Interleukin-32 a Proinflammatory Cytokine in Rheumatoid Arthritis

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Abstract

Objective: To investigate the expression of Interleukin (IL)-32 in the synovial tissue of patients with rheumatoid arthritis (RA) and to determine whether the expression of IL-32 is related to that of other proinflammatory cytokines, specifically tumor necrosis factor (TNF) α and IL-18, and to the degree of joint inflammation.

Methods: Biopsy specimens of knee synovial tissue either without synovitis or with moderate or severe synovitis (n=5, 4, and 13, respectively) were obtained from 22 patients with active RA. Paraffin-embedded, snap-frozen sections were used for immuno-histochemical detection of IL-32. TNF-α was measured in the serum (S) using RIA technique, while IL-18 was measured in both the S and synovial fluid (SF) by ELISA technique.

Results: IL-32 staining was detectable in 86.4% of the RA patients, in both the lining and sub lining of the knee synovial tissue. IL-32 expression in the lining highly correlated with microscopic inflammation scores, levels of S TNF-α, levels of S and SF IL-18 (p<0.0001) and also with the acute-phase reaction as measured by the erythrocyte sedimentation rate and C-reactive protein. Both TNF-α and IL-18 were higher in RA patients than in controls.

Conclusion: Our results show that the expression of IL-32 is associated with local inflammation in the synovial tissue of patients with RA, and it correlates with both the inflammatory cytokines and acute-phase response. These data indicate that IL-32 is a proinflammatory cytokine in RA.

Key Words: IL-32 – IL-18 – TNF-α – RA – Synovial tissue – Cytokines.

Introduction

RA is a persistent inflammatory arthritis and is known to be an autoimmune disease. Inflammation of the joints results in the destruction of cartilage and bone early in the course of the disease. Although the pathogenesis of RA is still unclear and may be heterogeneous, several proinflammatory cytokines participate in promoting the inflammation of the joints.

Since the development of TNF-α/IL-1/IL-6 anti-rheumatic biologics, several additional cytokines with pro-inflammatory activity as well as their receptors, which initiate upstream pathophysiologic effects, have been identified. In several cases, these cytokines have been cloned and sequenced. Thus, cytokines, such as IL-7, IL-12/IL-23, IL-15, IL-16, IL-17/IL-18, IL-19/IL-20/IL-22 and IL-32, have been implicated in arthritis development in animal models of RA and measurements in synovial tissue and synovial fluid obtained from patients with RA also suggested a role for them in RA disease progression, if not in its pathogenesis [1,2].

Human IL-32 has been reported as a novel cytokine [3,4]. IL-32 is produced mainly by T-, natural killer, epithelial cells and monocytes after stimulation by Interleukin-2, Interleukin-18 or IFN-γ Mabilleau and Sabokbar [5] and was formerly known as a natural killer cell transcript 4. IL-32 has proinflammatory effects both on myeloid and nonmyeloid cells [6]. After emigration from the bone marrow to the peripheral blood, monocytes enter tissues and differentiate into macrophages, the prototype scavenger of the immune system. IL-32 is a cytokine inducing proinflammatory cytokines and chemokines via p38-MAPK and NF-κB [1,7,8]. IL-32 induces differentiation of human blood monocytes as well as leukemic cells into macrophage-like cells with functional phagocytic activity for live bacteria [7]. IL-32-induced monocyte-to-macrophage differentiation is mediated through nonapoptotic, caspase-3-dependent mechanisms. Thus, IL-32 not only contributes to host responses through the induction of proinflammatory cytokines but also directly affects specific immunity by differentiating monocytes into macrophage-like
IL-32 induces the production of TNF-α, macrophage inflammatory protein (MIP)-2 and IL-8 in monocyte cell lines, indicating that this factor would be involved in the inflammatory responses. However, the biological activities of IL-32 on other cell types remained undetermined [8,9].

The gene encoding IL-32 resides in chromosome 16 p 13.3 and four mRNA transcripts, resulting from mRNA splicing are presently known, IL-32α, IL-32β, IL-32γ and, IL-32δ. IL-32α is present in intracellular locations, and IL-32β is secreted from the cells. IL-32α and IL-32β are thought to be the major expressed variants. The sequences of IL-32β and IL-32γ are quite similar [10]. Goda, et al. [11], demonstrated that there exist two more novel isoforms of IL-32 (ξ and ζ).

