Interleukin-33 promotes proliferation and inhibits apoptosis of fibroblast-like synoviocytes in rheumatoid arthritis

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Abstract

Objective

The aim of our study was to determine the effect of interleukin (IL)-33 on the proliferation, apoptosis, and secretion of inflammatory cytokines by fibroblast-like synoviocytes (FLSs) in rheumatoid arthritis (RA) and to investigate the underlying mechanisms.

Methods

Cultured RA FLSs and osteoarthritis (OA) FLSs were cocultured with different concentrations of IL-33. TUNEL assay and flow cytometry were used to detect apoptosis. Western blotting and Real-time (RT)-PCR were used to detect the expression levels of B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax), while the Cell Counting Kit-8 was used to determine cell proliferation in each cocultured group. Enzyme-linked immunosorbent assay was used to detect the expression levels of tumour necrosis factor (TNF)-α and IL-6 in the supernatant from each cell culture. Western blot analysis was used to determine the phosphorylated expression levels of the nuclear factor-kappa light chain enhancer of the activated B cells (NF-κB) pathway in each group.

Results

IL-33 inhibited RA FLS apoptosis, promoted FLS proliferation, increased Bcl-2 protein expression levels, and decreased Bax protein expression levels. It also increased the expression levels of inflammatory cytokines TNF-α and IL-6 and increased the expression levels of P-NF-κB in FLSs.

Conclusion

IL-33 inhibited apoptosis and promoted proliferation of FLSs; in addition, IL-33 increased the serum levels of inflammatory cytokines. The effect of IL-33 on RA FLSs was likely mediated via the NF-κB pathway.

Key words
Interleukin-33, rheumatoid arthritis, proliferation, NF-κB.

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that is often associated with increased production of inflammatory cytokines, immune disorders, and systemic complications. A variety of factors have been implicated in the pathogenesis and progression of this disease. The main pathological manifestation of RA is erosive injury to the cartilage and bone tissue caused by excessive proliferation of fibroblast-like synoviocytes (FLSs); the consequent joint damage negatively affects the quality of life of patients with RA.

Interleukins (ILs) are proteins that mediate cell-cell crosstalk. IL-1 family proteins, identified in 2005, comprise of 11 cytokines that play a central role in the regulation of immune and inflammatory responses to infection. IL-33 is a member of the IL-1 family. Other members of the IL-1 family include IL-1α, IL-1β, IL-18, IL-36, IL-37, and IL-38. ST2 is a receptor of IL-33 (1, 2). Recent studies have shown that rs3939286, an allele of IL-33, has a potential protective effect against subclinical atherosclerosis in patients with RA (3). IL-33 was shown to promote the secretion of insulin by inducing innate lymphoid cell 2 (ILC2) (4); in addition, it has protective effects against parasitic infections (5), viral infections (6), and Alzheimer's disease (7). However, IL-33 was also shown to aggravate asthma (8) and promote progression of RA (9, 10); in addition, IL-33 is associated with RA activity (11) and showed a positive correlation with RA onset. In a study by Palmer *et al*. (9, 12), intraperitoneal injection of IL-33 into mice with collagen-induced arthritis (CIA) was shown to aggravate the symptoms of CIA. Increased levels of ST2 in serum and synovial fluid were found to be associated with RA disease activity; in addition, IL-33 was found to induce inflammatory response (*e.g*. IL-6, IL-8, and tumour necrosis factor [TNF]-α) in RA synovial fibroblasts (13). IL-33 is mainly expressed in vascular endothelial cells, fibroblasts, and synovial-lining cells in patients with RA (14). Overexpression of IL-33 protein was also detected in the nucleus of RA FLSs (15). Jing Lu (11) found that IL-33 may also be involved in bone erosion in patients with RA; in addition, high expression of IL-33 in serum was found to be a marker of bone erosion in these patients (16). We speculated that IL-33 is involved in increased proliferation and reduced apoptosis of RA FLSs.

In the present study, we investigated the effects of IL-33 on the proliferation, apoptosis, and secretion of inflammatory cytokines in RA FLSs and explored the underlying mechanisms. Our findings may help characterise the role of IL-33 in inducing the imbalance between the proliferation and apoptosis of RA FLSs. Our overall aim was to further clarify the role of IL-33 in the pathogenesis of RA.

Materials and methods

Cells, reagents, and apparatus

Human RA FLSs and osteoarthritis (OA) FLSs were purchased from the Hongshun Company (Shanghai, China). The cells were cultured in high-glucose (H)-Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37°C in a 5% CO_2 humidified atmosphere. The cells were passaged from the third to the seventh generation and then used in the experiments.

