with corticosteroid treatment in all bDMARD-OP patients. After OP improved, bDMARD were readministered in 6 patients, and no OP recurrence was observed in any of these patients. Our multivariate analysis revealed that serum levels of interferon-α (IFN-α), interleukin (IL)-1β, IL-6, IL-8, and interferon-γ–inducible protein 10 were significantly associated with the development of OP, although these cytokines tended to be lower in the bDMARD-OP subset than in the csDMARD-OP subset.

Conclusion. OP is unlikely to be fatal in patients treated with bDMARD or csDMARD. IFN-α and proinflammatory cytokines are associated with the pathophysiology of OP in RA. (First Release February 1 2016; J Rheumatol 2016;43:738–44; doi:10.3899/jrheum.151019)

Key Indexing Terms:
ORGANIZING PNEUMONIA RHEUMATOID ARTHRITIS BIOLOGIC THERAPY
INTERFERON-α PROINFLAMMATORY CYTOKINES

Rheumatoid arthritis (RA) is an autoimmune disease characterized by inflammation of the synovial membrane. Occasionally, RA is complicated by extraarticular symptoms, including pulmonary disease, skin ulcers, and neuropathy. The major causes of death in RA are cardiovascular disease, pulmonary comorbidity, and malignancy[1,2]. Therefore, rheumatologists must carefully manage patients with RA who have those comorbidities.

The pulmonary lesions associated with RA include usual interstitial pneumonia (UIP), nonspecific IP (NSIP), organizing pneumonia (OP), diffuse alveolar damage, follicular bronchiolitis, rheumatoid nodules, pleurisy, and amyloidosis[3]. RA is often complicated by UIP or NSIP, and the occurrence of OP is also observed occasionally[4,5,6]. OP is a histopathological term that indicates that the bronchiole and alveolar ducts are obstructed by abnormal proliferation of the granulation tissue. OP is classified into cryptogenic OP (COP) and secondary OP[7]. Various causes of secondary OP have been reported, including infection, radiation therapy, drugs, hematological malignancy, hypereosinophilic syndrome, and connective tissue diseases such as RA[4,5,8], systemic lupus erythematosus[9], and Sjögren syndrome[10]. Drugs that induce OP in patients with RA include conventional synthetic disease-modifying antirheumatic drugs (csDMARD) such as bucillamine[11], salazosulfapyridine[12], methotrexate (MTX), and gold drugs[13].

Biologic DMARD (bDMARD), including tumor necrosis

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Supported in part by research grants from the Ministry of Health, Labor, and Welfare, Japan.

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Accepted for publication December 31, 2015.

factor (TNF) inhibitors (TNFi), anti-interleukin (IL)-6 receptor antibody, and T cell costimulation modulators, have been administered to patients with RA[14,15]. The bDMARD improve clinical symptoms and inhibit bone and joint destruction while preventing physical dysfunction in patients with RA. The management of RA has dramatically improved with the advent of bDMARD. Additionally, the safety and efficacy of bDMARD in treating patients with RA has been demonstrated[16,17]. Some case reports describe patients with RA who developed OP while being treated with bDMARD[18,19,20]. However, the clinical characteristics of OP in patients with RA treated with bDMARD remain unknown. Clinicians need to understand how patients with RA complicated with OP (RA-OP) should be managed while biologic therapy is being administered, and this issue will require further clarification. Moreover, the pathophysiology of RA-OP is not understood as clearly as that of COP, which is predominantly mediated by Th type 1 (Th1) responses[21].

In our report, we clarified the clinical characteristics of RA-OP in patients treated with bDMARD as compared with patients with RA-OP treated with csDMARD. In addition, to clarify the pathological mechanisms that lead to the development of RA-OP, we examined the cytokine profiles of patients with RA-OP for the first time. We also analyzed differences of the cytokine profiles between patients with RA-OP treated with bDMARD and those treated with csDMARD.

MATERIALS AND METHODS

Patients. Twenty-four patients with established RA were admitted to the Tokyo Women's Medical University Hospital between January 2008 and May 2014 with a diagnosis of OP. These 24 patients with RA with OP (OP subset) were enrolled in our case-controlled study. To compare the clinical characteristics between patients with RA with and those without OP, we also randomly selected outpatients with RA who had not developed OP (non-OP subset). They visited our hospital from November 2013 to January 2014. Written informed consent was provided by 29 patients without OP. We compared the clinical characteristics between the 2 subsets. In addition, we divided the OP subset into 2 subsets: patients treated with bDMARD (bDMARD-OP subset) and those treated with csDMARD (csDMARD-OP subset). We also compared the clinical characteristics between the 2 treatment subsets.