IL-32 is expressed in lymphoid tissues, such as the thymus, the spleen, and the intestines. Human natural killer cells increase the secretion of IL-32 by IL-18 and IL-12 stimulation. It was initially observed that T cells were the primary source of IL-32 because human peripheral blood mononuclear cells (PBMCs) which contain mostly T cells, produce IL-32 after stimulation with concanavalin A (Con A). Primary human blood monocytes stimulated with Toll-like receptor ligands synthesize the cytokine [9,12]. After interferon (IFN) -α or IL-1β stimulation, epithelial cell lines also produce IL-32. The fact that IL-32 is prominently induced by IFN-γ and that it stimulates TNF-α and IL-8 production, makes it clear that IL-32 exhibits the properties of a classical proinflammatory mediator [12].

The fact that IL-32 related cytokines, TNF-α and IL-18, show a close relation with arthritis [13] implies that IL-32 has a pathologic role in inflammatory diseases. IL-32 seems to be involved in a variety of diseases such as inflammatory bowel disease, myelodysplastic syndrome and chronic myelomonocytic leukaemia, HIV infection [5,14].

Moreover, it has been reported recently that in a cohort of patient suffering with rheumatoid arthritis, IL-32 was significantly increased in the synovial tissue and that its levels were strongly correlated with the severity of the disease [5,15].

To determine the participation of IL-32 in the inflammatory processes of RA, we investigated the expression of IL-32 in RA synovial tissue from patients with unaffected joints and with moderate or severe arthritis. We examined whether the local expression of IL-32 is related to that of other proinflammatory cytokines, specifically TNF-α and IL-18. Moreover, we assessed the relationship between IL-32 and the acute-phase reaction.

Patients and Methods

Twenty two female patients with RA were recruited from the outpatient clinic of Rheumatology and Rehabilitation department in Ain-Shams University, and were enrolled in this study. All patients met the American College of Rheumatology Criteria for RA [16]. Their mean age was 55.3 ± 15 years (range 40-70 years) and mean disease duration was of 12.2 ± 5.6 years.

The therapeutic regimens of all patients were recorded. Patients receiving prednisolone within 6 weeks or biologic therapies including anti-TNF-α or IL-1 receptor antagonist, before the study were not included.

The disease activity was calculated using a disease activity score (DAS) [17]. All patients were subjected to thorough clinical examination.

Ten age-matched females, who were subjected to knee joint arthroscopy due to traumatic joint lesion, with no history of acute or chronic inflammatory disease, were enrolled as controls. Their mean age was 51.2 ± 13 years (range 46-64 years).

Peripheral venous blood samples (10ml) were obtained under complete aseptic technique from patients and controls. 2ml of citrated blood were used for the determination of erythrocyte sedimentation rate (ESR) using the routine method in our laboratory (Westergren method), the remaining part of the sample was allowed to clot and the serum was separated by centrifugation aliquoted and kept frozen at −20°C till the day of determination of the other variables of the study.

The anti-cyclic citrullinated peptide antibodies (anti-CCP antibodies) was measured by the ELISA quantitative method as a routine in all RA patients, only anti-CCP +ve patients were included in this study, Schellekens, et al. [18].

C-reactive protein (CRP) was measured by the ELISA quantitative method (Chemicon, USA) following the manufacturer recommendations. The Rheumatoid factor (RF) was measured by the semi-quantitative latex testing following the manufacturer recommendations (RF slide, Diesse, Milan, Italy), with the sensitivity of detecting RF levels equal to or more than 15IU/ml. Positive specimens were serially diluted and the highest dilution titer was determined and reported.
Serum (S) and synovial fluid (SF) IL-18 was measured by the R&D ELISA technique (Minneapolis, USA) following the manufacturer recommendations [19].

Serum TNF-α was measured by an immunoradiometric assay developed by Medgenix Diagnostics (Brussels, Belgium). This method uses several monoclonal antibodies directed against distinct epitopes. The capture antibodies are attached to the plastic tubes and the signal antibody is labeled with 125I Leroux, et al. [20].

Percutaneous biopsies of the knee joint were performed with a Parker-Pearson needle after local administration of anesthesia. Before the biopsy, the ESR was measured, and knee joints were scored for the absence (score of 0) or presence of local pain, swelling, or effusion (score of 1, respectively for each of these). The three scores were added, and patients were classified as having no (score 0), moderate (score 1-2), or severe (score 3) knee joint arthritis.