Recombinant IL-33 (human) (batch 1106398B1419), Fas L (batch 0517498J0418) and TNF-α (batch 0218225K1119) were purchased from PeproTech Inc. (Rocky Hill, CT, USA); penicillin and streptomycin double antibiotic (batch 71766481) were purchased from the Federal Biological Reagents Co. Ltd. (Hangzhou, China), FBS (batch 1B010204) from Tianhang Biotechnology Co. Ltd (Hangzhou, China), H-DMEM medium (batch M10327) from Transgen Biotech (Beijing, China), the Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) apoptosis detection kit (batch 640914) from Biolegend (San Diego, CA, USA). TUNEL kit (batch 5J10K01910) was purchased from BioVision (Miliptas, USA); the Cell Counting Kit 8 (CCK-8) (batch 116G011) from Solarbio Reagent Co. Ltd. (Beijing, China), qPCR Master Mix (batch GC11901) and the IL-6 Enzyme-Linked Immunosorbent Assay (ELISA)

kit (batch B225583) from Biolegend (San Diego, CA, USA), the TNF-α ELISA kit (batch 158611029) from Invitrogen, anti-Bcl-2 (batch AF08268880) from Bioss (Beijing, China), anti-Bax (batch AH02288797) from Bioss (Beijing, China), and p-nuclear factor-kappa light chain enhancer of activated B cells (p-NF-κB) (batch 4338807) from eBioscience, Inc. (San Diego, CA, USA).

CCK-8 to evaluate the effect of IL-33 on the proliferation of FLSs

FLSs at a density of 5×10^3/mL were seeded into 96-well plates at 100 μL per well. The cells were grouped as follows: control (RA FLS); TNF-α, 20 ng/mL (RA-FLS); IL-33, 25 ng/mL (RA FLS); IL-33, 50 ng/mL (RA FLS); IL-33, 100 ng/mL (RA FLS); IL-33, 0 ng/mL (OA FLS); IL-33, 25 ng/mL (OA FLS); IL-33, 50 ng/mL (OA FLS); and IL-33, 100 ng/mL (OA FLS). TNF-α (20 ng/mL) was used as a positive control to promote the proliferation of RA-FLS. After 24 h, 48 h, and 72 h, 10 μL CCK-8 solution was added to each well, and the cells were incubated for 4 h at 37°C in a 5% CO_2 incubator. The absorbance at 450 nm was measured using the Anthos 2010 microplate reader (Biochrom Ltd., Cambridge, UK).

Flow cytometry to evaluate the effect of IL-33 on the apoptosis of FLSs

RA and OA FLS cells at 1×10^5/mL were seeded into six-well plates. Three different concentrations of IL-33 were added after cell adhesion, and the cells were grouped as follows: control (RA FLS); Fas L, 20 ng/mL+TNF-α, 10 ng/mL (RA-FLS); IL-33, 25 ng/mL+Fas L, 20 ng/mL+TNF-α, 10 ng/mL (RA FLS); IL-33, 50 ng/mL+Fas L, 20 ng/mL+TNF-α, 10 ng/mL (RA FLS); IL-33, 100 ng/mL+Fas L, 20 ng/mL+TNF-α, 10 ng/mL (RA FLS); IL-33, 0 ng/mL+Fas L, 20 ng/mL+TNF-α, 10 ng/mL (OA FLS); and IL-33, 100 ng/mL+Fas L, 20 ng/mL+TNF-α 10 ng/mL (OA FLS).

Fas L, 20 ng/mL was used to induce apoptosis of FLSs. TNF-α, 10 ng/mL was used to simulate the *in vivo* inflammatory environment. After incubating in IL-33 for 24 h and 48 h, the cells were collected, including those in the culture supernatant, washed with PBS, and the concentration adjusted to 1×10^6/mL. To analyse apoptosis, 100 μL cell suspension was added into a flow tube, incubated with 5 μL annexin V-FITC, and incubated with 10 μL PI solution for 15 min in the dark at room temperature. Subsequently, 400 μL cell staining buffer was added to detect the effect of different concentrations of IL-33 on the apoptosis of FLSs.

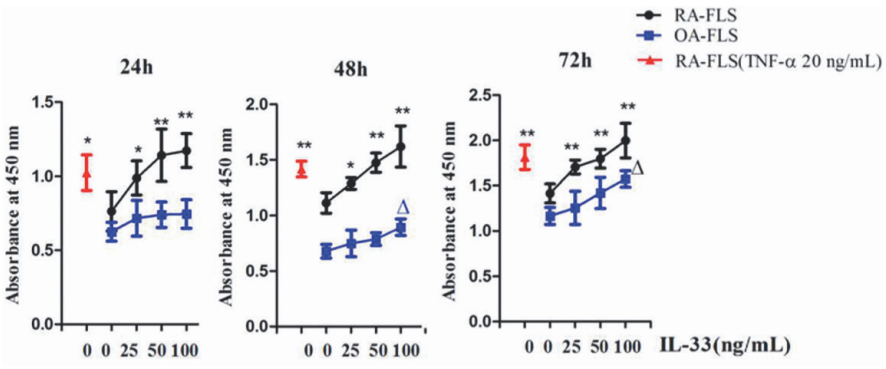

Fig. 1. Effects of different concentrations of interleukin-33 (IL-33) (0, 25, 50, or 100 ng/mL) on the proliferation of fibroblast-like synoviocytes (FLSs).
Data presented as mean ± standard deviation, n=4.
*p<0.05 *vs*. control (IL-33, 0 ng/mL, RA FLS); **p<0.01 *vs*. control (IL-33, 0 ng/mL, RA FLS); $^{\Delta}p$<0.01 *vs*. control (IL-33, 0 ng/mL, OA FLS).

