Concise report

Increased levels of soluble TNF-like cytokine 1A in ankylosing spondylitis

Maria Konsta¹, Giorgos Bamias¹, Maria G. Tektonidou², Panayiotis Christopoulos¹, Alexios Iliopoulos³ and Petros P. Sfikakis¹

Abstract

Objective. To determine the expression of soluble TNF-like cytokine 1A (sTL1A), a new member of the TNF superfamily, in patients with AS.

Methods. Seventy-five consecutive patients with AS [61 males, mean (s.d.) age: 47.2 (15.5) years, disease duration: 20.3 (13.9) years] were included in this study. Forty-four patients were anti-TNF treatment naïve, whereas the remaining patients were on infliximab (n=21), adalimumab (n=3) or etanercept (n=7). The patients’ perceived disease activity was recorded by BASDAI and AS DAS using serum CRP levels (ASDAS-CRP), whereas functional status was assessed by BASFI and measurements of spinal mobility (AS Metrology). Serum concentrations of TL1A were measured by ELISA. Twenty-five age- and sex-matched healthy individuals served as controls.

Results. Anti-TNF treatment-naïve patients demonstrated a 2.6-fold higher sTL1A average value [mean (s.e.m.) 581 (157.5) pg/ml] compared with healthy controls [226.7 (48.24) pg/ml, P=0.042]. The sTL1A levels of anti-TNF-treated patients [178 (42)] were significantly lower than anti-TNF treatment-naïve patients (3.3-fold decrease, P=0.0038) and comparable to those of healthy controls. No significant association was found between sTL1A level and functional status (BASFI score, AS Metrology parameters) or CRP measured in the same sera; however, a positive correlation was observed between individual levels of sTL1A and both BASDAI (P=0.008) and ASDAS-CRP (P=0.058) scores suggesting that sTL1A levels may reflect disease activity in patients with AS.

Conclusion. TL1A is up-regulated in AS, associates with disease activity and is influenced by anti-TNF treatment, suggesting that TL1A may be of pathogenic and potentially of therapeutic importance in AS patients.

Key words: TNF, TL1A, ankylosing spondylitis, anti-TNF therapy.

Introduction

The superfamilies of TNF-related proteins and their corresponding receptors (TNFSF and TNFRSF, respectively) are critically involved in the pathogenesis of AS [1]. Expression studies have shown that SI joint inflammation is associated with elevated levels of TNF [2]. More importantly, it is well known that treatment with anti-TNF agents leads to substantial clinical improvement in AS patients, providing direct evidence for the pivotal role of TNF in the chronic inflammation that characterizes AS [3].

In addition to TNF, other members of the TNF and TNF receptor (TNFR) superfamilies (TNFSF and TNFRSF) have also been implicated in chronic immune-mediated bone and joint inflammation [4]. Among them, the recently identified member of the TNF superfamily TNF-like cytokine 1A (TL1A; e.g. TNFSF15) and its functional receptor, death-domain receptor 3 (DR3, e.g. TNFRSF25) is of particular interest. Both TL1A and DR3 are expressed on immune cells, providing co-stimulatory signals that amplify inflammatory responses through increased cell proliferation, enhanced secretion of pro-inflammatory cytokines and regulation of apoptosis [5]. Recent studies...
have provided considerable evidence for the participation of these proteins in the pathogenesis of joint inflammation in animals with experimental arthritis, as well as in patients with RA [6–8].

Interestingly, TL1A and DR3 are also up-regulated in IBD and psoriasis [9, 10]. Both these conditions are frequently associated with the development of axial and peripheral joint inflammation present in the group of seronegative SpAs. The expression of TL1A in AS, the prototype of seronegative SpAs [11], has not been studied so far. Based on the previous associations, we hypothesized that patients with AS may have increased serum concentration of soluble (s)TL1A. In the present study, we examined whether TL1A is up-regulated in AS and we evaluated possible correlations between sTL1A concentration and various disease parameters, including treatment with anti-TNF agents.

Materials and methods

Seventy-five consecutive patients with AS (61 men) were included in this cross-sectional study. The mean (s.d.) age of the patients was 47.2 (15.5) years (range 18–78 years) and the mean (s.d.) disease duration was 20.3 (13.9) years (range 2–49 years). Thirty-one patients were treated with anti-TNF agents at the time of the study (21 with infliximab, 7 with etanercept and 3 with adalimumab) (anti-TNF-treated group). The remaining 44 patients have never received anti-TNF agents (anti-TNF treatment-naïve group). Twenty-five age- and sex-matched healthy individuals served as healthy controls.

