Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis

TALABOT FRISCHKNECHT-AYER, Dominique, et al.

Abstract

OBJECTIVES: Recent evidence suggests a role for interleukin (IL)-33 and its receptor ST2 in arthritis. In this study, we quantified IL-33 and soluble (s)ST2 levels in serum and synovial fluid (SF), and assessed synovial IL-33 expression levels and pattern in patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), or osteoarthritis (OA).

METHODS: Serum and SF IL-33 and sST2 levels were assessed by ELISA. IL-33 mRNA was quantified by RT-qPCR. Synovial IL-33 protein expression pattern was examined by immunohistochemistry.

RESULTS: Serum and SF IL-33 levels tended to be higher in RA than in OA patients. In contrast to RA, IL-33 was not detectable in PsA serum and SF. Serum sST2 levels were higher in RA than in OA. There was a wide variation of synovial tissue IL-33 mRNA expression within each disease group and IL-33 mRNA levels were not significantly different between the groups. A similar IL-33 protein expression pattern was observed in RA, PsA and OA synovium, with strong nuclear expression of IL-33 in endothelial cells and, in a subset of RA, PsA and OA patients, in cells morphologically consistent with [...]
Distinct serum and synovial fluid interleukin (IL-33) levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis

Dominique Talabot-Ayer\textsuperscript{a,b}, Thomas McKeec, Patricia Gindre\textsuperscript{c}, Sylvette Bas\textsuperscript{a}, Dominique L. Baetend, Cem Gabaya\textsuperscript{a,b}, Gaby Palmera,b,*

\textsuperscript{a} Division of Rheumatology, University Hospital, Geneva, Switzerland
\textsuperscript{b} Department of Pathology and Immunology, center medical university, University of Geneva School of Medicine, 1, rue Michel-Servet, 1211 Geneva 4, Switzerland
\textsuperscript{c} Division of Clinical Pathology, University Hospital, Geneva, Switzerland
\textsuperscript{d} Department of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Abstract

Objectives: Recent evidence suggests a role for interleukin (IL-33) and its receptor ST2 in arthritis. In this study, we quantified IL-33 and soluble (s)ST2 levels in serum and synovial fluid (SF), and assessed synovial IL-33 expression levels and pattern in patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), or osteoarthritis (OA).

Methods: Serum and SF IL-33 and sST2 levels were assessed by ELISA. IL-33 mRNA was quantified by RT-qPCR. Synovial IL-33 protein expression pattern was examined by immunohistochemistry.

Results: Serum and SF IL-33 levels tended to be higher in RA than in OA patients. In contrast to RA, IL-33 was not detectable in PsA serum and SF. Serum sST2 levels were higher in RA than in OA. There was a wide variation of synovial tissue IL-33 mRNA expression within each disease group and IL-33 mRNA levels were not significantly different between the groups. A similar IL-33 protein expression pattern was observed in RA, PsA and OA synovium, with strong nuclear expression of IL-33 in endothelial cells and, in a subset of RA, PsA and OA patients, in cells morphologically consistent with synovial fibroblasts.

Discussion/Conclusions: This study confirms increased circulating IL-33 levels in RA. In addition, we report that IL-33 is undetectable in the serum or SF of PsA patients. Local expression of IL-33 in the synovium was observed at similar variable levels in RA, PsA and OA, suggesting that inflamed joints do not represent the primary source of elevated serum and SF levels of IL-33 in RA.

© 2011 Published by Elsevier Masson SAS on behalf of the Société Française de Rhumatologie.

1. Introduction

Interleukin (IL)-33 (or IL-1F11) is the most recently discovered member of the IL-1 cytokine family, which was identified as a ligand for the T1/ST2 receptor (ST2; also called ST2L, IL-33Rα, or IL-1RL1) [1]. IL-33 is constitutively expressed in the nucleus of endothelial and epithelial cells [2,3]. In addition, IL-33 expression is induced in both resident and infiltrating cells in inflamed tissues [4–8]. Like IL-1α, IL-33 appears to act as a dual function cytokine with both nuclear and extracellular effects [1,9].

