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Biomedicine & Diseases: Review

Rheumatoid Arthritis and Interleukin-32

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Abstract. The inflammatory cytokine cascade plays a pivotal role in the pathogenesis of rheumatoid arthritis. Recently, a novel human cytokine, interleukin-32, was reported to induce tumor necrosis factor (TNF)-α. Interleukin-32 is expressed primarily in lymphoid tissues and leukocytes, but also in stimulated epithelial cells and synovial fibroblasts. Although the interleukin-32 receptor has not been reported, interleukin-32 can induce other inflammatory cytokines such as TNF-α, interleukin-1β, and interleukin-6 from monocytes/macrophages *in vitro* and *in vivo*, and it synergizes with signals from pattern-recognition receptors. Notably, in the inflamed synovial tissues from rheumatoid arthritis patients, interleukin-32 is prominently expressed and correlates with the severity of arthritis and the expression of other cytokines, including TNF-α and interleukin-1. In experimental mice models of arthritis, joint injection of interleukin-32 induces joint inflammation, and overexpression of interleukin-32β in hematopoietic cells exacerbates collagen-induced arthritis. Interleukin-32 can thus be seen to play an important role in the pathogenesis of rheumatoid arthritis.

Keywords. Rheumatoid arthritis, interleukin-32, tumor necrosis factor-α, interleukin-1β, interleukin-6.

Introduction

Rheumatoid arthritis (RA) is characterized by sustained inflammatory synovitis, and the persistent synovial inflammation results in cartilage damage and bone erosions [1, 2]. Although RA is a multisystem disease of unknown cause, inflammatory cytokines are involved in its pathogenesis. In particular, tumor necrosis factor (TNF)-α plays a crucial role in both the synovial inflammatory process and bone destruction. TNF-α is thought to be a primary mediator of the inflammatory cytokine cascade in the synovial inflammation of RA [3]. Indeed, the blockade of the effects of TNF-α by biological agents has elicted clearly good clinical responses as a novel RA therapy [1, 4]. Several other inflammatory cytokines such as interleukin (IL)-1 and IL-6 are involved in the pathogenesis of RA [5]. Major disturbances in the cascade or network of these inflammatory cytokines occur during the pathogenesis of RA, and precise elucidation of the relationship between inflammatory cytokines and arthritis is required.

IL-32 is a novel human cytokine that was reported by Kim. et al. in 2005 [6]. That study revealed that IL-32 can induce TNF-α expression and secretion in a mouse macrophage cell line and a human monocyte cell line [6]. IL-32 also induces other inflammatory cytokines, IL-1 and IL-6, from human peripheral blood mononuclear cells [7]. Because IL-32 has a potent TNF-α-inducing effect and a close relationship with other inflammatory cytokines, IL-32 suspected to

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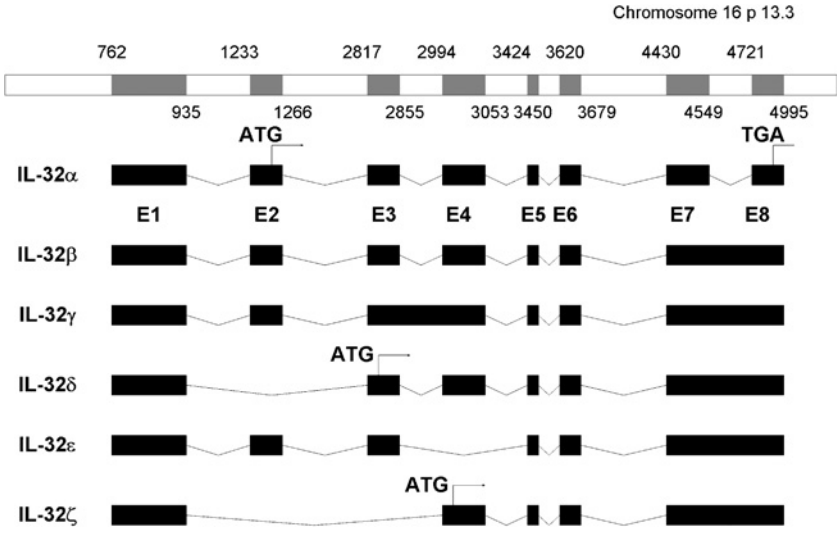

Figure 1. The six splice variants of IL-32. The human IL-32 gene is located on chromosome 16p13.3.

play a role in the inflammatory cytokine cascade and in the pathogenesis of RA. In this review, we summarize the previously reported functions of IL-32 and review the relationship between IL-32 and inflammatory arthritis.