The synovial tissue from controls was isolated during arthroscopic procedures by orthopedic surgeons.

Immunohistochemical analysis of RA synovial tissues:

Tissue samples were immediately fixed with 4% formaldehyde and embedded in paraffin. For staining, we used The DAKO EnVisionTM+ System, Peroxidase (Dako, California, USA). After dewaxing and dehydration, briefly, we first quenched endogenous peroxidase activity by incubating the specimen for 5 minutes with DAKO Peroxidase Block (included as part of the kit). Then the specimen was incubated with primary rabbit anti-IL-32 polyclonal antibody (ProSci, California, USA) or negative control reagent, followed by incubation with the labeled polymer, using two sequential 30-minute incubations. Staining was completed by a 5-10 minute incubation with 3,3’-diaminobenzidine (DAB)+ substrate-chromogen which results in a brown-colored precipitate at the antigen site (IL-32), and counterstained with hematoxylin for 30 seconds [10].

Sections were coded and randomly analyzed by two blinded observers. Inadequate sections, which lacked synovial lining, were left out of the analysis. Staining for IL-32 was semiquantitatively scored on a five-point scale (range 0-4) at X400 magnification; a score of 0 represented no or minimal staining, a score of 1 indicated 10-20% positive cells, a score of 2 indicated 30-40% positive cells, a score of 3 indicated 50-60% positive cells, and a score of 4 represented staining of >60% of the cells [21,22]. An Olympus (Melville, NY) microscope (BX50 model) equipped with a digital camera was used to prepare the microphotographs.

Statistical analysis:

Analysis was performed by using SPSS, version 11.0 Data are expressed as mean ± SD. Within groups comparisons were analyzed by Student’s t-test. Correlations are expressed by using the Spearmans’ rank correlation coefficient. Only p-values <0.05 were considered significant.

Results

Twenty two RA female patients with mean age of 55.3±15 years and with mean disease duration of 12.2±5.6 years were enrolled in this study. The results of lab works showed that 19 patients had positive RF (84.6%). Their mean ESR was 60.3±25mm/h, mean CRP was 8.8±4.7mg/dl and mean DAS was 4.5±1.3.

<table>
<thead>
<tr>
<th>IL-32 staining score</th>
<th>No inflammation (3/5)</th>
<th>Moderate inflammation (3/4)</th>
<th>Severe inflammation (13/13)</th>
<th>Controls (1/10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0 (no or minimum staining)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Score 1 (10-20% staining)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Score 2 (30-40% staining)</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Score 3 (50-60% staining)</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Score 4 (&gt;60% staining)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

IL-32 staining was detectable in 84.6% (19 of the 22 patients) of the synovial biopsies (Table 1). The cytokine is distributed in the lining, sub lining, and endothelial cells. Intense staining was found predominately in the lining layer of the synovium. The cells that were the most positive for IL-32 staining were macrophage-like cells (Figs. 1-4). The percentage of RA patients with IL-32-positive biopsies was lower among the group showing little clinical arthritis than in those with moderate or
severe visual knee inflammation (Graph 1). Only 3 RA patients showed no or minimal staining. On the other hand, only one control showed score 1 staining of the synovial tissue (Table 1). Statistical analysis showed a significant difference regarding the staining pattern between RA patients and controls.

Both the S and SF levels of IL-18 as well as the S level of TNF-α were higher in RA patients than in controls (Graph 2), and the differences were statistically significant in case of IL-18 (p<0.001), however, it was statistically insignificant for TNF-α (p>0.05). IL-18 levels inspite of being higher in the SF than in the S of RA patients, yet the difference was statistically insignificant (p>0.5) (Table 2).

In RA patients having severe synovitis, IL-32 staining score was significantly correlated with both the S and SF levels of IL-18, as well as with the S level of TNF-α. Similar findings were detected in RA patients having moderate synovitis (Table 3).

A significantly high correlation was found between the acute phase reactants, IL-32 staining scores and the clinically detected severity of joint inflammation (p<0.001) (Table 4).

Moreover, the semiquantitative scores for IL-32 in the lining were highly correlated with those for microscopic inflammation on hematoxylin and eosin (H&E) sections, and also with the acute-phase reaction as measured by the ESR.