Extracellular effects of IL-33 are mediated by binding to ST2 with subsequent recruitment of IL-1R accessory protein (IL-1RACP), leading to the activation of NF-κB and MAPK pathways and signaling similar to that induced by IL-1 [1,10,11]. ST2 is expressed on many different cells, mostly of hematopoietic origin, where IL-33 induces production of cytokines and chemokines, cell activation or chemotaxis [12–18]. Like other members of the IL-1 receptor family, ST2 also exists as a soluble form (sST2), generated by alternative mRNA splicing, which acts as a decoy receptor to inhibit IL-33 signaling [19]. Elevated serum concentrations of sST2 have been reported in patients suffering from various disorders, including systemic lupus erythematosus, atopic dermatitis, asthma, trauma, septic shock and myocardial infarction [20–23].

Over the last years, several studies suggested that IL-33 and ST2 are involved in the inflammatory process that leads to arthritis. Indeed, IL-33 and ST2 are expressed in human rheumatoid arthritis (RA) synovium [2,5,24]. IL-33 expression was observed in endothelial cells, but also in CD68+ inflammatory cells and in fibroblasts in RA synovial tissue. In vitro, expression of IL-33 is induced by pro-inflammatory stimuli in cultured human synovial fibroblasts [5,24–26]. In mice, several studies suggested a pro-inflammatory effect of IL-33 via ST2 signaling in different models of experimental arthritis [5,24,27–29]. Finally, in vitro data suggested a role for fibroblast-derived IL-33 in the induction of mast cell tryptase expression [26]. Taken together, these studies led to the hypothesis that release of locally produced IL-33, for instance by synovial...
fibroblasts, would contribute to the inflammatory process in the joint by activating mast cells or neutrophils [24,29]. Recently, two studies reported elevated IL-33 levels in serum and synovial fluid (SF) of RA, as compared to osteoarthritis (OA) patients [25,30]. However, to date, no information is available concerning IL-33 levels in inflammatory joint diseases other than RA. In addition, no study examined serum or SF sST2 and IL-33 levels in parallel. Finally, nothing is known concerning potential differences in IL-33 expression levels or pattern in synovial tissue in RA, as compared to other types of arthritis.

In the present study, we investigated IL-33 and sST2 levels in serum and SF in psoriatic arthritis (PsA), as compared to RA and OA samples. Histopathological features of PsA include the modification of synovial vascular morphology and angiogenesis [31]. In view of the well-described expression of IL-33 in endothelial cells, it seemed particularly interesting to evaluate IL-33 expression in PsA. Herein, we report elevated levels of IL-33 in the serum of RA patients. Interestingly and rather unexpectedly, IL-33 was not detectable in PsA serum or SF. IL-33 mRNA expression levels and protein expression patterns were similar in RA, PsA and OA synovium.

2. Methods

2.1. Patients and samples

Serum and SF were obtained from RA, PsA, and OA patients, who fulfilled appropriate classification criteria [32–34]. For RNA and protein expression studies, synovial tissue samples were obtained from RA, PsA, and OA patients either by needle arthroscopy of a swollen joint or during joint replacement surgery [35]. Synovial tissue was snap frozen for mRNA extraction or fixed in formaldehyde and mounted in paraffin for subsequent immunohistochemistry. Patient characteristics are summarized in Table 1. All study subjects gave written informed consent, as approved by the local Medical Ethics Committees.