The novel human cytokine IL-32

Human cytokine IL-32 was previously reported as NK cell transcript 4 (NK4), which is expressed in activated natural killer (NK) cells [8]. Kim et al. [6] revealed that NK4 expression is significantly increased by stimulation with IL-18, independent of IL-12 and IL-15 costimulation, in the human lung epithelial cell line A549. They also demonstrated that this molecule can induce TNF-α in some cell lines, and named this novel cytokine IL-32. IL-32 does not have sequence homology with other cytokines, and the receptor of IL-32 or mouse analog of IL-32 has not been reported so far. Six splice variants of human IL-32 have been reported, and it was IL-32γ that was originally reported as NK4 (Fig. 1). IL-32β is a dominant variant in activated T cells [9]. Kim et al. [6] reported that IL-32β is secreted out of cells, but that IL-32α remains intracellularly. However, the amounts of secreted IL-32β are also not very high, and these authors discussed the possibility that IL-32 could work intracellularly. Indeed, some splice variants of IL-32 lack the putative signal peptides existing in the original IL-32γ.

IL-32 is expressed in the secondary lymphoid tissues, including spleen, small intestine, colon, and peripheral blood leukocytes. IL-32 expression can be induced in concanavalin-A-stimulated human peripheral mononuclear cells and IL-12-plus-IL-18-stimulated NK cells [6]. What is more, many kinds of immune cells, both hematopoietic and non-hematopoietic, express IL-32. We demonstrated that activated T cells, B cells, and monocytes from human peripheral mononuclear cells can increase IL-32 expression. While B cells do not express significant amounts of IL-32, even when stimulated by anti-IgM or anti-CD40 [9], B cells and monocytes express IL-32 in the presence of activated T cells [10]. CD4-positive T cells highly express IL-32 when they are stimulated by anti-CD3 and anti-CD28 monoclonal antibodies and are considered a major source of IL-32 [10]. Th1 cells are known to produce interferon-γ and to be differentiated by stimulation with IL-12 and IL-18 [11]. Because this costimulation increases IL-32 expression, as do interferon-γ-secreting CD4-positive T cells, it follows that Th1 cells are one of the sources of IL-32 [10]. In addition, epithelial cells are widespread sources of IL-32. Although epithelial cells from healthy subjects express low levels of IL-32, IL-32 expression can be induced in human epithelial cells by interferon-γ [6]. Under pathological conditions such as Crohn's disease, IL-32 expression is increased remarkably [7]. Moreover, the synovial fibroblasts derived from RA patients express increased levels of IL-32 [10], which we discuss later. In this way, IL-32 is expressed mainly in leukocytes, but also in other kinds of cells, including epithelial cells and fibroblasts, particularly when they are stimulated by inflammatory cytokines (Table 1). However, the precise biological effects of IL-32 expression in a specific cell source should be examined further. Interestingly, there is a reciprocal induction system between IL-32 and TNF-α [10]. IL-32 induces the release of TNF-α from macrophages and monocytes. In turn, human CD4-positive T cells increase IL-32 expression by stimulation with TNF-α. IL-32 expression is also increased by TNF-α in human synovial

Table 1. Cellular sources and stimulation of human IL-32.

IL-32-expressing cells	Stimulation to induce IL-32	References
Hematopoietic cells		
T cell	T cell receptor signaling	9, 10
	IL-12 and IL-18	10
	TNF-α	10
B cell	activated T cell help	10
NK cell	IL-12 and IL-18	6
Monocyte	activated T cell help	10
Dendritic cell	TNF-α	10
Non-hematopoietic cells		
Epithelial cell	interferon-γ	6
Synovial fibroblast	TNF-α	10

Table 2. Immunological changes in response to overexpression of human IL-32 in mice (BM hIL-32 mice).

Types of assay	Immunological changes
Serum concentration	increased TNF-α levels
Splenocyte culture	
No stimulation	increased production of TNF-α (F4/80-positive macrophages)
LPS stimulation	increased production of TNF-α, IL-1β, and IL-6 (F4/80-positive macrophages)
	increased production of IL-1β (CD-11c-positive dendritic cells)
	no significant change in T cells and B cells
Disease models	
Collagen antibody-induced arthritis	exacerbation
Hapten-induced colitis	exacerbation increased expression of TNF-α in the lesions

Results from reference 10.

fibroblasts derived from RA patients *in vitro* [10]. These reciprocal induction systems between IL-32 and TNF-α could participate in the amplification and maintenance of the inflammation.

