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Original Research

Detection of the novel IL-1 family cytokines by QAH-IL1F-1 assay in rheumatoid arthritis

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Abstract: The interleukin (IL)-1 family of cytokines comprises 11 members, including 7 pro-inflammatory cytokines (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β,IL-36γ) and 4 anti-inflammatory cytokines (IL-1R antagonist (IL-1Ra), IL-36Ra, IL-37 and IL-38), and play central roles in mediating immune responses. In this study, we detected serum levels of IL-36 subfamily cytokines (including IL-36α, IL-36β, IL-36γ, IL-36Ra and IL-38), IL-37, IL-33 and aimed to investigate the roles of these cytokines in rheumatoid arthritis (RA) preliminarily. A total of 10 RA patients and 10 healthy controls (HCs) were involved in this study, we measured IL-36 subfamily cytokines, IL-37 and IL-33 levels in the serum of the experiment subjects by QAH-IL1F-1 assay. Clinical and laboratory data of the subjects were collected and analyzed by Spearman's rank test. Compared to that of HCs, IL-36α, IL-36β, IL-36Ra, IL-38 and IL-33 levels were significantly increased in RA patients. We also found RA patients with elevated IL-36Ra had a higher ESR and RF-IgM, and there was a positive correlation between increased IL-36α and CRP. Our study suggests that parts of the novel members of IL-1 family cytokines were involved in the pathogenesis of RA, and may provide a novel target for therapies of RA.

Key words: Rheumatoid arthritis, IL-1 family cytokines, QAH-IL1F-1 assay.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease with unknown aetiology, frequently leading to synovitis and progressive joint damage. However, inflammatory cytokines appear to be involved in the pathogenesis of RA, many of which had not been discovered until now (1). The inhibition of pro-inflammatory cytokines can reduce the manifestations of RA, improve function and retard radiological evidence of joint damage. Cytokine biomarkers may be a useful tool in estimation of the disease activity or joints' damage. Some of the proinflammatory cytokines, including IL-6, TNF-α and IL-17 are currently considered as potential biomarkers (2,3).

The interleukin (IL)-1 family comprises 11 members, namely IL-1α (IL-1F1), IL-1β (IL-1F2), IL-1R antagonist (IL-1Ra, IL-1F3), IL-18(IL-1F4), IL-36R antagonist (IL-36Ra, IL-1F5), IL-36α(IL-1F6), IL-37(IL-1F7), IL-36β(IL-1F8), IL-36γ(IL-1F9), IL-38(IL-1F10) and IL-33(IL-1F11). It is becoming clear that most members of the IL-1 family, such as IL-1α, IL-1β and IL-18, play important roles in immune regulation and inflammatory processes by inducing the expressions of other cytokines or matrix metalloproteinases (MMPs) and so on. While some members exert anti-inflammation activities, such as IL-1Ra and IL-36Ra (4,5). The roles of some of IL-1 family members in inflammation and immune modulation were almostly characterized, however, little is to be known about the effects and mechanism of the new members of IL-1 family cytokines, such as IL-36 subfamily cytokines (including IL-36α, IL-36β, IL-36γ, IL-36Ra and IL-38) and IL-37 in RA.

In this study, we detected the levels of these novel IL-1 family members and aimed to investigate the roles of these cytokines in RA preliminarily. We found IL-36α, IL-36β, IL-36Ra, IL-38 and IL-33 levels were significantly increased in RA patients compared to HCs. RA patients with elevated IL-36Ra had a higher erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF)-IgM, and there was a positive correlation between increased IL-36α and C- reactive protein.

Materials and Methods

Patients and controls

Serum samples were collected from 10 RA patients (Mean±SEM age 57.20±3.72 years) admitted to the ward of the Department of Rheumatology and Immunology, Ningbo No.2 Hospital from June 2014 to August 2014. All RA patients in this study fulfilled the 1987 revised criteria of the American College of Rheumatology (6). Age- and sex-matched healthy controls (HCs, n = 10, Mean±SEM age 56.10±3.94 years) were obtained from the medical examination center. Serum samples were stored at -80°C until used. The study protocol was approved by the ethics committee of Ningbo No.2 Hospital. All study subjects signed written informed consent before participating in the study. All patients had no other autoimmune or systemic diseases.