Patients had been diagnosed with RA according to the 1987 American College of Rheumatology classification criteria. Clinical data were obtained from the patients' medical records when OP developed in the OP subset or when they visited the hospital for the non-OP subset. Rheumatoid factor (RF) was measured using a latex agglutination assay. The upper limit for normal was < 15 U/ml RF. RA disease activity was evaluated with the Disease Activity Score at 28 joints-erythrocyte sedimentation rate (DAS28-ESR). This study was approved by the ethics committee of the Tokyo Women's Medical University (approval number: 3044) and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Definition of OP. OP was diagnosed when all the findings described below were clinically, microbiologically, and radiologically revealed in the patients with RA according to the American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias[22]. Clinically, the patients had subacute clinical courses and flu-like symptoms, such as fever, general fatigue, and cough. Their symptoms did not improve after treatment with antibiotics. Microbiologically, no pathogenic bacteria were cultured from sputum or bronchoalveolar lavage fluid (BALF). Focal airspace consolidations were present in lung computed tomography (CT) images[22]. Patchy consolidation and/or ground glass attenuations were observed with subpleural or peribronchial distributions. In some cases, the pulmonary pathological examinations revealed findings consistent with OP, which were confirmed by the presence of organizations within the alveolar ducts and alveoli[22].

Measurement of serum cytokines. Serum samples were stored at –80°C. Serum cytokine levels for interferon (IFN)-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-γ–inducible protein 10 (IP-10), and TNF-α were measured by the multiplex assays Milliplex MAP Human Cytokine/Chemokine Panel (EMD Millipore Corp.) and the Bio-Plex 200 System using the Bio-Plex Manager Software program, version 6.0 (Bio-Rad Laboratories Inc.). The levels of serum cytokines were estimated using nonlinear curve fitting.

Statistical analyses. Statistical analyses were performed using Fisher's exact test to compare frequencies and the Mann-Whitney U test to compare median values. Correlation coefficients were established with Spearman correlation coefficient. To identify which serum cytokines were associated with the development of OP, we conducted a multiple regression analysis. The data were analyzed using JMP software (SAS Institute). For the analyses presented here, statistical significance was declared for p values < 0.05, and no correction for multiplicity was used.

RESULTS

Clinical characteristics of OP in patients with RA. As shown in Table 1, 24 patients with RA were diagnosed with OP. OP was also diagnosed histologically by transbronchial lung biopsy in 7 patients. The median [interquartile range (IQR)] age at which OP developed was 63 years (56-70). The majority of the patients with OP were women (75%). The median RA disease duration for the patients who developed OP was 85 months (IQR 38–193). Nineteen patients (79%) had been taking MTX. The median dose of MTX was 8.0

Table 1. Comparison of clinical characteristics of OP and non-OP patients with RA. Statistical analyses were performed using Fisher's exact test to compare the frequencies and the Mann-Whitney U test for comparisons of median values. Values are median (interquartile range) unless otherwise specified.

Characteristics	Patients with OP, n = 24	Patients without OP, n = 29	p
Age, yrs	63 (56–70)	60 (48–69)	0.6
Female, n (%)	18 (75)	25 (86)	0.5
RA duration, mos	85 (38–193)	96 (33–187)	0.9
MTX, n (%)	19 (79)	23 (82)	1.0
MTX doses, mg/week	8.0 (0.5–10.0)	6.0 (4.0–8.0)	0.6
PSL, n (%)	10 (42)	8 (29)	0.4
Oral PSL doses, mg/day	0 (0–3.8)	0 (0–1.8)	0.2
bDMARD, n (%)	12 (50)	15 (52)	1.0
RF levels, IU/ml	99 (41–273)	48 (19–116)	0.14
DAS28-ESR	3.0 (2.1–4.0)	2.5 (2.0–3.7)	0.4
DAS28-ESR < 3.2, n (%)	13 (54)	19 (66)	0.6
ILD, n (%)	4 (17)	3 (11)	0.7