### Table (2): Comparison between RA patients and controls regarding the mean level of the studied cytokines.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Patients</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-18 (pg/ml)</td>
<td>358.12±210</td>
<td>150.25±87</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Synovial Fluid IL-18 (pg/ml)</td>
<td>416.71±215</td>
<td>165.50±98</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*p = Compared patients to controls. NS: Non significant.
** = Compared SF IL-18 to S IL-18. <0.001: Highly significant.

### Table (3): Relation between the clinical severity of RA patients, IL-32 staining scores (%) and the studied cytokines levels.

<table>
<thead>
<tr>
<th>Clinically detected synovitis</th>
<th>Serum TNF-α</th>
<th>Serum IL-18</th>
<th>Synovial IL-18</th>
<th>IL-32 Staining Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Synovitis (5/22)</td>
<td>Mean±SD</td>
<td>Risk</td>
<td>Risk</td>
<td>3/5</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>758±223</td>
<td>266±89</td>
<td>316±112</td>
<td>(2=1 score, and 1=2 score)</td>
</tr>
<tr>
<td>r=</td>
<td>0.32</td>
<td>0.41</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Moderate Synovitis (4/22)</td>
<td>Mean±SD</td>
<td>Risk</td>
<td>Risk</td>
<td>3/4</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>879±216</td>
<td>358±102</td>
<td>396±164</td>
<td>(1=2 score, and 2=3 score)</td>
</tr>
<tr>
<td>r&lt;</td>
<td>0.62</td>
<td>0.58</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Severe Synovitis (13/22)</td>
<td>Mean±SD</td>
<td>Risk</td>
<td>Risk</td>
<td>13/13</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1095±254</td>
<td>450±145</td>
<td>538±189</td>
<td>(1=1 s, 4=2 s, 5=3 s, and 3=4 s)</td>
</tr>
<tr>
<td>r&lt;</td>
<td>0.75</td>
<td>0.70</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

>0.05 : Is considered non significant.
<0.001: Is considered highly significant.

### Table (4): Relation between the clinical severity of RA patients, IL-32 staining score and the acute phase reactants.

<table>
<thead>
<tr>
<th>Clinically detected synovitis</th>
<th>ESR</th>
<th>CRP</th>
<th>IL-32 Staining Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Synovitis (5/22)</td>
<td>Mean±SD</td>
<td>45±13</td>
<td>7.8±7.3</td>
</tr>
<tr>
<td>r&lt;</td>
<td>0.59</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Moderate Synovitis (4/22)</td>
<td>Mean±SD</td>
<td>67±35</td>
<td>8.9±6.2</td>
</tr>
<tr>
<td>r&lt;</td>
<td>0.67</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Severe Synovitis (13/22)</td>
<td>Mean±SD</td>
<td>69±22</td>
<td>9.6±8.2</td>
</tr>
<tr>
<td>r&lt;</td>
<td>0.71</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

<0.001: Is considered highly significant.
Fig. (1): Shows the synovial tissue of a healthy control subject.

Fig. (2): Shows the synovial tissue of a patient with minimal inflammation having IL-32 expression graded as score 0.

Fig. (3): Shows the synovial tissue of a patient with moderate inflammation having IL-32 expression graded as score 2.

Fig. (4): Shows the synovial tissue of a patient with severe inflammation having IL-32 expression graded as score 4.

Graph (1): IL-32 staining score of both RA patients and controls.

Graph (2): The level of cytokines in RA patients and controls.
Interleukin-32 a Proinflammatory Cytokine in Rheumatoid Arthritis

Graph (3): The studied cytokines levels in relation to the severity of joint affection.

Discussion

The present study was conducted to assess the expression patterns of IL-32 in the synovial tissue from RA patients in the presence or absence of joint inflammation. Our aim was to analyze whether IL-32 expression correlates with the expression of other inflammatory mediators, like TNF-α and IL-18, and with the level of inflammation at the target organ.

Our results have shown that S and SF IL-18 as well as S TNF-α levels were higher in RA patients than in controls and the differences were statistically significant in case of IL-18 (p<0.01). Higher levels of IL-18 were found in the SF compared to the S of RA patients (p<0.01). Serum TNF-α as well as S and SF levels of IL-18 positively correlated with DAS. This is in agreement with the results of Petrovic-Rackov and Pejnovic [23], who also found that it correlates with suppression of the disease by therapy Rooney, et al. [24]. Sato, et al. [25] also found that IL-18 concentration was significantly higher in RA patients than in controls. This was also confirmed by Bokarewa and Hultgren [26] who found that IL-18 level was higher in erosive RA than in non-erosive RA, but it was not related to other markers of inflammation, duration of RA or to the treatment modality.