Although the functions of IL-32 remain unclear, the variable cytokine-inducing effects of IL-32 have been shown both *in vitro* and *in vivo*. In the *in vitro* assay, IL-32α and IL-32β induce human TNF-α and IL-8 in a THP-1 human monocyte cell line, as well as mouse TNF-α and macrophage inflammatory protein-2 in the Raw 264.7 macrophage cell line [6]. In addition, IL-32γ potently induces the inflammatory mediator prostaglandin E2 in these two cell lines [12]. We have also demonstrated the *in vivo* effects of IL-32β by the overexpression of IL-32β in model mice. We generated a model mouse with the overexpression of human IL-32β in bone marrow-derived cells (BM-hIL-32 mouse), as follows: the human IL-32β gene was transduced to bone marrow cells by retrovirus, and these cells were transplanted to lethally irradiated mice. In BM-hIL-32 mice, bone marrow-derived cells such as lymphocytes and monocytes expressed human IL-32β. The serum levels of mouse TNF-α were clearly elevated in BM-hIL-32 mice (Table 2), and splenic F4/80-positive macrophages increased the expression of TNF-α in BM-hIL-32 mice. Notably, CD4-positive T cells, CD8-positive T cells, and CD19-positive B cells from BM-hIL-32 mice showed no significant change in cytokine expression [10]. Therefore, the targets of IL-32 include monocytes and macrophages rather than lymphocytes, and one of the main functions of IL-32 is the induction of inflammatory cytokines, especially TNF-α, from monocytes and macrophages.

The intracellular signal pathways induced by IL-32 stimulation, have not been fully analyzed and remain largely unclear. Kim et al. have reported that IL-32 activates typical cytokine signal pathways of nuclear factor-kappa B and p38 mitogen-activated protein kinase. IL-32α stimulation induced the degradation of inhibitor of kappa B and the phosphorylation of p38 mitogen-activated protein kinase in the Raw 264.7 mouse macrophage cell line [6].

IL-32 sometimes receives post-transcriptional processing to increase its activity level. Novick et al. [13] have reported that IL-32α is a binding protein to proteinase 3, a neutrophil granule serine proteinase. They demonstrated that, after limited cleavage by proteinase 3, IL-32α is more active than intact IL-32α in inducing macrophage inflammatory protein-2 in the Raw 264.7 mouse macrophage cell line and also in inducing IL-8 in human peripheral blood mononuclear cells [13]. Neutrophil-derived proteinase 3 is a major and specific autoantigen for autoantibodies in Wegener's granulomatosis, and the relationship between proteinase 3 and IL-32 is worth noting.

Close relationship between IL-32 and inflammatory cytokines

Several inflammatory cytokines are involved in the pathogenesis of RA. These include TNF-α, IL-1, IL-6, and IL-18 [5–14], which also play important roles in the innate immune system. The innate immune system is ancient, and it recognizes pathogens by pattern-recognition receptors coded in the germ line and triggers a variety of protective mechanisms to eliminate pathogens [15]. The effector molecules consist of

a complement cascade, antimicrobial peptides, and inflammatory cytokines, which work as important mediators in the innate immunity system. Cells of the innate immune system include NK cells, monocytes, macrophages, dendritic cells, neutrophils and epithelial cells. The toll-like receptor (TLR) family members are known to consist of pattern-recognition molecules [16, 17]. Molecules of TLR family members are transmembrane proteins located on the cell surface membrane or the luminal side of the intracellular endosomes/lysosomes of monocytes, macrophages, dendritic cells, B cells, and non-hematopoietic epithelial cells. TLRs recognize a variety of microbial products derived from bacteria, viruses, and yeast, which exist outside the cells. TLR-mediated signals activate intracellular events and potently promote both the release of inflammatory cytokines and the presentation of antigens [15]. Importantly, lipopolysaccharide (LPS), a cell wall component of Gram-positive bacteria, binds to TLR4, leads to the activation of nuclear factor-kappa B, and releases large amounts of inflammatory cytokines, including TNF-α, IL-1, and IL-6 [18].

Although the molecules of the TLR family recognize pathogen-derived molecules outside cells, molecules of the nucleotide oligomerization domain (NOD) family are located in cytoplasm and recognize intracellular bacterial-derived molecules such as Gram-positive and Gram-negative peptidoglycans and their derived muropeptide components by leucine-rich repeat domains [19]. The signals from NOD1 and NOD2 activate nuclear factor-kappa B and induce the release of the inflammatory cytokines, including TNF-α, while NOD2 also activates caspase-1 [20], which processes pro IL-1β and IL-18 into effective IL-1β and IL-18 by limiting cleavage [21]. It has been reported that IL-32 is a novel cytokine that is induced by IL-18 stimulation and induces TNF-α in monocyte and macrophage cell lines, suggesting that it has a close relationship with these inflammatory cytokines and the innate immune system.

