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Original Article

TARC expression in the circulation and cutaneous granulomas correlates with disease severity and indicates Th2-mediated progression in patients with sarcoidosis

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Abbreviations used:
ACE, angiotensin-converting enzyme; BALF, bronchoalveolar lavage fluid; CCR4, CC chemokine receptor 4; CXCR3, CXC chemokine receptor 3; CD, cluster of differentiation; CRP, C-reactive protein; CT, high-resolution computed tomography; sIL-2R, soluble interleukin 2 receptor; TARC, thymus and activation-regulated chemokine; Th, T helper cell; WBC, White blood cell

ABSTRACT

Background: Sarcoidosis is a systemic disorder characterized by the accumulation of lymphocytes and monocyte/macrophage lineage cells that results in the formation of non-caseating granulomas. Thymus- and activation-regulated chemokine (TARC)/CCL17 is an important chemokine in the amplification of Th2 responses, which are achieved by recruiting CCR4-expressing $CD4^+$ T lymphocytes. TARC concentrations are known to increase in the serum of sarcoidosis patients; however, its role in the assessment of severity and prognosis of sarcoidosis remains unknown. The objective of this study is to elucidate the role of TARC in sarcoidosis by investigating its expression in peripheral blood and at inflammatory sites. We also examined its relationship with clinical features.

Methods: Serum levels of TARC, soluble interleukin 2 receptor, angiotensin-converting enzyme, and lysozyme were measured in 82 sarcoidosis patients. The Th1 and Th2 balance in circulating $CD4^+$ T cells was evaluated by flow cytometry. The immunohistochemical staining of TARC and CCR4 was performed in order to identify the source of TARC in affected skin tissues.

Results: TARC serum levels were elevated in 78% of patients and correlated with disease severity. The percentage of $CCR4^+$ cells and the $CCR4^+/CXCR3^+$ cell ratios were significantly higher in sarcoidosis patients than in normal subjects ($P = 0.002$ and $P = 0.015$, respectively). Moreover, TARC was expressed by monocyte/macrophage lineage cells within granulomas. The abundancy as well as distribution of TARC staining correlated with its serum levels.

Conclusions: The present results suggest that elevations in TARC drive an imbalanced Th2- weighted immune reaction and might facilitate prolonged inflammatory reactions in sarcoidosis.

Introduction

Sarcoidosis is a systemic granulomatous disorder that predominantly affects the lungs, intrathoracic lymph nodes, skin, and eyes. The hallmarks of sarcoidosis are non-caseating granulomas in which T lymphocytes and epithelioid cells accumulate.[1] Although the polarization into T helper type 1 (Th1) responses and Th1-associated cytokines reportedly play key roles in the pathogenesis of sarcoidosis, but efficacious treatments are not available for sarcoidosis due to the limited understanding of its etiology and the mechanisms responsible for granuloma formation.[2–8]

Chemotactic molecules contribute to the building of granulomatous structures.[9] Thymus- and activation-regulated chemokine (TARC), one of the CC chemokines and also known as CCL17, activates CC chemokine receptor 4 (CCR4) which is selectively expressed on T helper type 2 (Th2) lymphocytes. It recruits $CCR4^+$ T lymphocytes into inflamed sites, priming a Th2-type immune response.[10–13] Serum TARC levels have been considered as a useful laboratory marker for the diagnosis of atopic dermatitis and sharply reflect the disease activity, which is thought to be a Th2-dominant inflammatory skin disease, especially in the acute phase. In addition, serum

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TARC levels are also related to the disease activity of bullous pemphigoid and mycosis fungoid, but very high serum TARC levels are only seen in a limited number of various other skin diseases.[14] The balance between Th1 and Th2 cytokine expression patterns in sarcoidosis is critical factor for the clinical outcome of an immune response, in which Th1 response leads to an antigen/pathogen clearance and resolution, whereas the Th2 response results in an fibroproliferation and matrix deposition.[15] Recently, the serum level of TARC and the frequency of $CCR4^+$ $CD4^+$ T cells have been shown to be elevated in sarcoidosis.[2] In addition, Wahlström *et al.* reported an significant increase of proportion of circulating IL-4 producing T cells in sarcoidosis patients in comparison with healthy controls.[16]

The objective of present study was to evaluate the expression of TARC in both circulation and affected cutaneous tissues. Specifically, we wanted to study the clinical value of this Th2-chemokine in relation with severity and prognosis of the patients who suffered to a chronic inflammatory condition.

We herein demonstrated that elevated TARC serum levels were present in 78% of sarcoidosis patients. Patients with elevated TARC serum levels showed more severe clinical symptoms and significantly higher levels of other serum markers, indicating the severity of disease. TARC was detected in all affected cutaneous lesions, in which epithelioid cells and multinucleated giant cells were observed. High TARC expression levels within granulomas were associated with higher serum levels of TARC and other severity markers. Persistently elevated TARC serum levels during more than 3 years were associated with a higher frequency of pulmonary involvement, particularly the more advanced pulmonary stages. Based on the presence of this Th2 chemokine in the circulation and affected sites, we considered sarcoidosis to be driven by a Th2-type pattern of cytokine production, which plays a distinct role in the immunopathogenesis of this disease.