IL-32, previously termed NK transcript 4, is the newest inflammatory cytokine produced by mitogen-activated lymphocytes, IFN-γ activated epithelial cells, IL-12, IL-18 and IL-32-activated NK cells [8,27]. This induces TNF-α, IL-1β, IL-6 and 2 C-X-C chemokine family members involved in several autoimmune diseases. In addition, IL-32 activates arachidonic acid metabolism in peripheral blood mononuclear cells by stimulating the release of prostaglandins (PG) [28]. These properties suggested that IL-32 might play an important role in the amplification of inflammatory reactions [10,12].

The proinflammatory activity of IL-32 appears to take place after degradation of IκB, leading to activation of the typical cytokine signal pathways of nuclear factor-kappa B (NF-κB) as well as phosphorylation of mitogen-activated protein p38 [8,10]. Joosten, et al. [19], described an additional but unexpected proinflammatory effect of IL-32, namely the augmentation of cytokine production by muramyl peptides, which are present in all bacteria. In fact, the production of IL-6 by muramyl peptides depends on active IL-1ß release, which, in turn, requires caspase-1. This property of IL-32 to amplify the proinflammatory signals induced by the intracellular pattern recognition receptor of the nucleotide oligomerization domain (NOD)2 has particular importance because a mutation in NOD2 is known to be involved in the pathogenesis of Crohn's disease [9]. This data suggests that IL-32 is an important proinflammatory mediator possibly involved in the pathogenesis of several autoimmune diseases. This hypothesis agrees with our finding and is also supported by Joosten, et al. [19] and Mabileau and Sabokbar [8] who found that IL-32 is expressed in the synovial tissue from 84.6% and 83%, respectively, of the RA patients and that it correlates with the severity of inflammation in the synovial biopsies.

The source of IL-32 remains unresolved. Shoda, et al. [29], found that T cells played a principal role in IL-32 production. In contrast to B-cells and monocytes, T cells expressed IL-32 mRNA in a resting state. Moreover, activated T cells had the capacity to induce IL-32 mRNA expression in B cells and monocytes. In addition to T-cell receptor stimulation, IL-32 mRNA expression in CD4+ T cells is induced by various stimuli of inflammatory cytokines related to RA. IL-12 + IL-18 stimuli, IL-23 stimuli, and TNF-α stimuli increased IL-32 mRNA expression in CD4+ T cells. Since the mRNA expression of IL-32 was induced by either types of stimulation, IL-32 may be associated with the pathological roles of various dendritic cell-derived cytokines (namely IL-12, IL-18, and IL-23) in inflammatory diseases. These results suggested the capacity of CD4+ T cells to produce IL-32 in response to a wide range of stimuli, and thus exacerbate inflammatory arthritis. Notably, IL-32 mRNA expression was detected in the synovial-infiltrated lymphocytes. Shoda, et al. [29], suggested that IL-32-producing lymphocytes infiltrating the
inflamed synovium participate in the production of TNF-α in the RA synovium.

The importance of IL-32 for the induction of inflammation in the RA-affected joints is sustained by the observation that IL-32 expression was correlated with markers of inflammation such as ESR. In addition, IL-32 expression strongly correlated with the expression of other proinflammatory cytokines (TNF-α, IL-1β, and IL-18) in the synovial biopsies [19, Mabileau and Sabokbar [5]). Similar results were detected in our RA patients having severe and moderate synovitis, where IL-32 staining score significantly correlated with both the S and SF levels of IL-18 as well as with the serum level of TNF-α (p<0.01). In addition, IL-18 has been described by Joosten, et al. [30] to be an important proinflammatory mediator in RA. The effects of these cytokines on cell recruitment and activation, cartilage destruction, and bone destruction Nicklin, et al. [31] could in turn explain part of the effects of IL-32. Because IL-32 was strongly correlated with the expression of these cytokines in the synovial biopsies and because IL-32 induces these other cytokines in vitro, Joosten, et al. [19] concluded that IL-32 may participate in the positive-feedback mechanisms inducing inflammation.