IL-32 can induce several inflammatory cytokines. Netea et al. [7] have reported that IL-32γ stimulation induces the secretion of TNF-α, IL-1β, and IL-6 from peripheral blood mononuclear cells, especially monocytes rather than lymphocytes [7]. That study investigated the interaction of IL-32 with the TLR and NOD pathways. The secretion of TNF-α and IL-6 from peripheral blood mononuclear cells was measured after ligands of TLRs were added with or without IL-32γ. The results showed that IL-32γ has additive effects only on cytokine production induced via TLR signals. In contrast, the synergistic effects of IL-32 were observed with NOD pathways. They stimulated peripheral blood mononuclear cells with or without IL-32γ by Mur-Tri-DAP, which is a synthetic derivative of Gram-negative peptidoglycan and a ligand of NOD1, and by MDP, which is a synthetic derivative of Gram-positive peptidoglycan and a ligand of NOD2. Although IL-32 and muropeptides showed only additive effects on the release of TNF-α and IL-1α, IL-32 synergized with NOD signals for the release of IL-1β and IL-6 (3- to 10-fold increases). Indeed, this synergy between IL-32γ and the murine NOD1 ligand was absent in the peritoneal macrophages isolated from NOD1-deficient mice. Moreover, the synergistic effects of IL-6 production between IL-32γ and MDP were not observed in the peripheral blood mononuclear cells from patients with Crohn's disease, who were homozygous for the NOD2 loss-of-function mutation. Therefore, IL-32 amplifies the NOD signals in a synergistic manner [7]. These authors also investigated the mechanism underlying this synergizing effect of IL-32. The synergistic induction of IL-6 from peripheral blood mononuclear cells by IL-32γ and MDP was found to be restricted by the pan-caspase inhibitor and the specific caspase-1 inhibitor. The synergistic effects of IL-32/MDP were also reduced by IL-1 receptor antagonist, which inhibited IL-1 activity, but not by IL-18-binding protein, which inhibited IL-18. The authors therefore concluded that the synergistic stimulation of IL-6 by IL-32/MDP is due to an intermediary release of endogenous IL-1β in a caspase-1-dependent manner [7].

In accordance with the *in vitro* data, we demonstrated the *in vivo* effects of IL-32 by an overexpression mouse model of human IL-32β [10]. Freshly isolated splenocytes of BM-hIL-32 mice showed increased expression and secretion of TNF-α compared to those of control mice. No increased expression or secretion of IL-1β or IL-6 was observed in freshly isolated splenocytes of BM-hIL-32 mice. The serum concentrations of TNF-α protein were elevated significantly in BM-hIL-32 mice. Serum concentrations of IL-1β or IL-6 protein were not detected in BM-hIL-32 or control mice. Cell-sorting analysis of splenocytes of BM-hIL-32 mice revealed that the expression of TNF-α was increased in freshly isolated F4/80$^+$ macrophages. We next examined the response of splenocytes of BM-hIL-32 mice to LPS stimulations. LPS-stimulated splenocytes of BM-hIL-32 mice showed markedly increased expression and secretion of TNF-α and IL-1β. Both F4/80$^+$ macrophages and CD11c$^+$ dendritic cells showed increased expression of TNF-α and IL-1β after LPS stimulation in the splenocytes of BM-hIL-32 mice. We also observed that LPS-stimulated splenocytes of BM-hIL-32 mice showed an additively increased secretion of IL-6 protein, and F4/80$^+$ macrophages showed an increased expression of IL-6. These results suggested that the function of *in vivo*-ex-