TUNEL assay to evaluate the effect of IL-33 on the apoptosis of FLSs

RA-FLSs and OA FLSs were cultured in 96-well plates and grouped as described above. IL-33 and FLS were co-cultured for 48 hours to detect apoptosis. According to the manufacturer's instruction for TUNEL assay, the supernatant was removed and the cells washed with PBS three times. Subsequently, 100 μL of fixative solution was added to each well and the cells incubated for 15 min at RT in dark. The supernatant was removed and the cells washed with Wash Buffer. 100 μL of permeabilisation buffer was added and the cells incubated for 10 min at RT. The supernatant was removed and the cells washed twice in 100 μL of dH_2O. 50 μL of TUNEL reaction cocktail was used per well for 1 h at 37°C. Subsequently, the cells were washed and 100 μL EZClick reaction cocktail was added for 30 min at RT. After wash, DNA staining was performed for 20 min at RT. After the final wash, the cells were imaged with a fluorescence microscope. TUNEL positive cells were stained red, the nuclei were stained green, and the apoptotic cells were stained orange. The rate of apoptosis was counted in each group.

Western blotting to determine the effect of IL-33 on the expressions of FLS Bax and Bcl-2

FLSs were cultured as described above and grouped as follows: control (RA FLS); TNF-α, 10 ng/mL (RA-FLS); IL-33, 25 ng/mL+TNF-α, 10 ng/mL (RA FLS); IL-33, 50 ng/mL+TNF-α, 10 ng/mL (RA FLS); IL-33, 100 ng/mL+TNF-α, 10 ng/mL (RA FLS); IL-33, 0 ng/mL+TNF-α, 10 ng/mL (OA FLS); and IL-33, 100 ng/mL+TNF-α, 10 ng/mL (OA FLS). TNF-α, 10 ng/mL was used to simulate the *in vivo* inflammatory environment. Total FLS protein was extracted from each group after 48 h. Coomassie brilliant blue was used to stain the cells and determine the total protein concentration. The protein loading amount was set to 30 μg/well. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted, and the cells were then transferred to polyvinylidene fluoride membranes. The membranes were incubated overnight with the primary antibody (1:1000) at 4°C and then with the secondary antibody (1:2000) at room temperature for 1 h. After washing the membranes, the images were collected in a gel imaging analysis system, and the gray values of the target protein band and the internal control protein

