Cytokine 81 (2016) 10–14

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Circulating levels of Th1 and Th2 chemokines in patients with ankylosing spondylitis

Jianing Wang [a,1], Qi Zhao [a,1], Gaoya Wang [a], Chunshu Yang [b], Yong Xu [a], Yujia Li [a], Pingting Yang [a,*]

[a] Department of Rheumatology and Immunology, First Affiliated Hospital, China Medical University, Shenyang 110001, People's Republic of China
[b] Department of 1st Cancer Institute, First Affiliated Hospital, China Medical University, Shenyang 110001, People's Republic of China

ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form 21 January 2016
Accepted 21 January 2016

Keywords:
Ankylosing spondylitis
Chemoattractants
TNF blockade
Disease activity index
Biomarker

ABSTRACT

Objective: Although chemokines are critical elements for the selective attraction and activation of various leukocyte subsets in the inflammatory process, there are few findings concerning T helper (Th) 1 or Th2 chemokines in ankylosing spondylitis (AS). This study was designed to determine whether serum levels of chemokines that are preferentially chemotactic for Th1 (IFN-gamma-inducible protein-10, IP-10/CXCL10) and Th2 (thymus and activation regulated chemokine, TARC/CCL17) and (macrophage derived chemokine, MDC/CCL22) cells were elevated and whether they correlated with the clinical features in patients with AS.

Methods: Forty-two patients with axial AS and 25 healthy controls were enrolled into the study. Serum levels of chemokines (IP-10, TARC and MDC) and cytokines (IFN-γ, TNF-α and IL-4) were examined using ELISA. The disease activity was evaluated by Ankylosing Spondylitis Disease Activity Score (ASDAS). Serum levels of IgG, IgA, IgM, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured.

Results: Serum chemokine levels of IP-10, TARC and MDC were significantly higher in patients with AS than those in healthy controls. Serum cytokine levels of IFN-γ, TNF-α were also significantly increased, but the levels of IL-4 were not. Furthermore, IP-10 levels in AS patients correlated with ESP, CRP and ASDAS, while the levels of TARC and MDC did not correlate with these clinic indexes. Correlation analysis between the levels of chemokines and cytokines revealed a positive correlation between IP-10 and TNF-α. The levels of both Th1 and Th2 chemokines decreased under blockade of TNF-α.

Conclusion: Our results suggest that both a Th1 chemoattractant IP-10 and Th2 chemoattractants, TARC and MDC, cooperatively play a role in the development of AS.

1. Introduction

Ankylosing spondylitis (AS), a chronic inflammatory rheumatic disease, is characterized by persistent inflammation, typically affecting the axial skeleton joints and resulting in pain, functional impairment, and structural changes [1]. The immunopathological mechanism underlining the joint chronic inflammation remains unclear. Accumulating evidence suggest that infiltration of T lymphocytes into the entheses might play an important role in the tissue damage of enthesopathies in AS patients. For example, magnetic resonance imaging indicates that severe osteitis occurred at the bone–cartilage interphase in AS patients [2–5]. And, histologic investigations from the sacroiliac joint indicate that mononuclear cells invade and erode the cartilage in early phases of AS [2,5,6].

To date, little has been known about the process of leukocytes infiltration in AS. It has been well established that the recruitment of T cells into the inflamed tissue is highly dependent on chemokines [7,8]. T helper (Th) cells are divided into Th1 and Th2 subsets based on their cytokine production profiles. Th1 cytokines (IFN-γ and TNF-α) may promote the development of autoimmune disorders, whereas the Th2 cytokines (IL4 and IL10) attenuate these diseases [9]. Chemokines play key roles in regulating the migration of specific T cells during immune and inflammatory responses. For example, IFN-γ-inducible protein-10 (IP-10/CXCL10) displays a strong chemoattractant activity for Th1 lymphocytes, which is

Abbreviations: IP-10, IFN-gamma-inducible protein-10; TARC, thymus and activation regulated chemokine; MDC, macrophage derived chemokine; ASDAS, Ankylosing Spondylitis Disease Activity Score; IFN, interferon; TNF, tumor necrosis factor.

* Corresponding author.
E-mail address: yangpingtingting@163.com (P. Yang).

[1] These two authors equally contributed to this work.

http://dx.doi.org/10.1016/j.cyto.2016.01.012
1043-4666/

considered as a reliable marker of aggressive Th1-mediated autoimmune disease [10]. On the other hand, bind thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22) play an important role in the selective migration of Th2 cells [11,12]. Thus, revealing the possible role of circulating Th1- and Th2-chemokines in AS is particularly helpful for understanding the immunopathology mechanism of AS.

However, to date, few studies have focused on this issue. In particular, regarding IP-10 and TARC, there is only one study reported that the serum levels of IP-10 and TARC were not significantly elevated in patients with AS ($n = 12$) comparing to healthy controls ($n = 27$) [13], while, the serum levels of IP-10 and TARC decreased after the patients received successful treatment with a TNF-α blocking agent. The aims of our study are: (1) the evaluation of serum levels of preferential Th1 chemoattractants (IP-10) and Th2 chemoattractants (TARC and MDC) in a larger series of AS patients, and (2) the assessment of possible relationships between chemokine levels and the clinical or laboratory findings.