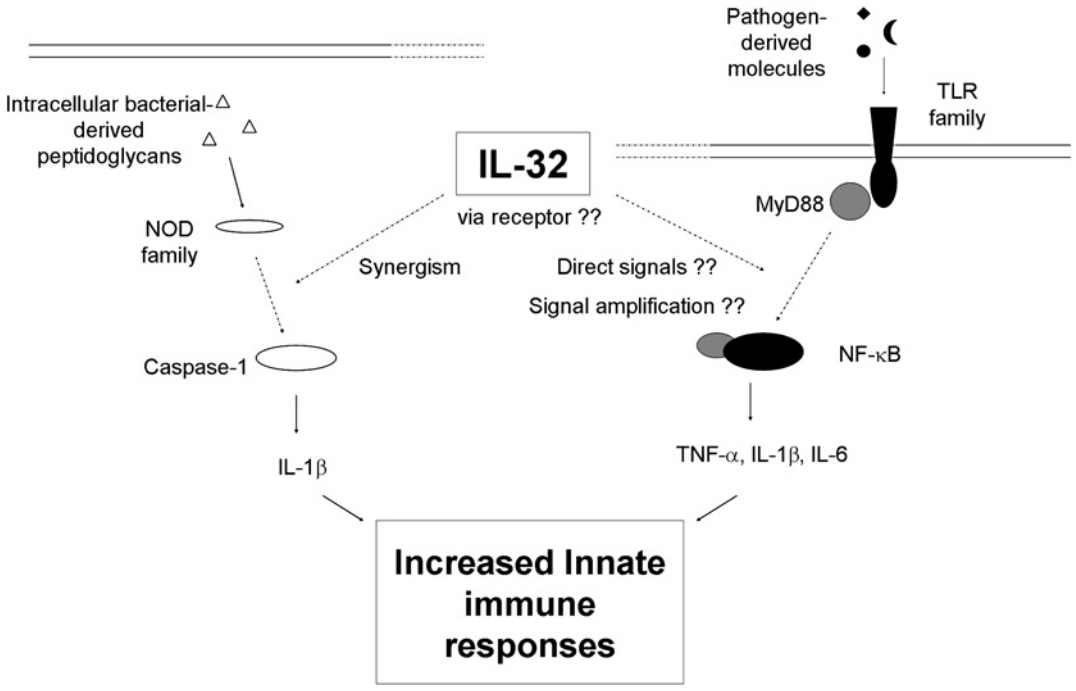

Figure 2. Scheme for the hypothetical correlations of IL-32 with the signals mediated by pattern-recognition receptors. NOD, nucleotide oligomerization domain; TLR, toll-like receptor; NF-κB, nuclear factor-kappa B; TNF, tumor necrosis factor; IL, interleukin.

pressed IL-32β was focused on the induction of TNF-α production, especially in the macrophages. Our results also suggested that *in vivo*-expressed IL-32β collaborates with TLR4 signals to induce inflammatory cytokine production in macrophages and, in part, dendritic cells [10].

In this way, IL-32 is a novel inducer of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 from macrophages and monocytes. Because IL-32 can induce TNF-α and IL-1β from TLR4-deficient peritoneal macrophages [12], IL-32 itself can induce these inflammatory cytokines independent of TLR signals. In addition, the synergistic effects of IL-32 and NOD signals were present, although only the additive effects of IL-32 and TLR signals were observed. The receptor or precise signal pathways of IL-32 are still being investigated. However, the relationship between IL-32 and inflammatory cytokine induction is clearly demonstrated, and IL-32 would be a 'tuner' of the innate immune response by regulating the expression levels of the inflammatory cytokines (Fig. 2).

IL-32 and inflammatory arthritis

Although RA is caused by multisystem disorders, dysregulation of the balance between pro-inflammatory cytokines and anti-inflammatory cytokines is presumed to be a major mechanism in the development of inflammatory arthritis. The pro-inflammatory cytokines related to RA include TNF-α, IL-1β, IL-6, and IL-18 [5, 14]. Since IL-32 mutually induces TNF-α and is closely related to these inflammatory cytokines, it is suspected that IL-32 comprises part of the cytokine network in the inflammatory lesions, and also plays an important role in the pathogenesis of RA. Indeed, microarray analysis revealed that IL-32 gene expression in cultured synovial fibroblasts obtained from RA patients is significantly increased compared to those from patients with osteoarthritis (OA) [22]. Cagnard et al. [22] have studied the expression of 171 cytokine and chemokine genes of synovial fibroblasts and compared their expression between RA and OA patients. They detected only four genes with significant differences: CCL2 (also called monocyte chemoattractant protein-1), PF4F1, GDF10, and IL-32. In particular, IL-32 was reported to be the most prominently differentially expressed gene, with higher expression in the synovial fibroblasts of RA patients than in those of OA patients ($p<0.0073$). Our group also determined IL-32 mRNA expression in the inflamed synovial tissues obtained from RA patients by *in situ* hybridization. In that study, synovial-infiltrating lymphocytes prominently expressed IL-32 mRNA (Fig. 3a). We also examined IL-32 mRNA expression in the cultured synovial fibroblasts obtained from RA patients. After TNF-α treatment, IL-32 mRNA expression was potently induced in the synovial fibroblasts from RA patients (Fig. 3b) [10].