OP: organizing pneumonia; RA: rheumatoid arthritis; MTX: methotrexate; PSL: prednisolone; bDMARD: biologic disease-modifying antirheumatic drugs; RF: rheumatoid factor; DAS28: Disease Activity Score at 28 joints; ESR: erythrocyte sedimentation rate; ILD: interstitial lung disease.

mg/week (IQR 0.5–10.0). Prednisolone (PSL) was orally administered to 10 patients (42%). The median PSL dose was 0 mg/day (IQR 0–3.8). Twelve patients (50%) received bDMARD. The median DAS28-ESR score was 3.0 (IQR 2.1–4.0). OP developed in 13 patients with RA (54%) who had low disease activity or were in remission for RA disease (DAS28-ESR < 3.2). Interstitial lung disease (ILD) was found in 4 patients (17%).

In addition, we compared the clinical characteristics between the OP subset (n = 24) and the non-OP subset (n = 29) in patients with RA. As shown in Table 1, there were no significant differences between the 2 subsets.

Clinical characteristics of OP in patients with RA treated with bDMARD. We next examined the clinical characteristics of patients with RA-OP treated with bDMARD. The clinical information of 12 patients with RA-OP treated with bDMARD is shown in Table 2. MTX had been administered to most of these patients. All patients except 1 were treated with TNFi [infliximab (IFX) and etanercept (ETN) were administered to 6 and 5 patients, respectively]. Anti-IL-6 receptor monoclonal antibody, tocilizumab (TCZ), was given to only 1 patient (patient 5). Four patients (patients 1, 4, 7, and 8) developed OP within 2 months of initiating bDMARD treatment. In the other 8 patients, OP developed more than 1 year after bDMARD were administered. The RA disease activity when OP developed was low or in remission (DAS28-ESR < 3.2) for 5 patients (42%). Comorbid pulmonary disease was found in 2 patients. These 2 patients (7 and 8) were elderly and developed OP within 1 month of bDMARD administration.

The bDMARD and csDMARD were discontinued for each patient when OP developed and oral PSL therapy (0.8–1.0 mg/kg/day) was initiated. Overall, the treatment responses to OP were good and the respiratory symptoms and radiological findings improved in all of the patients. There were no respiratory failure deaths caused by OP. The same bDMARD as before were readministered to 5 patients (patients 1, 2, 3, 5, and 9) after OP improved. In 1 patient (patient 8), ETN was switched to TCZ after the development of OP. The other 6 patients were treated with csDMARD alone and did not receive bDMARD again. No recurrence of OP was observed in any of the patients.

Comparison of clinical characteristics between patients with RA-OP treated with bDMARD and csDMARD. We compared the clinical characteristics between the bDMARD-OP and csDMARD-OP subsets in Table 3. The RA disease durations tended to be shorter and the RA disease activities were higher in the bDMARD-OP subset than in the csDMARD-OP subset, although the differences were not statistically significant. MTX doses were significantly lower in the bDMARD-OP subset than in the csDMARD-OP subset. There were no differences in the age, sex, PSL doses, RF levels, and the rate of pulmonary comorbidity between the 2 subsets.

Serum cytokine profiles in patients with RA-OP. We next measured serum cytokines in patients with RA-OP to investigate the pathophysiology of RA-OP. We analyzed the differences in serum cytokine levels between the OP and non-OP subsets in patients with RA. Serum samples were obtained from 15 of the 24 enrolled patients with OP. We adjusted for the clinical manifestations between the 2 subsets, including age, sex, treatment, and disease activity of RA because many clinical background features such as the arthritis disease status and content of treatment could affect the serum cytokine results. Therefore, 19 non-OP patients with RA were selected from the enrolled 29 non-OP patients with RA and matched to the 15 patients with OP.

Table 2. Clinical characteristics of patients with RA-OP treated with bDMARD.