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Determination of IL-36 subfamily cytokines, IL-37 and IL-33 levels using the QAH-IL1F-1 assay

Serum IL-36 subfamily cytokines, IL-37 and IL-33 levels in all samples were measured with QAH-IL1F-1 assay (Raybiotech, USA) on a GenePix 4000B Microarray Scanner (1311 Orleans Drive Sunnyvale, CA 94089-1136 United States) according to the manufacturer's instructions. The sensitivity was 1 pg/ml.

Clinical and laboratory data analysis

All patients were obtained clinical data on age, sex, disease duration and ESR, CRP, anti-cyclic citrullinated peptides (CCP) antibodies and RF-IgM. ESR was evaluated by the Westergren method. Values ≤ 20.00 mm/h were considered normal. CRP was examined by the immunonephelometry method and a value > 10.00 mg/l was considered positive. Anti-CCP antibody and RF-IgM were tested by enzyme linked immunosorbent assay (ELISA), Anti-CCP antibody with nomal ranges of 0-25.00 U/ml, and 0-15.90 IU/ml for RF-IgM.

Statistical analysis

Means ± standard error of the mean (SEM) were calculated for all conditions, and differences between means were analyzed using Student's t-test and clinical features in RA patients were analyzed by Spearman's rank test. All statistical analyses were performed using GraphPad Prism software (Graph-Pad, San Diego, CA, USA). A P-value < 0.05 was considered significantly different.

Results

Patient clincal characteristics

A total of 10 RA patients were recruited into the study. And they didn't use disease modifying anti-rheumatic drugs(DMARDs). Detailed clinical characteristics and laboratory features of RA were shown in **Table 1**.

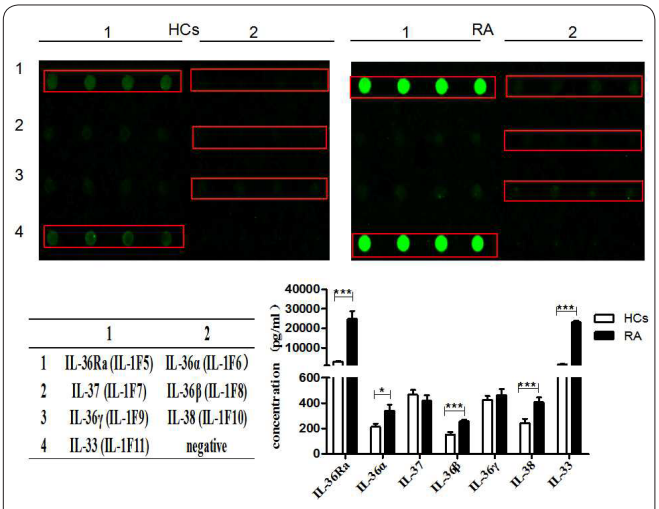

Figure 1. Serum levels of IL-36 subfamily cytokines, IL-37 and IL-33 in RA patients were detected by QAH-IL1F-1 assay. Compared to that of HCs, IL-36α, IL-36β, IL-36Ra, IL-38 and IL-33 levels were significantly increased in RA patients. *p<0.05, **p<0.01, ***p<0.001 (in comparison with HCs).