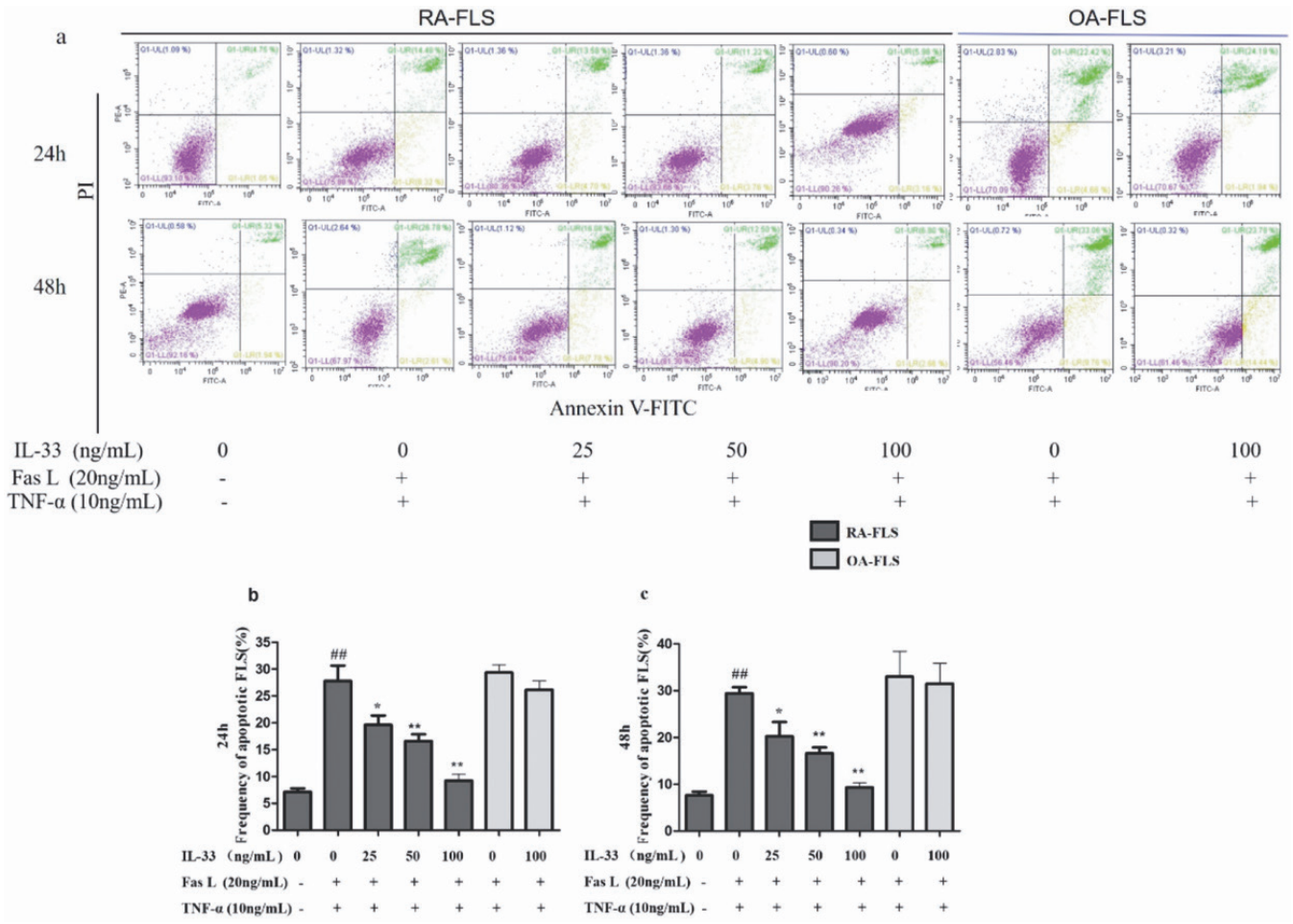

Fig. 2. Effect of interleukin-33 (IL-33) on the apoptosis of fibroblast-like synoviocytes (FLSs).
a: Results of flow cytometry showing FLS apoptosis in response to different concentrations of IL-33 (0, 25, 50, or 100 ng/mL).
b: Analysis of apoptotic rate at 24 h.
c: Analysis of apoptotic rate at 48 h.
Data presented as mean ± standard deviation, n=4.
$^{\#\#}p<0.01$ *vs*. control (IL-33, 0 ng/mL, RA FLS); $^{*}p<0.05$ *vs*. TNF-α, 20 ng/mL (RA-FLS); $^{**}p<0.01$ *vs*. TNF-α, 20 ng/mL (RA-FLS).

band were determined. The ratio of the grey value of the target protein band to the internal control protein band was used as the protein expression level.

RT-PCR to detect the effect of IL-33 on the expressions of FLS Bax and Bcl-2

FLSs were cultured and grouped as described above. Total RNA was extracted using the animal tissue/cell total RNA extraction kit according to the manufacturer's instructions. RNA was reverse transcribed into complementary DNA using a reverse transcription kit. PCR reactions were set up using a qPCR kit. The reaction conditions were as follows: 94°C for 2 min, followed by 40 cycles of melting at 94°C for 20 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s. The primer sequences used for RT-PCR were as follows: 5′-TGCCCGTTCATCTCAGTCC-3′ (forward) and 5′ -AGCACTCCCGCCACAAA-3′ (reverse) for Bax; 5′-AACATCGCCCTGTGGATGAC-3′ (forward) and 5′ -AGAGTCTTCAGAGACAGCCAGGAG-3′ (reverse) for Bcl-2. 5′-GCACCGTCAAGGCTGAGAAC-3′ (forward) and 5′ -TGGTGAAGACGCCAGTGGA-3′ (reverse) for GAPDH. The relative expressions of Bax and Bcl-2 were normalised to that of the GAPDH as the internal control.

ELISA was used to assess the expressions of IL-6 and TNF-α in the FLS culture supernatant

FLSs were seeded into a 96-well plate. After adhesion, the cells were treated with different concentrations of IL-33 for 24 h, 48 h, and 72 h. IL-1β (10 ng/mL) was used as a positive control. Subsequently, the culture supernatant was collected to assess the expression levels of IL-6 and TNF-α, according to the manufacturer's instructions.

Effect of IL-33 on the FLS NF-κB signal transduction pathway

Using the methods described above to culture FLSs, the cells were grouped as follows: FLS control, FLS+IL-33, and FLS+IL-33+dexamethasone (an NF-κB inhibitor, 40 ng/mL, Lot DXM-38-01, San Diego, USA).