No.	Age/sex	RA Duration, Mos*	csDMARD	PSL	bDMARD	bDMARD Duration, Mos*	RF, IU/ml	DAS28-ESR	Comorbidity of Pulmonary Disease
1	53/F	27	MTX	+	IFX	2	6	3.3	—
2	56/F	35	MTX	–	IFX	12	45	4.3	—
3	60/F	36	MTX	–	ETN	14	195	4.4	—
4	31/F	28	MTX	–	ETN	1	45	2.1	—
5	66/F	102	MTX, TAC	+	TCZ	15	112	2.1	—
6	66/M	84	MTX	–	IFX	18	122	3.8	—
7	79/F	144	—	–	ETN	1	< 5	2.8	ILD
8	78/F	8	SASP	+	ETN	0	307	5.1	ILD
9	42/F	106	MTX	+	IFX	62	< 3	3.4	—
10	41/F	258	LEF	+	ETN	60	29	1.4	—
11	63/M	84	MTX, SASP	–	IFX	26	86	3.0	—
12	56/M	86	MTX	–	IFX	27	403	4.8	—

* RA disease durations and biological therapy durations were defined as periods at the development of OP. RA: rheumatoid arthritis; OP: organizing pneumonia; DMARD: disease-modifying antirheumatic drugs; bDMARD: biologic DMARD; csDMARD: conventional synthetic DMARD; PSL: prednisolone; RF: rheumatoid factor; DAS28: Disease Activity Score at 28 joints; ESR: erythrocyte sedimentation rate; MTX: methotrexate; TAC: tacrolimus; SASP: salazosulfapyridine; LEF: leflunomide; IFX: infliximab; ETN: etanercept; TCZ: tocilizumab; ILD: interstitial lung disease.

Table 3. Comparison of clinical characteristics of patients with RA-OP treated with bDMARD or csDMARD. Statistical analyses were performed using Fisher's exact test to compare the frequencies and the Mann-Whitney U test for comparisons of median values. Values are median (interquartile range) unless otherwise specified.

Characteristics	bDMARD-OP, n = 12	csDMARD-OP, n = 12	p
Age, yrs	58 (45–66)	68 (58–75)	0.2
Female, n (%)	9 (75)	9 (75)	1.0
RA duration, mos	84 (30–105)	134 (74–241)	0.1
MTX, n (%)	9 (75)	10 (83)	1.0
MTX doses, mg/week	1.3 (0–7.5)	9.0 (0–12.5)	0.04
PSL, n (%)	5 (42)	5 (42)	1.0
Oral PSL doses, mg/day	0 (0–6.3)	0 (0–4.5)	0.8
RF levels, IU/ml	99 (41–223)	99 (36–456)	0.7
DAS28-ESR	3.4 (2.3–4.4)	2.6 (1.9–3.5)	0.1
DAS28-ESR < 3.2, n (%)	5 (42)	7 (58)	0.4
ILD, n (%)	2 (17)	2 (17)	1.0

RA: rheumatoid arthritis; OP: organizing pneumonia; DMARD: disease-modifying antirheumatic drugs; bDMARD: biologic DMARD; csDMARD: conventional synthetic DMARD; bDMARD-OP: patients with RA who developed OP during treatment with bDMARD; csDMARD-OP: patients with RA who developed OP during treatment with csDMARD; MTX: methotrexate; PSL: prednisolone; RF: rheumatoid factor; DAS28: Disease Activity Score at 28 joints; ESR: erythrocyte sedimentation rate; ILD: interstitial lung disease.

As shown in Table 4, we matched the clinical manifestations between the OP (n = 15) and non-OP subsets (n = 19). Serum levels were significantly higher in the OP subset than in the non-OP subset for IFN-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IP-10, and TNF-α. Further, we analyzed the serum levels of cytokines between the OP and the non-OP subsets in patients treated with csDMARD (n = 15) and those with bDMARD (n = 19), respectively. In patients treated with csDMARD, the levels were significantly higher in the OP subset (n = 7) than the non-OP subset (n = 8) for IFN-α, IL-1β, IL-2, IL-6, IL-8, IL-10, and TNF-α. The median levels of IFN-γ, IL-12, and IP-10 were higher in the OP subset, although there were no statistical significances. On the other hand, the levels of IL-10 and IP-10 were significantly higher in the OP subset (n = 8) than the non-OP subset (n = 11) in patients treated with bDMARD. The levels of IFN-α, IFN-γ, IL-1β, IL-2, IL-6, IL-12, and TNF-α were higher in the OP subset, although statistically significant differences were not found (data not shown). To clarify which cytokines are closely associated with the development of OP, we conducted a multivariate analysis. As shown in Table 5, serum levels were significantly associated with the development of OP for IFN-α ($p < 0.001$), IL-1β ($p = 0.006$), IL-6 ($p = 0.002$), IL-8 ($p < 0.001$), and IP-10 ($p < 0.001$).