Analysis of serum IL-36 subfamily cytokines, IL-37 and IL-33 levels in RA patients

We measured serum levels of IL-36 subfamily cytokines, IL-37 and IL-33 in RA patients and age-matched HCs by QAH-IL1F-1 assay. The results showed that compared to that of HCs, serum levels of IL-36α (RA 339.32±49.07, HCs 214.70±23.57), IL-36β (RA 258.20±13.66, HCs 154.00±20.62), IL-36Ra (RA 24815.62±4123.20, HCs 3022.50±363.63), IL-38 (RA 408.85±35.82, HCs 234.70±33.52) and IL-33 (RA 23004.29±958.11, HCs 1571.00±132.49) were significantly increased in RA patients. The serum of IL-37 (RA 418.31±44.88, HCs 466.20±38.53) and IL-36γ (RA 463.45±48.21, HCs 426.10±30.72) did not differ between RA patients and HCs (**Fig. 1**). Our results suggest that parts of the novel members of IL-1 family cytokines might be associated with the pathogenesis of RA.

Table 1. Clinical and laboratory features in 10 patients with RA.

NO.	Age (yrs)	Sex	Disease duration (yrs)	ESR (mm/h)	CRP (ug/ml)	Anti-CCP (U/ml)	RF -IgM (IU/ml)	Medication within three months
1	76	Female	8	39.00	30.00	783.50	357.00	Meloxicam(15mg/d)
2	72	Female	30	120.00	74.20	1584.00	611.00	Traditional Chinese medicine
3	58	Female	20	30.00	14.20	160.19	11.10	None
4	57	Female	8	98.00	30.00	176.50	399.00	diclofenac slow release tablet(150mg/d)
5	40	Male	5	45.00	45.10	1387.46	621.00	diclofenac slow release tablet(150mg/d)
6	66	Female	1	77.00	30.00	1600.00	90.40	diclofenac slow release tablet(150mg/d) Traditional Chinese medicine
7	53	Female	2	38.00	50.10	45.12	11.10	None
8	41	Female	4	44.00	12.60	11.71	33.60	Ointment for external use
9	53	Female	8	37.00	16.30	456.00	43.30	Traditional Chinese medicine
10	56	Female	2	51.00	8.70	88.70	38.10	Meloxicam(15mg/d)

Table 2. Correlations of the novel IL-1 family cytokines with disease activity and auto-antibody production in RA patients.

	IL-36Ra		IL-36α		IL-37		IL-36β		IL-36γ		IL-38		IL-33	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
ESR	0.82	0.006**	0.25	0.492	0.44	0.204	-0.26	0.470	0.60	0.073	0.22	0.537	0.25	0.492
CRP	0.10	0.785	0.71	0.027*	0.47	0.166	-0.33	0.349	0.36	0.296	0.58	0.081	0.06	0.892
Anti-CCP	0.28	0.427	0.18	0.632	0.42	0.233	0.22	0.537	0.18	0.632	0.24	0.514	0.14	0.707
RF-IgM	0.74	0.017*	0.47	0.179	0.26	0.470	-0.01	0.973	0.08	0.838	0.41	0.233	0.11	0.759

Positive correlations of increased IL-36Ra with ESR and RF-IgM and IL-36α with CRP in RA patients

In our study, the correlations of the increased novel IL-1family cytokines with clinical activity and auto-antibody levels were analyzed. We found that RA patients with elevated IL-36Ra had higher ESR and RF-IgM, and there was a positive correlation between increased IL-36α and CRP. But no other correlations were found (Table 2).

Discussion

Cytokines are important mediators in inflammation and immune responses. An imbalance in the cytokine network may lead to inflammatory response and subsequent tissue damage in autoimmune diseases. It is widely recognized that TNF-α, IL-1β and IL-6 are involved in the pathogenesis of RA and have become main therapeutic targets (7-9). In view of growing need to search for new RA biomarkers, novel cytokine profiles could be used as a powerful predicting tool for indicating the progress of rheumatic disorders (10,11). In this study, we detected serum IL-36 subfamily cytokines, IL-37 and IL-33 levels in RA patients and found that compared to that of HCs, IL-36α, IL-36β, IL-36Ra, IL-38 and IL-33 levels were significantly increased in RA patients. Moreover, we found positive correlations of increased IL-36Ra with ESR and RF-IgM, and IL-36α had a positive correlation with CRP.