The FLS+IL-33+dexamethasone group was treated with the inhibitor for 3 h, and then the FLS+IL-33 and FLS+IL-33+dexamethasone groups were treated with 50 ng/mL IL-33 for 24h. Western blotting was performed to evaluate the level of p-NF-κB protein in each group.

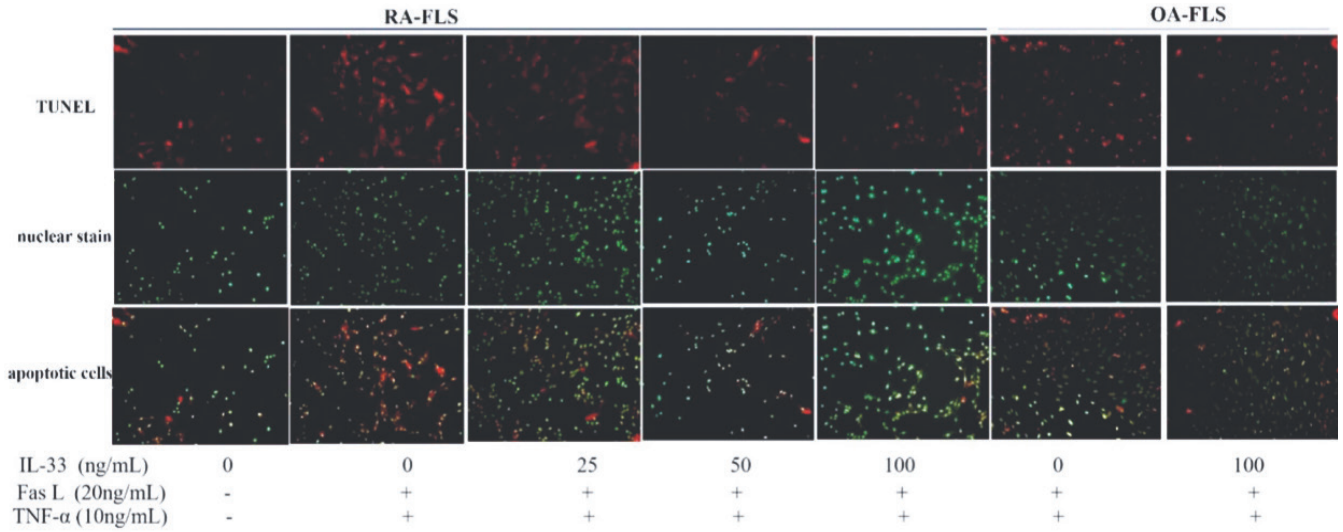

Fig. 3. Results of TUNEL assay showing the effect of IL-33 on the apoptosis of FLSs. TUNEL positive cells are stained red, cell nuclei are stained green; the apoptotic cells are stained orange.

Statistical analyses

All data analyses were performed using the SPSS 17.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance was used to assess the differences among the groups, LSD-*t* test was used for pair-wise comparisons. *p*-values <0.05 were considered indicative of statistical significance. The figures were created using GraphPad Prism 8 (https://www.graphpad.com/scientific-software/prism/).

Results

IL-33 increased the proliferation of RA FLSs

The 24h, 48h, and 72h RA FLS (TNF-20 ng/mL) groups showed significant differences from the control group (IL-33, 0 ng/mL, RA FLS) ($p<0.05$, $p<0.01$, and $p<0.01$, respectively). The 24 h and 48 h IL-33, 25 ng/mL (RA FLS) groups showed significant differences from the control group (IL-33, 0 ng/mL, RA FLS) ($p<0.05$); 72 h IL-33, 25 ng/mL (RA FLS) group was also significantly different from the control group (IL-33, 0 ng/mL, RA FLS) ($p<0.01$). Compared with the control group (IL-33, 0 ng/mL, RA FLS), the 24 h, 48 h, 72 h IL-33, 50 ng/mL (RA FLS) and IL-33, 100 ng/mL (RA FLS) group showed significant differences ($p<0.01$). Compared with the IL-33, 0 ng/mL (OA FLS) group, the 48 h and 72 h, IL-33, 100 ng/mL (OA FLS) groups were significantly different ($p<0.01$). Compared with IL-33, 0 ng/mL (OA FLS), other OA FLS groups were not significantly different $p>0.05$). The above results indicate that RA-FLSs are more sensitive to the proliferative effect of IL-33 than OA-FLSs (Fig. 1).