Disease activity was assessed using the self-reported questionnaire BASDAI and the AS DAS using serum CRP levels (ASDAS-CRP). The patient’s perceived functional limitation was recorded by the BASFI. The objective functional status was evaluated by measurement of spinal mobility (AS Metrology): cervical rotation and flexion, occiput-to-wall distance, chest expansion, modified Scho¨ber test and lateral spinal flexion. Serum concentrations of TL1A were measured by ELISA as described in detail elsewhere [7]. The study was approved by Laikon Hospital Ethics Committee and all subjects gave informed consent.

Statistical analysis

Mann–Whitney two-sample statistics was used for group comparisons. Spearman’s coefficients were used to test for correlation between variables. An α-level of <0.05 was considered significant.

Results

Baseline characteristics of patients with AS, including a subgroup analysis of the anti-TNF treatment-naïve and anti-TNF-treated individuals, are shown in Table 1. Patients treated with anti-TNF agents demonstrated significantly lower CRP serum levels, as well as lower BASDAI, ASDAS-CRP and BASFI scores, findings that are compatible with suppressed inflammatory activity in this group. Along this line, active disease (BASDAI ≥ 4) was evident in 31 of 44 anti-TNF treatment-naïve patients vs only 3 of the 31 patients receiving anti-TNF treatment.

Measurement of sTL1A protein showed no significant difference between patients with AS and controls (Fig. 1). Nevertheless, subgroup analysis revealed that anti-TNF treatment-naïve patients demonstrated a 2.6-fold higher sTL1A average value [mean (s.e.m.) 581 (157.5) pg/ml] compared with healthy controls [226.7 (48.24) pg/ml, P = 0.042]. Interestingly, anti-TNF-treated patients had sTL1A levels [178 (42)] that were significantly lower as compared with the anti-TNF treatment-naïve group (3.3-fold decrease, P = 0.0038), and comparable to those of healthy controls. Moreover, patients with BASDAI ≥ 4 demonstrated a 3.4-fold higher sTL1A levels [mean (s.e.m.) 676 (201) pg/ml] compared with the remaining patients [197 (33) pg/ml, P = 0.02]. Patient gender, HLA-B27 status or the level of response to anti-TNF treatment, either by BASDAI or ASDAS-CRP, did not correlate with sTL1A levels (data not shown).

Table 1 Characteristics of AS patients and healthy controls [mean values (s.d.)]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy controls (n = 25)</th>
<th>All patients (n = 75)</th>
<th>Anti-TNF naïve (n = 44)</th>
<th>Anti-TNF treated (n = 31)</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>46 (13.3)</td>
<td>47.2 (15.5)</td>
<td>46.5 (15.5)</td>
<td>48.2 (15.6)</td>
<td>0.6</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>20.3 (13.9)</td>
<td>19.9 (14.9)</td>
<td>19.4 (14.9)</td>
<td>20.8 (12.5)</td>
<td>0.47</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>2.7 (0.9)</td>
<td>13.4 (16.3)</td>
<td>17.5 (19.8)</td>
<td>7.9 (6.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>BASDAI score</td>
<td>3.48 (2.2)</td>
<td>4.6 (1.9)</td>
<td>4.8 (1.9)</td>
<td>1.8 (1.6)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>ASDAS-CRP</td>
<td>2.74 (1.2)</td>
<td>3.38 (1.1)</td>
<td>3.38 (1.1)</td>
<td>1.8 (0.9)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>BASFI score</td>
<td>3.48 (2.2)</td>
<td>4.0 (2.1)</td>
<td>4.0 (2.1)</td>
<td>2.8 (2.2)</td>
<td>0.008</td>
</tr>
<tr>
<td>Chest expansion, cm</td>
<td>3.2 (1.8)</td>
<td>3.2 (1.6)</td>
<td>3.2 (1.6)</td>
<td>3.3 (2.1)</td>
<td>0.9</td>
</tr>
<tr>
<td>Schöber test, cm</td>
<td>2.9 (1.9)</td>
<td>3.0 (1.9)</td>
<td>3.0 (1.9)</td>
<td>3.0 (1.9)</td>
<td>0.87</td>
</tr>
<tr>
<td>Occiput to wall distance, cm</td>
<td>7.7 (8.3)</td>
<td>7.9 (7.8)</td>
<td>7.9 (7.8)</td>
<td>7.5 (9.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Cervical rotation, in degrees</td>
<td>56.7 (30.7)</td>
<td>57.5 (31.2)</td>
<td>57.5 (31.2)</td>
<td>55.7 (30.4)</td>
<td>0.7</td>
</tr>
<tr>
<td>Cervical flexion, in degrees</td>
<td>26.2 (28.2)</td>
<td>27.4 (30.6)</td>
<td>27.4 (30.6)</td>
<td>24.7 (25.5)</td>
<td>0.9</td>
</tr>
<tr>
<td>Lateral spinal flexion, cm</td>
<td>9.5 (7.4)</td>
<td>9.5 (7.2)</td>
<td>9.5 (7.2)</td>
<td>9.6 (7.6)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