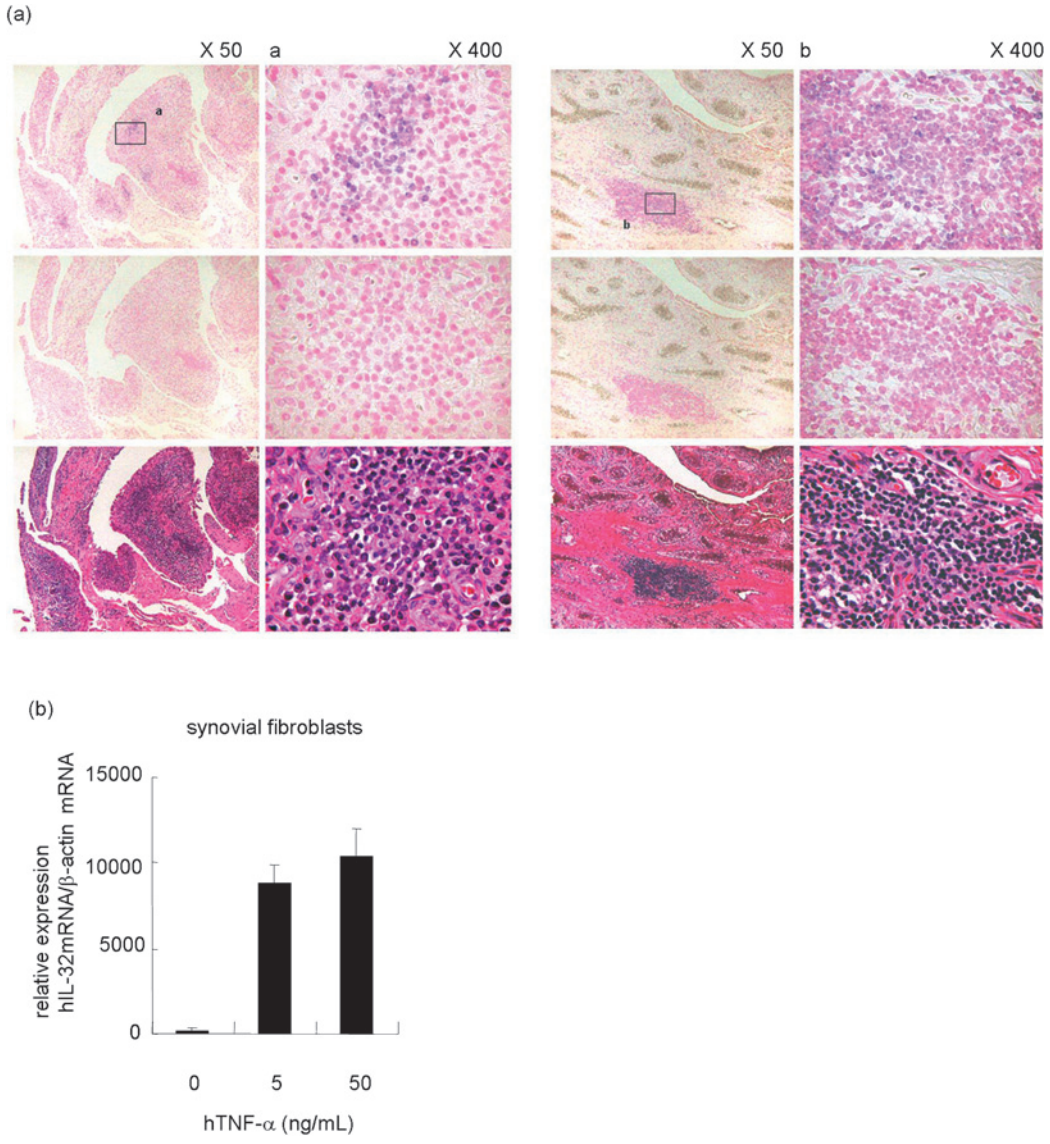

Figure 3. IL-32 mRNA expression in the inflamed synovial tissues of RA patients. (*a*) IL-32 mRNA is detected in the synovial tissues of RA patients by *in situ* hybridization. IL-32 mRNA was detected in the synovial-infiltrating lymphocytes. (*b*) IL-32 mRNA expression in the synovial fibroblasts. The synovial fibroblasts derived from RA patients were cultured with human TNF-α for 48 h, and IL-32 mRNA expression was determined by quantitative PCR. These figures are cited from reference 10.