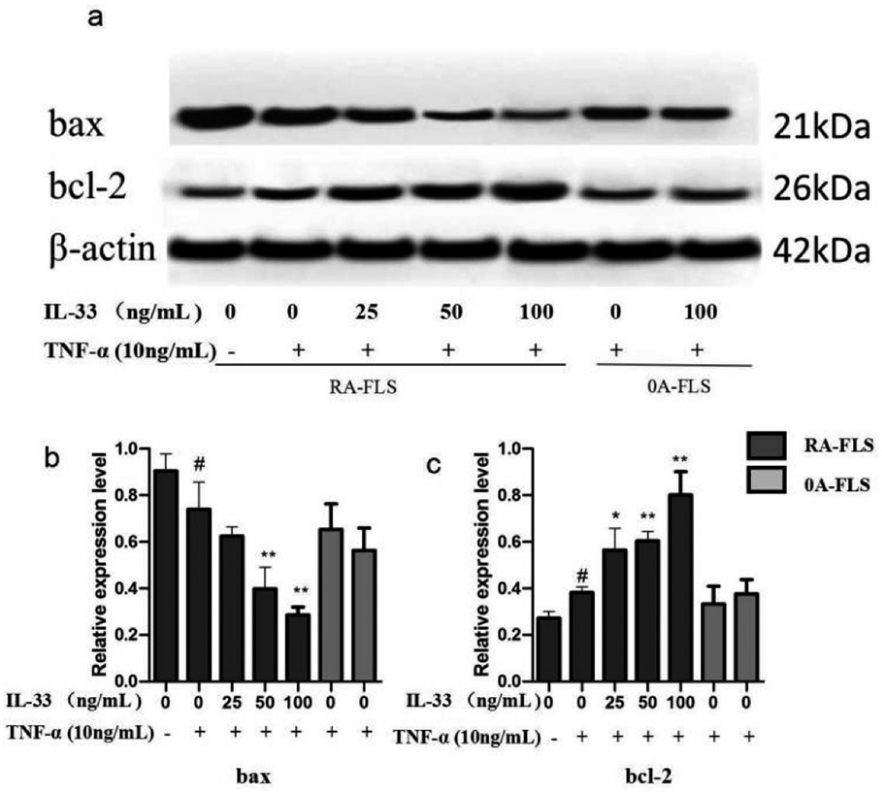

Fig. 4. Effect of interleukin-33 (IL-33) on fibroblast-like synoviocyte (FLS) B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax).
a: Results of Western blotting showing the expressions of FLS Bcl-2 and Bax proteins in response to different concentrations of IL-33 (0, 25, 50, or 100 ng/mL).
b: Relative expression levels of Bax protein.
c: Relative expression levels of Bcl-2 protein.
Data presented as mean ± standard deviation, n=3.
#$p<0.05$ *vs.* control (IL-33, 0 ng/mL, RA FLS); *$p<0.05$ *vs.* TNF-α, 10 ng/mL control group (RA-FLS); **$p<0.01$ *vs.* TNF-α, 10 ng/mL control group (RA-FLS).

IL-33 inhibited the apoptosis of RA FLSs

The rate of apoptosis of RA FLSs in the Fas L, 20 ng/mL+TNF-α, 10 ng/mL (RA-FLS) group was significantly greater than that in the IL-33 groups (RA-FLS); this indicated that IL-33 inhibited the apoptosis of RA FLS in a dose-dependent manner. IL-33 did not inhibit the apoptosis of OA FLSs (Fig. 2).

Results of TUNEL assay showing the effect of IL-33 on the apoptosis of FLSs

Apoptotic cells were stained orange. The rate of RA FLS apoptosis in the Fas L, 20 ng/mL+TNF-α, 10 ng/mL (RA-FLS) was significantly greater than that in the IL-33 groups (RA-FLS); this indicated that IL-33 inhibited RA FLS apoptosis in a dose-dependent manner. IL-33 was not found to inhibit the apoptosis of OA FLSs (Fig. 3).

Effect of IL-33 on expression levels of Bcl-2 and Bax in FLSs detected using Western blotting

The expression levels of RA FLS Bax protein in the TNF-α, 10 ng/mL control group were significantly greater than those in the IL-33 groups at 50 and 100 ng/mL; the expression levels of RA FLS Bcl-2 protein in the TNF-α, 10 ng/mL control group was significantly lesser than that in the IL-33 groups, and the differences were dose dependent. This indicated that IL-33 increased the expression of anti-apoptotic protein Bcl-2 and decreased the expression of pro-apoptotic protein Bax. IL-33 showed no significant effect on the expressions of Bcl-2 and Bax in OA-FLSs (Fig. 4).

Effect of IL-33 on the expression levels of Bcl-2 and Bax in FLSs detected using RT-PCR

The expression level of RA FLS Bax mRNA in the TNF-α, 10 ng/mL control group was significantly greater than that in the IL-33 groups at 50 and 100 ng/mL. The expression levels of RA FLS Bcl-2 mRNA in the TNF-α, 10 ng/mL control group was significantly lesser than those in the IL-33 groups, and the differences were dose-dependent. This indicated that IL-33 increased the expression of anti-apoptotic mRNA Bcl-2 and decreased the expression of pro-apoptotic mRNA Bax. IL-33 had no significant effect on the Bcl-2 and Bax mRNA expressions in OA-FLSs (Fig. 5).