2.2. Determination of serum and SF IL-33, sST2 and IL-6 concentrations by ELISA

IL-33, sST2 and IL-6 levels were assessed by ELISA in serum and SF of patients with RA (serum, n = 11; SF, n = 10), PsA (serum, n = 9; SF, n = 9) and OA (serum, n = 9; SF, n = 7) using commercial DuoSet detection systems (R&D Systems, Abingdon, UK) according to the manufacturer’s instructions. Serum and SF samples were diluted (1:2 for IL-33; 1:2 and 1:50 for sST2; 1:2 for IL-6 in serum; 1:10 and 1:100 for IL-6 in SF) in sample dilution buffer. Since SF was found to affect detection of recombinant IL-33 and sST2, standard curves for determinations in SF were prepared in IL-33 and sST2 negative SF, diluted 1:2 with sample dilution buffer. Potential interference of rheumatoid factors (RF) with ELISA results was assessed by spiking of recombinant IL-33 or sST2 into RF positive and negative sera and by addition of purified RF to IL-33 or sST2 positive sera or to recombinant IL-33 or sST2 diluted in serum or in sample dilution buffer. No interference of RF was observed in any of these tests. To assess potential interference of sST2 with IL-33 detection, recombinant sST2 (10 ng/ml) was added to IL-33 positive serum or SF or to recombinant IL-33 diluted in sample dilution buffer. The addition of sST2 did not affect detection of IL-33. Finally, to exclude the presence of a potential inhibitory factor, which might interfere with detection of IL-33 in PsA samples, we verified correct detection of recombinant IL-33 spiked into PsA serum.

2.3. Determination of IL-33, ST2L and sST2 mRNA levels in synovial tissue by RT-qPCR

Expression of mRNA encoding IL-33, the long signaling form of ST2L (ST2L), and sST2 was quantified by RT-qPCR in synovial biopsies obtained from patients with RA (n = 10), PsA (n = 10), and OA (n = 8). Total RNA was extracted from 100 mg of synovial tissues by Trizol (Invitrogen AG, Basel, Switzerland) extraction according to manufacturer’s instructions. Total RNA (500 ng) was reverse-transcribed using the Superscript II reverse transcriptase (Invitrogen). IL-33, ST2L, sST2 and GAPDH mRNA levels were examined by quantitative PCR using the iCycler iQ Real time PCR Detection System (Bio-Rad Laboratories AG, Reinch, Switzerland) and a SYBR Green master mix (Eurogentec, Seraing, Belgium). IL-33, ST2L and sST2 mRNA levels were determined by a standard curve method and normalized for GAPDH levels. Primer sequences were as follows:

- IL-33 forward 5′-GGGCTTTACTGAGTATCTAGG-3′;
- IL-33 reverse 5′-GGACCTCACCAGAGGTGTCTTG-3′;
- ST2L forward 5′-TGGGGGCACTGGTCTAGAACCA-3′;
- ST2L reverse 5′-AGGGCCACAGTGTTCAAGG-3′;
- sST2 forward 5′-AGGCTTTCATCCTGGTCA-3′;
- sST2 reverse 5′-CACTGACACAAGGACTTGTCATAATGG-3′;
- GAPDH forward 5′-TGAACACCAACTGCTAGAGC-3′;
- GAPDH reverse 5′-GCCATGGACTGTTCTAGAGC-3′.

The absence of DNA contamination in RNA preparations was tested by including RNA samples that had not been reverse-transcribed.

2.4. Immunohistochemistry

IL-33 protein expression pattern was examined by IHC in the synovial tissue from patients with RA ($n=8$), PsA ($n=7$) and OA ($n=4$) using a polyclonal goat anti–IL-33 antibody (AF3625, R&D Systems). Formalin-fixed, paraffin-embedded sections were deparaffinized and antigen retrieval was performed in a pressure chamber (Pascal; Dako, Baar, Switzerland) in EDTA buffer (pH 7.0) at 125°C for 30 sec. Slides were blocked for endogenous peroxidase activity and incubated with anti–IL-33 antibody (4 µg/ml final concentration) in TBST (50 mM Tris pH 7.6, 150 mM NaCl, 0.05% Tween 20) containing 1% BSA for 1 h at RT. Subsequently, slides were incubated with anti-goat IgG-biotin conjugate and streptavidin–HRP (Dako) in TBST and developed with diaminobenzidine. Specificity of staining was assessed by preincubation of the antibody with 2 µg/ml of recombinant human IL-33 (Enzo Life Sciences AG, Lausen, Switzerland) for 1 h at 37°C before addition to the slides.