In the inflamed synovial samples of RA patients, IL-32 is expressed in several kinds of cells such as synovial-infiltrating lymphocytes and synovial lining cells. Joosten et al. [12] have demonstrated that the staining of IL-32 in the lining layers correlates with topical and systemic inflammation by immunohistochemistry. They also showed the presence of IL-32 in lymphocytes that infiltrated severely inflamed synovial tissues [12]. They reported that IL-32 is highly expressed in RA synovial tissue samples by immunohistochemistry. In contrast to RA patients, synovial tissue samples from patients with OA did not express IL-32. The levels of IL-32 staining correlated with the severity of synovial inflammation, RA disease activity and the expression of other inflammatory cytokines [12]. In that study, 25 of the 29 synovial biopsy samples from patients with RA were positive for IL-32 staining by immunohistochemistry. The intensity of synovial staining of IL-32 correlated with microscopic synovial inflammation (r=0.80) as well as with the erythrocyte sedimentation rate, a marker of systemic inflammation (r=0.63). The presence of synovial staining of IL-32 also strongly correlated with the presence of synovial staining of other inflammatory cytokines, TNF-α (r=0.68), IL-1β (r=0.79), and IL-18 (r=0.82). These results suggest a close association between IL-32 and other inflammatory cytokines [12]. IL-32 appears to play an important role both in the

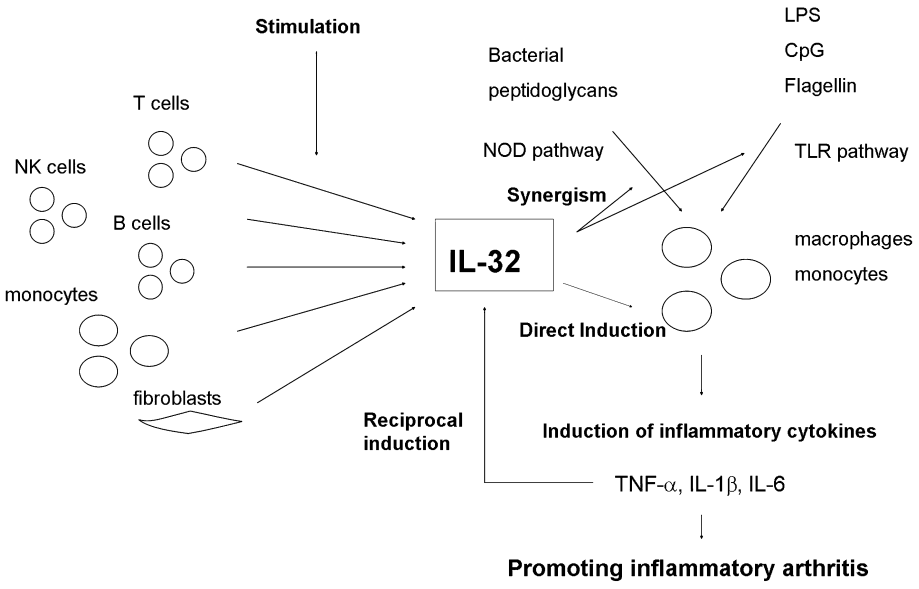

Figure 4. Scheme of the hypothetical roles of IL-32 in the pathogenesis of arthritis. NOD, nucleotide oligomerization domain; TLR, toll-like receptor; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin.

inflammatory cytokine network and in the pathogenesis of RA, since IL-32 is prominently expressed in the inflamed synovial tissues from RA patients, and since the intensity of IL-32 staining correlates with the severity of arthritis and the expression of other inflammatory cytokines.

In experimental mice models of inflammatory arthritis, some data supported the pathological roles of IL-32. Joosten et al. [12] have reported that recombinant human IL-32γ injection to knee joints of naïve mice induces joint swelling in clinical observation, and infiltration of inflammatory cells and cartilage damage were observed histologically. In TNF-α-deficient mice, IL-32γ injection could not induce joint swelling, and cellular infiltration was markedly reduced. However, TNF-α deficiency did not affect the loss of proteoglycan [12]. These data suggest that the role of IL-32 in evoking joint inflammation is, in part, dependent on TNF-α, and the authors suspect that the loss of proteoglycan following the injection of IL-32γ might be due to IL-1β activity.