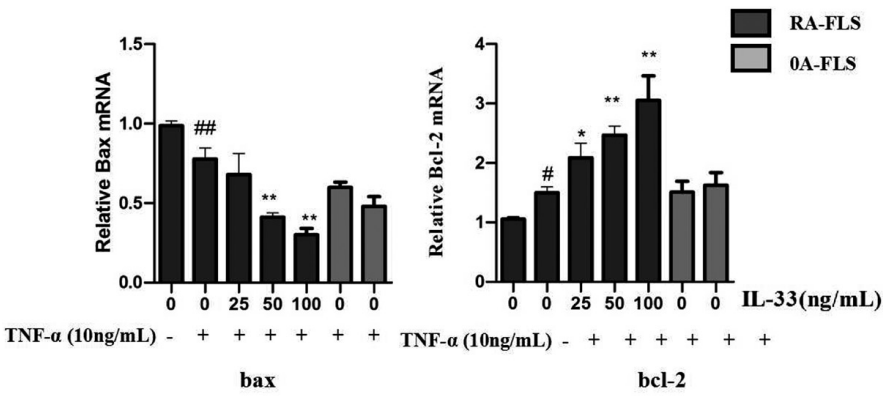

Fig. 5. Effect of interleukin-33 (IL-33) on the expressions of B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) in fibroblast-like synoviocytes (FLS).
Data presented as mean ± standard deviation, n=3;
$^{\#}p<0.05$ *vs*. control (IL-33, 0 ng/mL, RA FLS); $^{\#\#}p<0.01$ *vs*. control (IL-33, 0 ng/mL, RA FLS); * <0.05 *vs*. TNF-α, 10 ng/mL control group (RA-FLS); $^{**}p<0.01$ *vs*. TNF-α, 0 ng/mL control group (RA-FLS).

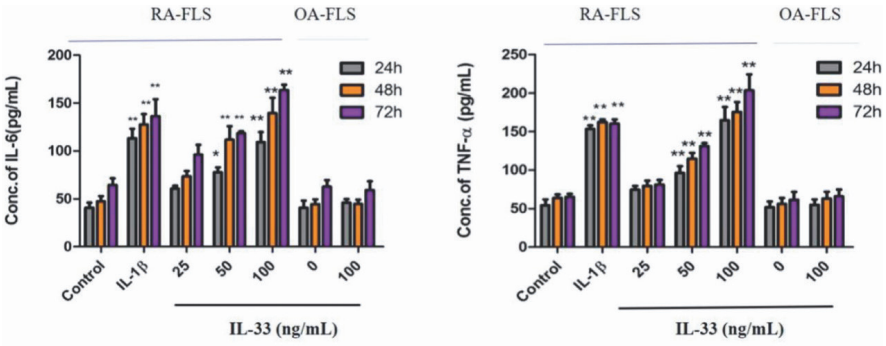

Fig. 6. Effects of different concentrations of interleukin-33 (IL-33) (0, 25, 50, or 100 ng/mL) on the secretion of IL-6 and tumour necrosis factor (TNF)-α by fibroblast-like synoviocytes (FLSs).
Data presented as mean ± standard deviation, n=5.
$^{*}p<0.05$ *vs*. control group; $^{**}p<0.01$ *vs*. control group.

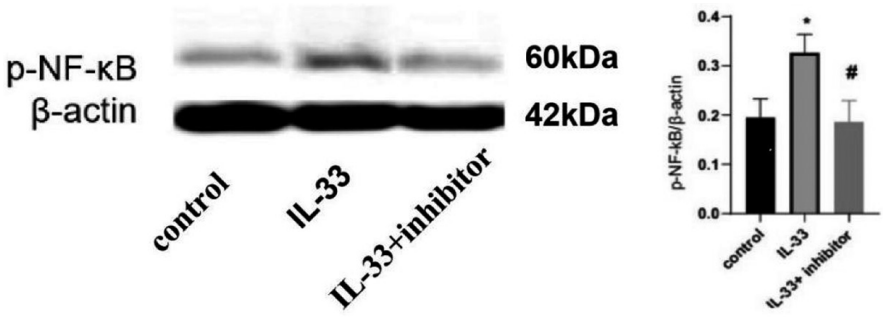

Fig. 7. Interleukin+33 (IL-33) activated nuclear factor-kappa light chain enhancer of activated B cells (NF-κB) in fibroblast-like synoviocytes (FLSs).
Data presented as mean ± standard deviation, n=4.
$^{*}p<0.05$ compared to control FLS, $\#p<0.05$ compared to FLS treated with IL-33.