In addition, we examined associations between serum cytokine levels and arthritis disease activity in the 34 patients with RA (the OP, n = 15, and non-OP subsets, n = 19). We investigated correlations between each serum cytokine level and DAS28-ESR scores using Spearman correlation coefficient, and there were no significant correlations between any of the tested serum cytokine levels and DAS28-ESR scores (data not shown).

Comparison of cytokine profiles in patients with RA-OP treated with bDMARD and csDMARD. We hypothesized that there were pathophysiological differences in the development of OP in patients with RA treated with bDMARD and those treated with csDMARD because bDMARD have the ability to regulate inflammatory cytokines more directly, in particular TNF. We compared serum cytokine levels between patients with RA-OP treated with bDMARD (n = 8) and those treated with the csDMARD (n = 7).

As shown in Table 6, serum median levels of IL-10 and TNF-α were higher in the bDMARD-OP subset than in the csDMARD-OP subset, although the differences did not reach statistical significance. Serum levels of the other cytokines were lower in the bDMARD-OP subset than in the csDMARD-OP subset. There was also no significant difference between the 2 subsets except in the levels of IL-8.

DISCUSSION

In our report, we described the clinical characteristics and cytokine profiles of patients with RA-OP. Mori, *et al* have reported the clinical features of 25 patients with RA-OP who were treated with csDMARD[6]. In almost all of the cases, the RF levels were elevated. The authors' results suggested that high levels of RF might be a risk factor for the development of OP in patients with RA. On the other hand, OP occasionally precedes arthritis and is the first clinical symptom of RA[23,24]. OP is considered an extraarticular manifestation of RA[4,5,8]. Our study showed that RF levels and DAS28-ESR scores tended to be higher in patients with RA who had OP than in those without OP. In addition, we found that the clinical manifestations of OP are not very different between patients with RA treated with bDMARD and those treated with csDMARD. Controlling RA disease activity with csDMARD or bDMARD might prevent the development of OP.

However, our present study also revealed that OP developed in patients with low disease activity or while in remission when treated with csDMARD or bDMARD. These findings indicate that OP develops not only as a result of RA disease activity, but also because of other factors, including infection and drugs. In our present study, OP was diagnosed in 4 patients (patients 1, 4, 7, and 8) within 2 months after treatment with bDMARD was initiated. This result may indicate that biologic therapy is, to some extent, involved in the development of OP. Two (patients 7 and 8) of the above 4 patients were elderly and presented with ILD. Pulmonary infection is associated with aging and comorbidities of pulmonary disease in patients with RA treated with bDMARD[25]. In some patients with RA, pulmonary infection may induce the development of OP. It is important to determine whether clinicians should restart biologic therapy to regulate RA disease activity after the improvement of OP.

Table 4. Comparison of serum cytokines in patients with RA with and without OP. Statistical analyses were performed using Fisher's exact test to compare the frequencies and the Mann-Whitney U test for comparisons of median values. Units of all cytokines are pg/ml. Values are median (interquartile range) unless otherwise specified.