IL-1 family of cytokines can be divided into IL-1 subfamily, IL-18 subfamily and IL-36 subfamily. The IL-36 subfamily comprised of IL-36α, β and γ as well as IL-36 Ra and IL-38 (12). These IL-36 cytokines were mainly expressed in keratinocytes and monocytes / macrophages (13,14). Moreover, IL-36α and IL-36β were expressed on T cells (15,16). It has been found that IL-36 can enhance the expression and function of Th17 cytokines in an autocrine manner (17) and promote dendritic cells (DC) to produce inflammatory cytokines at a higher level than other IL-1 family members (18), suggesting its potential role in immunity and inflammatory responses. A recent study found the overexpression of IL-36α by synoviocytes from patients with RA or psoriatic arthritis (19), Magne (20) showed that joint and serum IL-36β levels elevated in several samples from RA patients, indicating that IL-36β can contribute to joint inflammation in RA. Moreover, recent data showed plasma concentrations of IL-36γ was significantly higher in active systemic lupus erythematosus (SLE) patients than those in HCs (21). In the present study, we found increased serum IL-36α and IL-36β levels in RA patients, and there was a positive correlation between increased IL-36α and CRP, suggesting that IL-36α/β exert their inflammatory effects in RA.

IL-36Ra acts similar to IL-1Ra, and could exert its antagonistic effects through binding with IL-36R. IL-38 is a recently identified IL-1 family antagonist that functioned similarly to IL-36Ra (22). It was predominantly expressed in the skin and proliferating B-cells of the tonsil (23). Previous studies showed IL-38 gene polymorphisms were associated with psoriatic arthritis (PsA), ankylosing spondylitis (AS) (24-26) and it was showed to be able to suppress IL-17 and IL-22 secretion (22). Interestingly, IL-36Ra also did the same activity as IL-38, suggesting that IL-38 and IL-36Ra are partial receptor antagonists. Consistent with previous reports, in our results, there were higher serum levels of IL-36Ra and IL-38 in RA patients. And we found positive correlation of increased IL-36Ra with ESR and RF-IgM in RA patients. As IL-1 family members, higher IL-38 and IL-36Ra suggest they might be a negative feedback increase to inhibit inflammatory responses in RA.

IL-37 was expressed in monocytes, macrophages and epithelial cells (27). Zhao et al. reported that plasma IL-37 level was significantly higher in RA patients, and positively correlated with IL-17A, TNF-α and disease activity (28). But in our experiment, perhaps because of limited samples, no significant difference of serum IL-37 levels was found between RA patients and HCs. More patients need be detected to testify the exact role of IL-37 in RA.

IL-33 was widely expressed in different cell types, such as macrophages, DC, fibroblasts, endothelial cells. IL-33 binding with its ligand ST2L on mast cells, macrophages and activated neutrophils, enhanced joint inflammation by inducing cytokines such as TNF-α, IL-1β, IL-6, IL-17, and interferon (IFN)-γ in arthritis models (29,30). Yeon-Sik Hong (31) found that serum level of IL-33 correlated with that of IL-1β and IL-6. Serum IL-33 and sST2 levels decreased together with CRP, after DMARDs therapy in patients with treatment-naive RA. Another study observed that IL-33 induced neutrophil migration by activating synoviocytes and macrophages and could directly attract neutrophils to the site of inflammation (32). Here we also confimed that IL-33 were significantly increased in RA patients which might explain the pro-inflammatory role of IL-33 in rheumatic disorders.

In this study, we preliminary revealed the association between the new members of IL-1 family cytokines and RA patients. However, our study had several limitations. First, the sample size was not large. Another limitation was the heterogeneity of our patients. Therefore, more patients need to be examined to confirm the roles of these cytokines in RA patients. Further studies of the new member of IL-1 family cytokines would be interesting to help with understanding the pathogenesis of RA.

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