aComparison between anti-TNF-naïve and anti-TNF-treated groups, Mann–Whitney test.
Data are presented as mean (S.E.M.) values. Therefore, reduction of circulating TL1A following anti-TNF treatment may reflect a suppressed inflammatory activity in this group of AS patients. Previous in vitro experiments have showed that TNF directly stimulates TL1A expression [6]; thus up-regulation of TL1A in AS may in fact be driven by TNF. Several recent studies have shown that interaction between TL1A and its functional receptor DR3 modifies immune responses in multiple ways [13]. Involvement of these molecules in the pathogenesis not only of RA [7, 13] but also of IBD and psoriasis has been proposed [9, 14]. As the latter conditions belong to the spectrum of seronegative SpAs [15], it is of particular interest that in our present study we report increased expression of TL1A in AS. Although the exact pathophysiological mechanisms remain to be elucidated, the high incidence of concurrent IBD, arthritis and/or psoriasis in the spectrum of SpAs along with increased TL1A expression in all cases, raises the possibility that TL1A may be a common denominator of gut, joint and skin inflammation. Along this line, transgenic mice that overexpress TL1A develop chronic intestinal inflammation that in a minority of animals is associated with skin lesions and arthritis [14, 16]. Further studies including parallel data on the expression of pro-inflammatory cytokines such as IL-6 or TNF would help to interpret the clinical significance of sTL1A levels in AS and further explore its potential use as a possible therapeutic target.

In conclusion, TL1A is up-regulated in AS, associates with disease activity and is clearly influenced by anti-TNF treatment, suggesting that TL1A may be of pathogenic importance in AS.

Concentration of sTL1A protein was measured by ELISA in healthy controls (n = 25), anti-TNF treatment-naive (n = 44) and anti-TNF-treated (n = 31) patients with AS. Data are presented as mean (s.e.m.) values. P-values denote differences using Mann-Whitney two-sample statistics HC: healthy controls; NS: not significant.

We further investigated whether sTL1A concentration correlated with established disease indices for AS. No significant association was found between sTL1A levels and functional status parameters such as BASFI score (Spearman’s r = 0.1, P = 0.38) and AS Metrology (P > 0.05, for all the above-mentioned measurements of spinal mobility) or CRP measurement in the same sera (Spearman’s r = −0.08, P = 0.47). On the other hand, a positive correlation was observed between individual levels of sTL1A and both the corresponding BASDAI (Spearman’s r = 0.3, P = 0.008) and ASDAS-CRP (Spearman’s r = 0.22, P = 0.058) scores. These results indicate that sTL1A levels may reflect disease activity in our population of AS patients.

**Discussion**

Our results have shown that serum levels of TL1A may correlate with disease activity in AS. First, anti-TNF treatment-naïve patients with AS had increased sTL1A concentrations in comparison with matched healthy controls. Secondly, patients on anti-TNF treatment had TL1A concentrations comparable to levels of healthy controls. Thirdly, individual levels of sTL1A correlated positively with both BASDAI and ASDAS-CRP scores, which represent reliable indicators of disease activity [11, 12]. Therefore, reduction of circulating TL1A following anti-TNF treatment may reflect a suppressed inflammatory activity in this group of AS patients. Previous *in vitro* experiments have showed that TNF directly stimulates TL1A expression [6]; thus up-regulation of TL1A in AS may in fact be driven by TNF.