Variables	OP, n = 15	Non-OP, n = 19	p
Clinical manifestations			
Age, yrs	66 (56–76)	68 (54–78)	0.8
Female, n (%)	12 (80)	16 (84)	1.0
RA duration, mos	74 (33–182)	108 (32–192)	0.7
MTX, n (%)	11 (73)	15 (79)	0.7
MTX doses, mg/week	7.5 (0–9.0)	6.0 (4.0–10.0)	0.8
PSL, n (%)	5 (33)	6 (32)	1.0
PSL doses, mg/day	0 (0–3.0)	0 (0–2.0)	0.7
bDMARD, n (%)	8 (53)	11 (58)	1.0
RF levels, IU/ml	114 (52–273)	43 (13–370)	0.13
DAS28-ESR	3.3 (2.1–4.1)	3.3 (2.3–3.8)	0.8
DAS28-ESR < 3.2, n (%)	7 (47)	9 (47)	1.0
ILD, n (%)	4 (27)	3 (16)	0.7
Serum cytokine			
IFN-α	45.2 (1.3–614.4)	< 2.5 (< 2.5 to < 2.5)	0.002
IFN-γ	44.1 (4.1–142.2)	< 2.9 (< 2.9–27.9)	0.01
IL-1β	1.6 (< 2.9–74.6)	< 2.9 (< 2.9 to < 2.9)	0.02
IL-2	0.8 (< 2.8–58.6)	< 2.8 (< 2.8 to < 2.8)	0.02
IL-4	< 3.2 (< 3.2–93.4)	< 3.2 (< 3.2 to < 3.2)	0.013
IL-6	15.2 (4.3–95.5)	< 2.6 (< 2.6–31.6)	0.017
IL-8	12.1 (8.3–18.2)	4.6 (< 3.2–22.0)	0.2
IL-10	4.6 (< 3.1–48.8)	< 3.1 (< 3.1 to < 3.1)	0.0002
IL-12	5.1 (< 3.2–199.3)	< 3.2 (< 3.2–6.1)	0.0013
IL-13	< 2.5 (< 2.5–46.3)	< 2.5 (< 2.5 to < 2.5)	0.13
IL-17	< 3.1 (< 3.1–61.3)	< 3.1 (< 3.1–3.2)	0.5
IP-10	1726.0 (1398.3–2706.8)	572.2 (343.3–909.9)	0.0004
TNF-α	22.5 (12.8–40.0)	9.7 (5.1–21.1)	0.02

RA: rheumatoid arthritis; OP: organizing pneumonia; MTX: methotrexate; PSL: prednisolone; bDMARD: biologic disease-modifying antirheumatic drugs; RF: rheumatoid factor; DAS28: Disease Activity Score at 28 joints; ESR: erythrocyte sedimentation rate; ILD: interstitial lung disease; IFN: interferon; IL: interleukin; IP-10: IFN-γ–inducible protein 10; TNF-α: tumor necrosis factor-α.

Table 5. Associations between serum cytokine levels and the development of OP. Statistical analyses were performed using multiple regression analysis.

Serum Cytokines	Chi-square Value	p
IFN-α	11.095	< 0.001
IFN-γ	< 0.001	1.0
IL-1β	7.556	0.006
IL-2	< 0.001	1.0
IL-4	< 0.001	1.0
IL-6	9.465	0.002
IL-8	269.849	< 0.001
IL-10	< 0.001	1.0
IL-12	< 0.001	1.0
IL-13	0.002	1.0
IL-17	0.355	0.6
IP-10	136.827	< 0.001
TNF-α	0	1.0

OP: organizing pneumonia; IFN: interferon; IL: interleukin; IP-10: IFN-γ–inducible protein 10; TNF-α: tumor necrosis factor-α.

In our study, OP did not relapse in any of the 6 patients who were given bDMARD after their OP improved. On the other hand, the other 6 patients in our study were not readministered bDMARD. The decision of whether bDMARD were readministered after OP improved should be prudently made based on general condition, disease activity of RA, and pulmonary status in each patient, although OP is treatable and has a relatively good prognosis.

COP is characterized histopathologically by granulation tissue in the alveoli, alveolar ducts, and bronchiole[13,26]. In the BALF obtained from patients with COP, the proportions of lymphocytes, neutrophils, and eosinophils are increased[26,27]. The pathophysiology of COP is predominantly mediated by Th1 responses[21]. In contrast, the pathophysiology of RA-OP has not been adequately characterized. In our study, the levels of IFN-γ, IL-2, IL-12, and IP-10 were significantly higher in the OP subset in patients with RA. In general, dendritic cells, macrophages, and monocytes synthesize IL-12[28]. Naive T

Table 6. Serum cytokine levels in bDMARD-OP and csDMARD-OP patients. Units of all cytokines are pg/ml. Statistical analyses were performed using the Mann-Whitney U test for comparisons of median values. Values are median (interquartile range) unless otherwise specified.