2.5. Statistical analysis

The significance of differences was assessed by ANOVA, followed by Bonferroni’s multiple comparisons test for normally distributed values. Data with non-Gaussian distribution were analyzed by Kruskal-Wallis test, followed by Dunn’s multiple comparisons test. Paired samples were analyzed using the Wilcoxon matched pairs test. A difference between groups was considered significant if $P<0.05$. Spearman’s rank correlation coefficient was used to assess relationships between two variables. Correlation was considered significant if $P<0.05$.

3. Results

3.1. IL-33 and sST2 levels in serum and SF of RA, PsA and OA patients

IL-33 and sST2 levels were assessed in the serum and SF of patients with RA, PsA and OA by ELISA (Fig. 1). Serum and SF levels of IL-33 tended to be higher in RA than OA patients (Fig. 1A). In matched serum–SF samples of RA patients, we observed comparable concentrations of IL-33 in the serum and SF (Fig. 2), although levels tended to be higher in SF. In contrast to RA, IL-33 was not detectable in any of the PsA serum and SF samples (Fig. 1A). In addition, we analyzed two matched serum and SF samples obtained from patients with nonpsoriatic spondyloarthritis, in which IL-33 was also undetectable.

The levels of sST2 were higher in RA than in OA serum, while SF sST2 levels were not different between the groups (Fig. 1B). In matched serum and SF samples, sST2 levels were weakly correlated ($r^2=0.255$, $P=0.0119$). In contrast, there was no correlation between IL-33 and sST2 levels in serum or SF samples (data not shown). SF IL-33 or sST2 levels were also not correlated to SF leukocyte counts (data not shown).

Finally, we measured serum and SF IL-6 concentrations to evaluate the inflammatory status of RA and PsA patients. As expected, we detected elevated serum levels of IL-6 in a subset of RA and PsA, but not OA patients. Similarly, high levels of SF IL-6 ($\geq 15$ ng/ml) were detected exclusively in RA and PsA patients (Fig. 1C). There was no correlation between IL-33 or sST2 levels and IL-6 concentrations in serum or SF (data not shown). Values for serum and SF IL-33, sST2 and IL-6 levels for each individual patient are indicated in Table 2.

3.2. Expression of IL-33 mRNA and protein in RA, PsA and OA synovium

We examined expression of IL-33 mRNA by RT-qPCR in synovial tissue obtained from RA, PsA, and OA patients (Fig. 3). We observed a wide variation of synovial tissue IL-33 mRNA expression levels within each disease group and IL-33 mRNA levels were not significantly different between the groups. In contrast, HLA-DR mRNA
no significant differences were observed between the groups.

Finally, we investigated the expression pattern of IL-33 protein by IHC in RA, PsA and OA synovium (Fig. 4). In all pathologies, we consistently detected strong nuclear IL-33 immunoreactivity in endothelial cells. These observations are in line with our previous report describing IL-33 expression in synovial endothelial cells, which were identified by co-staining with anti-CD34 and D2-40 antibodies, in order to label both vascular and lymphatic endothelium [5]. In addition, IL-33 expression was also observed in the nucleus of cells morphologically consistent with synovial fibroblasts in a subset of synovial tissues (RA, n = 2/8; PsA, n = 3/7; OA, n = 3/4). Local expression of IL-33 mRNA and protein in the synovium was thus observed at similar variable levels and with a similar pattern in RA, PsA and OA.

4. Discussion

In the present study, we investigated the expression of IL-33 and ST2 in different types of arthritis. Serum and SF IL-33 levels tended to be higher in RA than in OA patients, although, with the relatively low number of samples analyzed in the present study, these differences did not reach statistical significance. Our data thus confirm two previous studies reporting elevated serum and SF IL-33 in RA [25,30]. In this study, we observed comparable IL-33 concentrations in RA serum and SF, although IL-33 levels tended to be slightly higher in SF. In two previous reports [30], IL-33 levels were reported to be either comparable or higher in RA SF as compared to serum samples [25,30].