IL-33 promotes the secretion of IL-6 and TNF-α by FLSs

There was no significant difference in the expression levels of IL-6 and TNF-α between the control group and the IL-33 group at 25 ng/mL (RA FLS). However, IL-33 at 50 and 100 ng/mL significantly increased the secretion of IL-6 and TNF-α in the culture supernatant. IL-33 had no significant effect on the expressions of IL-6 and TNF-α in OA-FLSs (Fig. 6).

Effect of IL-35 on the FLS NF-κB signal transduction pathway

The level of p-NF-κB in the IL-33 group at 50 ng/mL was higher than that in the control and FLS+IL-33+dexamethasone groups ($p<0.05$). There was no significant difference between the level of p-NF-κB in the FLS+IL-33+ dexamethasone group and that in the control group ($p>0.05$) (Fig. 7).

Discussion

RA is a common systemic autoimmune disease. Both innate and adaptive immune responses are implicated in the causation of this disease (17). The condition is characterised by pathological tumour-like hyperplasia of FLSs that leads to synovitis and vasospasm, which play a role in bone destruction. A complex network of inflammatory cytokines (such as IL-6, TNF-α, and IL-17) also plays an important role by promoting the production of receptor activator of nuclear factor-kappa B ligand (RANKL) by the FLSs. This further induces osteoclastogenesis and bone destruction and accelerates the destruction of joint structure (18). Therefore, proliferation and apoptosis of RA FLSs play a key role in the progression or regression of RA.

IL-33, a member of the IL-1 family (19), is localised in the nucleus during the early stages of infection or inflammatory disease response. It was originally described as a cytokine that is passively released by the necrotic epithelial and endothelial cells; however, expressions of IL-33 were subsequently found in macrophages, dendritic cells, mast cells, smooth muscle cells, fibroblasts, glial cells, osteoblasts, synovial cells, and adipocytes. Patients with RA have increased expression of IL-33 in the serum and synovial tissues; in addition, there is a high expression of IL-33 mRNA in FLSs (20-22). IL-33 and its receptor ST2 are expressed in both osteoclasts and osteoblasts, which play a significant role in bone metabolism. Moreover, IL-33 was shown to activate osteoclastogenesis by inducing RANKL and macrophage colony-stimulating factor in the extracellular milieu (23, 24) and to promote bone resorption (25). IL-33 is also involved in the RA inflammatory environment; it was shown to activate mast cells, promote neutrophil migration and production of chemotactic factors, and to induce production of proinflammatory cytokines by macrophages (26). IL-33 was found to regulate T helper 1 (Th1)/Th17 differentiation in CIA (27); in addition, IL-33 was shown to induce the production of TNF-α and IL-8 in human synovial tissue mast cells (28), while IL-10 was found to inhibit the development of CIA through IL-33 signalling (29). These findings indicate the involvement of IL-33 in the progression of RA.

To study the effects of IL-33 on the proliferation and apoptosis of FLSs, we cultured RA FLSs and used OA FLSs as control. We found that IL-33 promoted the proliferation of RA FLSs and inhibited apoptosis in a concentration-dependent manner. IL-33 reduced the expression of pro-apoptotic Bax and increased the expression of anti-apoptotic Bcl-2 in FLSs, thereby promoting the proliferation of RA FLSs. Synovial thickening in the RA joint is the result of hyperplasia and hypertrophy of the synovial-lining cells; FLS is the main cell type in that layer. Our study demonstrated that IL-33 can aggravate the thickening of RA synovium by promoting proliferation and inhibiting apoptosis of FLSs. Hyperproliferation of FLSs can induce vasospasm, which can accelerate the degradation of cartilage and bone in patients with RA. Our results also show that IL-33 can increase the expressions of IL-6 and TNF-α in FLSs. In an inflammatory environment, IL-6, TNF-α, and IL-17 can promote the production of RANKL by FLSs, which is an important factor that promotes osteoclastogenesis. These results suggested that IL-33 may cause bone destruction in RA patients by promoting the proliferation of FLSs. In our study, high concentrations of IL-33 were found to promote the proliferation of OA FLS at 48 and 72 hours; however, there was no significant effect on the apoptosis of OA FLSs, expressions of Bax and Bcl-2, and the secretion of inflammatory cytokines IL-6 and TNF-α. This indicates that IL-33 has no significant effect on OA FLSs.

IL-33 may be a therapeutic target for treatment of RA and may stop associated bone destruction. Our study also found that IL-33 exerts a biological effect on FLSs by activating the NF-κB signalling pathway; this indicates that the effects of IL-33 may be mediated through the RANKL-RANK-osteoprotegerin system. However, additional studies are required to further explore this aspect.

Conclusion

IL-33 inhibited the apoptosis of RA FLSs by activating the NF-κB signalling pathway and promoted the proliferation of FLSs. Use of new biologics that can block IL-33 is a potential novel approach for clinical treatment of RA.

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