Serum Cytokines	bDMARD-OP, n = 8	csDMARD-OP, n = 7	p
IFN-α	13.6 (< 2.5–218.3)	219.1 (< 2.5–2654.0)	0.5
IFN-γ	33.0 (4.4–102.1)	76.9 (< 2.9–1074.9)	0.7
IL-1β	0.8 (< 2.9–17.4)	7.6 (< 2.9–487.7)	0.5
IL-2	0.4 (< 2.8–31.5)	4.2 (< 2.8–410.9)	0.5
IL-4	< 3.2 (< 3.2–23.7)	< 3.2 (< 3.2–477.3)	0.7
IL-6	9.0 (3.6–81.1)	17.9 (8.4–198.1)	0.5
IL-8	8.5 (4.5–14.9)	16.8 (12.1–25.3)	0.02
IL-10	21.4 (0.1–98.3)	3.4 (< 3.1–34.1)	0.5
IL-12	4.1 (0.03–168.7)	24.9 (< 3.2–2424.4)	0.6
IL-13	< 2.5 (< 2.5–94.7)	< 2.5 (< 2.5–44.2)	0.8
IL-17	< 3.1 (< 3.1–4.2)	4.4 (< 3.1–76.8)	0.2
IP-10	1594.2 (1406.5–2534.3)	1833.0 (656.9–2866.8)	0.9
TNF-α	24.7 (18.0–37.9)	13.2 (12.1–355.7)	0.5

DMARD: disease-modifying antirheumatic drugs; bDMARD: biologic DMARD; OP: organizing pneumonia; csDMARD: conventional synthetic DMARD; bDMARD-OP: patients with RA who developed OP during treatment with bDMARD; csDMARD-OP: patients with RA who developed OP during treatment with csDMARD; RA: rheumatoid arthritis; IFN: interferon; IL: interleukin; IP-10: IFN-γ–inducible protein 10; TNF-α: tumor necrosis factor-α.

cells are stimulated by IL-12, and differentiate to Th1 cells that produce IL-2 and IFN-γ. IFN-γ activates macrophages[29] and induces the production of IP-10 by a wide variety of cell types, including lymphocytes, neutrophils, eosinophils, and monocytes[30]. IP-10 chemoattracts CXCR3-positive cells, including macrophages, dendritic cells, natural killer cells, and activated T lymphocytes toward inflamed areas. IL-10 and TNF-α were also higher in the OP subset in our present study. Cai, *et al* reported that alveolar macrophages collected by bronchoalveolar lavage from patients with OP produced higher levels of IL-10 and TNF-α in culture[26]. Therefore, our findings suggest that Th1-associated cells and alveolar macrophages could be involved in the pathogenesis of RA-OP, as well as COP.

We found that IFN-α, IL-1β, IL-6, and IL-8 were significantly associated with the development of RA-OP. In general, IFN-α is expressed in response to viral infection. Porcine circovirus type 2 (PCV2) infection caused the development of OP in swine[31]. In swine infected with PCV2, serum levels of IFN-α, IL-1β, and IL-6 all increased. The pathohistology of the lung in the swine infected with PCV2 was similar to the pathohistological findings of OP in humans. Taken together, the findings support the model that RA-OP may develop as the result of viral infection.

Our present study demonstrated that, in general, serum cytokines other than TNF-α tended to be lower in the bDMARD-OP subset than in the csDMARD-OP subset, although there were no statistically significant differences. This finding may be associated with the effect of anticytokine therapy. Increased levels of TNF-α may be prompting the use of TNFi[32,33].

We acknowledge that our study has some limitations. First, there might be a selection bias in selecting a part of the non-OP patients from our outpatients with RA. Another limitation was that serum samples were not obtained from all of the patients with OP. Serum cytokine data could be affected by many factors including content of treatment such as anticytokine biologic agents, timing of blood drawing, and duration of the treatment. The interpretation of cytokine profiles data should be considered carefully. Third, evaluations on cytokine expression using BALF samples and lung tissue specimens are important to clarify pathophysiology of RA-OP more precisely. However, in our present study, we could not collect a sufficient number of these samples. Finally, in Japan, IFX and ETN were in widespread usage earlier than other bDMARD such as adalimumab, TCZ, abatacept, golimumab, and certolizumab. Pharmaceutical market trends may be contributing to the rate of OP development in the patients with RA treated with bDMARD in our study population.

Clinicians should consider OP as a potential pulmonary complication in patients with RA treated with bDMARD or csDMARD. Response to treatment and OP clinical outcomes are favorable in patients treated with bDMARD or csDMARD. The pathophysiology of RA-associated OP is associated with IFN-α and proinflammatory cytokines such as IL-1β and IL-6.

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