Methods

Study participants

The study group consisted of 82 sarcoidosis patients (27 men and 55 women; 42–83 years of age, mean 65.8 years) who were admitted to the Dermatology Department of Kansai Medical University Hospital (Osaka, Japan) between December 2008 and June 2017. None of the patients had received systemic and topical treatments within 1 month prior to their first visit. All of study subjects had no history of asthma or allergic diseases. The participants gave written informed consent and patient anonymity was preserved using methods approved by the Ethics Committee. The study protocol was approved by the Internal Review Board of Kansai Medical University (approval number 2016610).

Complete medical histories, clinical examinations, and laboratory tests were conducted for all patients at the first visit, as described previously.[17–20] Skin lesions were assessed by biopsy; chest radiogram and high-resolution computed tomography (CT) were used to assess pulmonary involvement; eye involvement was evaluated by split-lamp and funduscopic examinations. The panda sign and lambda sign as well as other involved organs were examined by imaging with ^{18}F-fluorodeoxyglucose positron emission tomography (FDG PET) and/or ^{67}Ga scintigraphy uptake. Patient characteristics are summarized in Table 1.

Laboratory analyses

Blood samples were collected for assays of TARC levels with routine analyses at the first visit and at several time points thereafter. TARC levels were assessed with a commercially available enzyme-linked immunosorbent assay, and its normal range was 0–450 pg/mL. Angiotensin-converting enzyme (ACE), soluble interleukin-2 receptor (sIL-2R), lysozyme, C-reactive protein (CRP), white blood cell (WBC) and lymphocyte counts, and the percentage of peripheral monocytes and eosinophils were measured in the clinical laboratory (normal values: ACE, 8.3–21.4 U/L; lysozyme, 5–10.2 μg/mL; sIL-2R, 122–466 U/mL; CRP, 0–0.3 mg/dL; WBC count, 3500–8500/μL; lymphocyte count, 1000–3000/μL; monocytes, 3–9%; eosinophils, 0.5–6%). All of blood samples were strived to take in the same time zone at early afternoon.

Table 1
Demographic characteristics of sarcoidosis patients.

Number, no.	82
Age, years	65.8 (1.2)
Male: female, no.	27:55
Duration of disease, years	5.33 (0.46)
Pulmonary involvement, no. (%)	43 (52.4)
Chest radiographic stage, no. (%)	
0	39 (47.6)
I	25 (30.5)
II	10 (12.2)
III, IV	8 (9.8)
Laboratory markers	
TARC (pg/mL)	2196.5 (459.4)
sIL-2R (U/mL)	787.7 (87.9)
ACE (U/L)	17.81 (0.81)
Lysozyme (μg/mL)	11.16 (1.00)
CRP (mg/dL)	0.203 (0.04)
WBC count (cells/μL)	5182 (178.1)
Lymphocyte count (cells/μL)	1350 (55.5)
Eosinophils (%)	3.47 (0.37)
Monocytes (%)	6.90 (0.39)

Values are means (SEM). BHL, bilateral hilar lymphadenopathy; TARC, thymus and activation-regulated chemokine; sIL-2R, soluble interleukin-2 receptor; ACE, angiotensin-converting enzyme; CRP, C-reactive protein; WBC, white blood cells; SEM, standard error of the mean.

Flow cytometric analysis

After written informed consent had been obtained, 2 mL of EDTA blood samples was collected from 22 patients with sarcoidosis and 12 healthy subjects. One hundred microliters of whole blood was incubated with Fc blocking for 10 min and then for 15 min with mixed antibodies. Cells were then lysed and fixed for 10 min, washed and suspended in sheath buffer, and analyzed with FACS Canto II (BD Biosciences, San Jose, CA). A forward scatter (FSC) and side scatter (SSC) were used to select the lymphocyte-containing population, lymphocytes were then gated based on the expression of CD3 and CD4. The proportion of $CCR4^+$ and $CXCR3^+$ cells was calculated among $CD4^+$ lymphocyte population. A list of reagents and their working concentrations is shown in Supplementary Table 1.

Immunohistochemical staining

Paraffin-embedded sections from 24 patients were cut, deparaffinized, and rehydrated through graded alcohol. A heat-induced epitope retrieval method was performed in Retrieval Solution pH 9 at 115 °C for 10 min. Tissues were treated with blocking buffer at room temperature (RT) for 1 h and incubated in the first antibody (anti-TARC and anti-CCR4 antibodies) at 4 °C overnight. After blocking endogenous peroxidase activity with 0.3% H_2O_2, immunohistochemical detection was performed using Envision + Duolink System-HRP at RT for 1 h. The reaction was developed with the DAB Peroxidase Substrate Kit for a maximum of 3 min. Sections were finally counterstained with hematoxylin and mounted. All images

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