In order to evaluate local IL-33 production in the joint, we investigated IL-33 mRNA and protein expression in synovial tissue. IL-33 mRNA expression levels in the synovium displayed important intersample variability, but were not different in RA, PsA or OA tissues. Similar expression patterns were also observed for IL-33 protein in RA, PsA and OA synovium, with strong nuclear expression of IL-33 in endothelial cells. In addition, variable IL-33 expression was observed in synovial fibroblasts in RA, PsA and OA synovium.


Table 2
Serum and SF IL-33, sST2 and IL-6 levels for individual patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>IL-33 (ng/ml)</th>
<th>sST2 (ng/ml)</th>
<th>IL-6 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>SF</td>
<td>Serum</td>
</tr>
<tr>
<td>RA1</td>
<td>nd</td>
<td>nd</td>
<td>0.374</td>
</tr>
<tr>
<td>RA2</td>
<td>nd</td>
<td>nd</td>
<td>1.09</td>
</tr>
<tr>
<td>RA3</td>
<td>0.858</td>
<td>13.288</td>
<td>0.796</td>
</tr>
<tr>
<td>RA4</td>
<td>0.07</td>
<td>0.516</td>
<td>1.34</td>
</tr>
<tr>
<td>RA5</td>
<td>0.114</td>
<td>nd</td>
<td>0.392</td>
</tr>
<tr>
<td>RA6</td>
<td>0.262</td>
<td>0.478</td>
<td>nd</td>
</tr>
<tr>
<td>RA7</td>
<td>0.616</td>
<td>–</td>
<td>0.558</td>
</tr>
<tr>
<td>RA8</td>
<td>nd</td>
<td>nd</td>
<td>0.14</td>
</tr>
<tr>
<td>RA9</td>
<td>0.39</td>
<td>1.224</td>
<td>61.6</td>
</tr>
<tr>
<td>RA10</td>
<td>0.166</td>
<td>0.184</td>
<td>0.274</td>
</tr>
<tr>
<td>RA11</td>
<td>nd</td>
<td>nd</td>
<td>2.872</td>
</tr>
<tr>
<td>PsA1</td>
<td>nd</td>
<td>nd</td>
<td>0.392</td>
</tr>
<tr>
<td>PsA2</td>
<td>nd</td>
<td>nd</td>
<td>0.392</td>
</tr>
<tr>
<td>PsA3</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>PsA4</td>
<td>nd</td>
<td>nd</td>
<td>0.092</td>
</tr>
<tr>
<td>PsA5</td>
<td>nd</td>
<td>nd</td>
<td>0.866</td>
</tr>
<tr>
<td>PsA6</td>
<td>nd</td>
<td>nd</td>
<td>0.47</td>
</tr>
<tr>
<td>PsA7</td>
<td>nd</td>
<td>nd</td>
<td>0.358</td>
</tr>
<tr>
<td>PsA8</td>
<td>nd</td>
<td>nd</td>
<td>0.732</td>
</tr>
<tr>
<td>PsA9</td>
<td>nd</td>
<td>nd</td>
<td>1.54</td>
</tr>
<tr>
<td>OA1</td>
<td>0.056</td>
<td>0.038</td>
<td>1.256</td>
</tr>
<tr>
<td>OA2</td>
<td>nd</td>
<td>nd</td>
<td>0.072</td>
</tr>
<tr>
<td>OA3</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>OA4</td>
<td>nd</td>
<td>nd</td>
<td>0.23</td>
</tr>
<tr>
<td>OA5</td>
<td>nd</td>
<td>nd</td>
<td>0.994</td>
</tr>
<tr>
<td>OA6</td>
<td>nd</td>
<td>–</td>
<td>0.12</td>
</tr>
<tr>
<td>OA7</td>
<td>nd</td>
<td>–</td>
<td>0.15</td>
</tr>
<tr>
<td>OA8</td>
<td>0.168</td>
<td>–</td>
<td>nd</td>
</tr>
<tr>
<td>OA9</td>
<td>nd</td>
<td>–</td>
<td>0.0</td>
</tr>
<tr>
<td>OA10</td>
<td>–</td>
<td>–</td>
<td>1.706</td>
</tr>
<tr>
<td>OA11</td>
<td>–</td>
<td>nd</td>
<td>0.662</td>
</tr>
</tbody>
</table>

nd: not detectable; –: no sample available.