2. Materials and methods

2.1. Patients

Forty-two patients with definite axial AS were recruited into the study. Diagnosis of AS of was made according to the modified New York criteria [14] in the Department of Rheumatology and Immunology of the First Affiliated Hospital of China Medical University from May 2012 to August 2015. All the patients did not receive specific treatment of AS before the experiment. Fifteen of the forty-two patients received etanercept 25 mg subcutaneous injection two times every week. The concomitant treatment included non-steroidal anti-inflammatory drugs. Patients with current pregnancy or lactation, a history of neoplasm, recent acute infection, or history of any other chronic inflammatory disease were excluded from the study. The same exclusion criteria were applied for 25 healthy controls matched for age and sex. After the subjects had accepted an informed consent, clinical and laboratory measures were routinely assessed and peripheral blood was drawn for further analyses as approved by the local ethics committee. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were stored at −80 °C prior to use.

2.2. Clinical assessment

The serum IgG, IgA, IgM, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were measured at the time of the assessment. Serum levels of IgG, IgA and IgM were quantified by turbidimetric immunoassay, using an IMMAGE 509 analyzer (Beckman Coulter Inc, Brea, CA, USA). ESR was measured immediately after blood collection using a manual Westergren method (BD vacutainer systems and BD seditainer™, Becton Dickinson, France). CRP levels were measured by turbidimetric immunoassay, using an IMMAGE 508 analyzer (Beckman Coulter Inc., Brea, CA, USA). HLA-B27 was determined by flow cytometry analysis with an anti-HLA-B27 reagent kit (Becton Dickinson). Disease activity measures were assessed using the Ankylosing Spondylitis Disease Activity Score (ASDAS), which includes the CRP level [15].

2.3. ELISA for chemokines

Serum levels of chemokines (IP-10, TARC, and MDC) were determined by sandwich ELISA developed by R&D Systems (Minneapolis, MN, USA) according to the manufacturer's protocol. Briefly, 96-well plates were coated with capture antibodies (anti-human IP-10, TARC or MDC monoclonal antibodies) at room temperature overnight. After washing and blocking, the serum samples diluted to 1:2 and standard dilutions of each recombinant human chemokine were added to duplicate wells for 2 h at room temperature. After washing, the detection antibodies (biotinylated anti-human IP-10, TARC, or MDC antibodies) were added for 2 h at room temperature. After washing four times, the bound antibodies were detected with streptavidin–horseradish peroxidase, using tetramethylbenzidine and H_2O_2 as substrate. The optical density at 450 nm was subsequently measured with a microplate reader. Serum levels of IFN-γ, TNF-α, and IL-4 were also determined by ELISA as mentioned above.

2.4. Statistical analysis

Normally distributed variables were compared using an independent *t*-test, and non-normally distributed variables were compared using the Mann–Whitney *U* test. Categorical variables were assessed by the chi-square test. Spearman's rank correlation coefficient was used to examine the relationships between 2 continuous variables. A *P*-value < 0.05 was considered statistically significant. All data were shown as mean ± SD.

3. Results

The sample general characteristics are presented in Table 1. The distributions of serum IP-10, TARC and MDC in patients with AS and healthy controls are presented in the box-and-whisker plot in Fig. 1. Serum IP-10 levels in most of patients with AS were significantly higher than those in normal controls (Fig. 1A). Mann–Whitney test revealed a significant difference between them (575 ± 261 vs. 70 ± 29 pg/ml, $p < 0.01$). Serum TARC level was also significantly elevated in patients with AS (383 ± 338 pg/ml, $p < 0.01$) compared with normal controls (155 ± 59; Fig. 1B). Though the distribution of serum MDC levels in AS patients had an overlap with that in normal controls, there was a substantial part of patients showed a higher MDC level than normal controls (Fig. 1C). The difference between the two groups was also statistically significant (256 ± 111 vs. 151 ± 83 pg/ml, $p < 0.01$).

We next analyzed the correlation between serum chemokine levels and disease activity variables of AS patients before therapy. As shown in the scatter plots in Fig. 2, ESR, CRP and ASDAS increased with the increase of IP-10 level. Table 2 lists the *r* and *p* values of the correlation analysis. Consistent with the visual observation in Fig. 2, IP-10 showed a significant correlation ($p < 0.05$) with ESR, CRP and ASDAS. However, serum IgG, IgA, IgM did not correlate with IP-10 level. However, TARC and MDC did not correlate with any of the measured disease activity variables. Also the serum levels of IP-10, TARC and MDC were mutual independent; there was no significant correlation between any pairs of the three chemokine levels.

Another interesting issue is whether the serum chemokine levels are correlated with the Th1 cytokines (IFN-γ, TNF-α) and Th2 cytokine (IL-4) in AS patients. The results of correlation analysis are present in Table 2. We only found a significant positive correlation ($p < 0.05$) between the serum levels of IP-10 and TNF-α.

To determine the therapeutic effect on the chemokine levels, we measured the serum levels of IP-10, TARC and MDC after 8 weeks etanercept treatment, and compared them with the serum levels measured before the treatment. The serum levels of IP-10, and TARC significantly decreased by blockade of TNF-α (Fig. 3A and B, $p < 0.01$ and $p = 0.03$, paired *t*-test), whereas the decrease of serum level of MDC after treatment was not significant (Fig. 3C, $p = 0.20$, paired *t*-test). Thus both type 1 and type 2 chemokines were affected by blockade with TNF-α. After the therapy, the serum level