It is generally accepted that TNF-α and IL-18 are the master cytokines in the process of chronic joint inflammation Vilcek and Feldmann [32]; Dinarello [33] and that TNF-α is the pivotal cytokine that drives joint swelling in acute joint inflammation [34]. Arthritis could be elicited by local injection of recombinant cytokines into the joint, and chronic erosive arthritis develops spontaneously in mice overexpressing human TNF-α Zwerina, et al. [35] or lacking the natural inhibitor of IL-1, namely the IL-1 receptor antagonist [36]. Indeed, when TNF-α-deficient mice were injected with recombinant human IL-32γ, the joint swelling induced by a single intra-articular injection of IL-32 was fully absent, demonstrating that this particular proinflammatory effect of IL-32 is due to intermediary production of TNF-α. Moreover, Shoda, et al. [29] demonstrated a reciprocal relationship between TNF-α and IL-32. TNF-α induced the reciprocal expression of IL-32 in various kinds of cells. They suggested that there is a positive feedback system between TNF-α and IL-32 which promotes tissue inflammation in the synovium. Interestingly, the influx of proinflammatory cells was partly TNF dependent. The IL-32-driven cell influx might be a result of the direct effect of IL-32 and IL-32-induced TNF-α production. Both IL-32 and TNF-α are potent inducers of MIP-2 and IL-8 in macrophages [10]. The latter cytokines attract inflammatory cells to sites of inflammation [37,38]. Thus, injection of IL-32 induced a marked recruitment of inflammatory cells in the joints, which is a crucial event in the inflammatory reaction [18].

Moreover, a substantial body of evidence exists that Prostaglandin E2 (PGE2) exerts pathological roles in the pathogenesis of RA, especially through its effects on cartilage and bone destruction [39,40]. Low concentrations of IL-32 induced production of PGE2 from human monocytes and mouse macrophages, which is an additional likely mechanism through which IL-32 exerts its arthritogenic effects [712]. Consistent with these in vitro data is the observation that IL-32 induced depletion of proteoglycans from the cartilage layer 4 days after the intra-articular injection. Interestingly, cartilage proteoglycan depletion was not TNF-α-dependent, because only a minor reduction of proteoglycan loss was noted in TNF-α-deficient mice after IL-32 injection [19].

The importance of IL-32 for the inflammatory reactions in RA gains further support from two other studies. In the first study done by Cagnard, et al. [41], IL-32 was the gene most strongly correlated with the inflammation of RA. In that study, the expression of a large number of proinflammatory genes was analyzed by microarray comparing gene expression profiles in cultured fibroblast-like synoviocytes from eight patients with RA to those in nine patients with osteoarthritis. IL-32 was the most prominently differentially expressed gene in RA but not in osteoarthritis. A second study done by Shoda, et al. [42], demonstrated that overexpression of human IL-32 promotes the development of murine type II collagen-induced arthritis. This was confirmed by Shoda, et al. [29], who found that in vivo expression of the novel cytokine human IL-32 induced TNF-α production, and that overexpressed IL-32β significantly exacerbated the mouse model of arthritis. Consistent with the study done by Joosten, et al. [19], it was shown that IL-32 promotes proliferation and expression of proinflammatory cytokines in murine macrophages. These results were later supported with similar results by Mabileau and Sabokbar [5], who also reported that in a cohort of patient suffering with rheumatoid arthritis, IL-32 was significantly increased in the synovial tissue and that its levels were strongly correlated with the severity of the disease.

In an attempt to find the receptor for IL-32, it has been shown that the serine protease PR3 expressed by neutrophils binds and cleaves IL-32α from a 20KDa protein forming two cleavage prod-
products of 16KDa and 13KDa [34]. Cleavage of IL-32 by PR3 was also shown to exacerbate the induction of MIP-2 and IL-8 in mouse RAW264.7 cells. Inhibition of PR3, using serine protease inhibitors, is therefore an attractive potential target [44].

In conclusion, the previous studies, as well as this study, implicate IL-32 as a proinflammatory cytokine participating in the synovitis of RA and possibly of the destruction component on cartilage. In experimental mice models of arthritis, joint injection of interleukin-32 induces joint inflammation.

Hence, the present findings suggest that IL-32 may be a newly identified prognostic biomarker in RA, thereby adding valuable knowledge to the understanding of this disease. Because IL-32 expression correlates with clinical and histological markers of disease severity as well as the presence of cytokines known to be important in the pathogenesis of RA, reducing IL-32 activity may provide benefit to patients with RA. Certainly, IL-32 is another potent proinflammatory cytokine, however, the specific role of this newly-discovered protein in the network of cytokine biology remains to be determined and the impact of its reduction on disease activity is still to be further investigated.

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