Fig. 3. IL-33 mRNA expression was quantified by RT-qPCR on total RNA extracted from synovial tissues obtained from patients with RA (squares; n = 10), PsA (triangles; n = 10) and OA (inverted triangles; n = 8). The amount of GAPDH mRNA was monitored as an internal control and IL-33 mRNA levels were corrected for GAPDH expression. Results are shown as individual values (symbols) and means (lines) for each group of patients. No significant differences were observed between the groups.
OA patients. These observations confirm previous descriptions of IL-33 expression in endothelial cells and synovial fibroblasts in RA [2,5,24]. Interestingly, nuclear expression of IL-33 in activated fibroblasts was also described recently in ulcerative colitis lesions, and proposed to be associated with wound-healing, suggesting that induction of IL-33 expression in fibroblasts might be commonly observed in inflamed or damaged tissues [8].

In contrast to RA, and rather unexpectedly, IL-33 was not detectable in PsA serum or SF. Nevertheless, IL-33 mRNA and protein expression in the synovium was similar in PsA and in RA or OA samples, indicating that circulating and SF IL-33 levels are not correlated to local IL-33 expression in the joint. These observations further suggest that the inflamed synovial tissue does not represent the primary source of elevated serum and SF levels of IL-33 in RA, which remains to be identified. The difference in circulating IL-33 levels in RA and PsA might thus point to differences in disease mechanism involved in the pathogenesis of these arthropathies. The presence of elevated levels of IL-6 in serum and SF indicate that the variations of IL-33 levels in RA and PsA cannot be explained by differences in the magnitude of the inflammatory process. However, as endothelial cells constitutively express IL-33, one possible hypothesis could be that in the case of autoimmune disorders such as RA, but not in PsA, the presence of immune complex deposits on endothelial cells could activate the release of IL-33.

Serum levels of sST2 were higher in RA than in OA, while SF sST2 levels were similar in RA, PsA and OA. Although both IL-33 and sST2 were elevated in RA serum, we could not detect any correlation between circulating IL-33 and sST2 levels. Finally, comparable concentrations of sST2 were found in the serum and SF and local sST2 mRNA expression was undetectable in synovial tissue samples, arguing against a contribution of synovial tissue-derived sST2 to circulating sST2 levels. Of note, IL-6 levels were much higher in the SF than in the serum, indicating that in contrast to IL-33 and sST2, inflamed joints constitute the primary source of circulating IL-6 in arthritis.

In conclusion, this study confirms increased circulating IL-33 levels in RA. In addition, we report that IL-33 is undetectable in the serum of PsA patients. Local expression of IL-33 in the synovium was observed at similar variable levels in RA, PsA and OA, suggesting that the inflamed rheumatoid synovium does not represent the primary source of elevated serum and SF levels of IL-33 in RA.

**Disclosure of interest**

None of the authors has any conflicts of interest to declare.

**Acknowledgements**

We would like to thank Jean-Marc Walburger and Axel Finckh for helpful discussions. This work was supported by grants from the Swiss National Science Foundation (320000–120319 to GP, 320000–119728 to CG), the Rheumasearch Foundation and the Loterie Romandie.

**References**


Palmer G, Lipsky BP, Smithgall MD, et al. The IL-1 receptor accessory protein (AcP) is required for IL-33 signaling and soluble AcP enhances the ability of soluble ST2 to inhibit IL-33. Cytokine 2008;42:358–64.