Our group examined the pathological roles of IL-32 in collagen antibody-induced arthritis and collagen-induced arthritis mice [10]. We first tested the severity of collagen antibody-induced arthritis in BM-hIL-32 mice. BM-hIL-32 mice, in which human IL-32β was overexpressed in hematopoietic cells, showed increased serum levels of TNF-α, and the splenocytes from BM-hIL-32 mice increased the expression and secretion of TNF-α, IL-1β, and IL-6 in response to LPS *in vitro*. After the transfer of collagen antibodies and the injection of LPS, these BM-hIL-32 mice developed significantly more severe arthritis than control mice. Next, we examined the roles of IL-32-producing lymphocytes in the inflamed synovial tissues. In the synovial tissues from RA patients, both the lining cells and lymphocytes expressed IL-32, and the synovial-infiltrating lymphocytes were found to consist primarily of CD4-positive T cells [23]. To test the pathological effects of IL-32-producing CD4-positive T cells in collagen-induced arthritis, we transduced the human IL-32β gene to splenic CD4-positive T cells by retrovirus, and then transferred these cells to the collagen-immunized mice before the onset of arthritis. The transfer of IL-32β-producing CD4-positive T cells significantly exacerbated collagen-induced arthritis compared to the transfer of control CD4-positive T cells. Microscopic cellular infiltration was also increased in mice with transferred IL-32β-producing CD4-positive T cells. Notably, TNF-α blockade by etanercept reduced the exacerbating effects of IL-32β-producing CD4-positive T cells [10]. These results suggest that IL-32, which was produced by CD4-positive T cells, contributed to the pathogenesis of inflammatory arthritis. Moreover, TNF-α partially mediates the pro-inflammatory effects of IL-32 *in vivo*. However, BM-hIL-32 mice did not develop arthritis spontaneously, despite the elevated serum levels of TNF-α. We could not fully explain this phenomenon, but suspect that IL-32 expression in the epithelial cells and fibroblasts, as well as lymphocytes, would play important roles in the development of arthritis (Fig. 4).

Some data imply a close relationship between the development of RA and tuberculosis infection. A Spanish group has shown that the relative risk of tuberculosis in Spanish RA patients is 3.68 [24], and another group has demonstrated a ten-fold increased risk of tuberculosis infection in Japanese male RA patients [25]. Notably, the anti-cyclic citrullinated

peptide antibody, known as a remarkably specific serum antibody for the diagnosis of RA, was also detected from the sera of active lung tuberculosis patients [26]. A recent report showed that *Mycobacterium tuberculosis*-derived antigens can induce IL-32 synthesis in human monocytes and macrophages [27]. The authors also showed that other heat-killed organisms, such as *Staphylococcus aureus, Candida albicans*, and *Aspergillus fumigatus*, do not induce IL-32 release. That report suggested that IL-32 plays a role in protecting against tuberculosis infection, and that the *M. tuberculosis*-derived antigens could have an association with the pathogenesis of RA by inducing IL-32 and other inflammatory cytokines. It is necessary to keep in mind that the neutralization of IL-32 could render patients more susceptible to tuberculosis infection, as does the blockade of TNF-α.

Conclusion

Human novel cytokine IL-32 participates in the induction of inflammatory cytokines, and is closely related to the signals from pattern-recognition receptors. IL-32 is expressed in inflamed synovial tissues obtained from RA patients, and the intensity of IL-32 staining correlates with the severity of RA and the expression of TNF-α, IL-1, and IL-18. IL-32-overexpressed mice showed increased expression of TNF-α and the exacerbation of experimental arthritis, which was inhibited by the blockade of TNF-α. IL-32 therefore plays an important role in the innate immune system and the pathogenesis of inflammatory arthritis. However, many questions about IL-32 remain unresolved. A receptor of IL-32 has not yet been reported, and the precise signal pathways activated by IL-32 have only been partially revealed. We do not know if the blockade of IL-32 could ameliorate arthritis, or if IL-32 participates in the generation of osteoclasts. Nonetheless, IL-32 is thought to be a good candidate for a new therapeutic target of RA by virtue of its close relationship with TNF-α and synovial inflammation. Therapies for RA are being developed, and biological agents against inflammatory cytokines have achieved good clinical responses. However, even when infliximab, a monoclonal chimeric antibody to human TNF-α, is administered, only half of the patients achieve 50% improvement as defined by the American College of Rheumatology [28]. It therefore remains necessary to clarify the mechanisms of arthritis and to develop novel therapeutic agents for RA. Since IL-32 possesses several pathways for activating pro-inflammatory mechanisms beyond TNF-α-inducing effects, such as the synergy with NOD signals to promote IL-1β and IL-6 release, IL-32 could be a potential therapeutic target of inflammatory arthritis, and is worth investigating further.

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