Activin A Expressed in Rheumatoid Synovial Cells Downregulates TNF α -Induced CXCL10 Expression and Osteoclastogenesis

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Keywords

Rheumatoid arthritis · Activin A · CXCL10 · Osteoclastogenesis

Abstract

Objective: Activin A is known to be highly expressed in rheumatoid synovium. In the present study, we investigated the effect of inflammatory cytokines on activin A production and its role in rheumatoid inflammation using freshly prepared rheumatoid synovial cells (fresh-RSC). **Methods:** Fresh-RSC from patients with rheumatoid arthritis were obtained and stimulated with multiple cytokines for activin A production. Gene expression levels of activin A and inflammatory cytokines were determined by quantitative PCR (qPCR) analysis. An enzyme-linked immunosorbent assay (ELISA) was used to measure activin A and CXCL10 in culture supernatants. The osteoclasts generated from human peripheral monocytes by RANKL stimulation were identified by tartrate-resistant acid phosphatase staining and bone resorption assay using Osteo plate. The expression levels of NFATc1 and cathepsin K, critical intracellular proteins for osteoclastogenesis, were determined by Western blotting. **Results:** Activin A produc-

tion in fresh-RSC was markedly enhanced by the synergistic effect of TGF- β_1 with inflammatory cytokines, including TNF α , IL-1 β , and IL-6. Activin A inhibited TNF α -induced CXCL10, an important chemoattractant for pathogen-activated T cells and monocytes of osteoclast precursors, but it did not affect the expression of inflammatory cytokines and chemokines. In addition, activin A directly inhibited the expression of NFATc1 and cathepsin K, as well as osteoclast formation in human samples. **Conclusion:** Our data indicated that TGF- β_1 is involved in the expression of activin A at inflamed joints. Activin A mainly exerts an anti-inflammatory action, which prevents joint damage via the regulation of CXCL10 and osteoclastogenesis.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease with hyperplasia of synovial cells and infiltration of inflammatory cells at the inflamed joint, and persistent inflammation results in progressive destruction of the articular cartilage and the subchondral bone [1, 2]. Al-

though the bone erosion of inflammatory joints is caused by osteoclast progenitor cells and mature osteoclasts [3, 4], a variety of cytokines and growth factors involved in the inflammatory process play important roles in the differentiation of osteoclasts [5, 6].

Activin A, a homodimer structurally composed of 2 β A subunits (β A β A), is expressed in a number of tissues including ovaries, pituitary gland, brain, placenta, and bone marrow [7]. Interestingly, activin A is also expressed by cells involved in rheumatoid inflammation, such as bone marrow macrophages, monocytes, osteoblasts, osteoclasts, and endothelial cells [7–11]. Additionally, activin A is highly expressed in the inflamed synovium of patients with RA [9, 10]. Activin A is reported to be expressed in fibroblast-like synoviocytes (FLS) of the lining and sublining layers, and in macrophages of the active rheumatoid synovium [10]. Furthermore, the activin A level in the synovial fluid of RA patients has been shown to be higher than that of systemic lupus erythematosus or osteoarthritis [12].

The pathophysiological role of activin A in RA inflammation remains controversial. In murine models, administration of activin A induces the release of IL-6 [13, 14], whereas neutralizing activin A modulates the release of TNF α , IL-1 β , and IL-6 [15]. In addition, activin A can regulate human macrophage polarization, directing them toward an inflammatory M1 phenotype [16]. On the other hand, activin A has been reported to inhibit the production of MMP-3 [17] and inflammatory cytokines such as IL-1 β , IL-6, and IL-18 in a variety of cell types [18–20].

The functional role of activin A in osteoclastogenesis also remains unclear. Activin A has been shown to stimulate RANKL-induced osteoclast differentiation. Inhibition of activin A by its soluble receptor can inhibit osteoclast differentiation [8, 21, 22]. In contrast, activin A has been shown to act as a local regulator during osteoclast development by exerting a dominant negative effect on the motility and lifespan of osteoclasts [23]. Furthermore, activin A acts as a negative regulator of osteoclast motility via activation of SMAD2 and AKT1 [23].

These conflicting data prompted us to clarify the regulation of expression and the pathophysiological role of activin A in RA. In the present study, to understand the role of activin A in osteoclastogenesis, we evaluated the effect of inflammatory cytokines on activin A production in freshly prepared rheumatoid synovial cells (fresh-RSC). In addition, we examined the effect of activin A on cytokine expression in fresh RSC and RANKL-induced osteoclastogenesis in human monocytes.

Materials and Methods

Preparation of Fresh-RSC

A total of 6 patients with RA diagnosed based on the revised 1987 American College of Rheumatology criteria for RA were recruited. Synovial tissues were collected from 6 patients who underwent arthroplastic joint surgery and synovectomy.

Synovia obtained from RA patients were aseptically dissected from the surrounding tissues, minced, and enzymatically digested with 2 mg/mL *Clostridium* collagenase (Wako Pure Chemical Industries, Ltd, Osaka, Japan) and 5–10 μ g/mL deoxyribonuclease 1 (Sigma Chemical Co., St. Louis, MO, USA) for 2–3 h. After digestion, the resulting single cell suspension was washed, filtered through sterile gauze and nylon mesh, washed thoroughly again, and finally resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum. The cells were then cultured overnight to allow the cells to adhere to the culture plate. After washing the plate to remove non-adherent cells, the remaining adherent cells were cultured with the indicated cytokines, and adherent cells of 2–3 passages were further used in this study. The recombinant human cytokines used in this study were as follows: IL-1 β , TNF α , IL-6, IL-6Ra, IL-17A, M-CSF (all BioLegend, San Diego, CA, USA), TGF- β ₁, activin A, and RANKL (all Wako Pure Chemical Industries).

Preparation of Peripheral Blood Monocytes

Mononuclear cells from the peripheral blood of healthy donors were separated by Lympholyte[®]-H (Cedarlane, NC) density gradient centrifugation. Highly purified monocytes were separated using MACS microbeads (Pan Monocyte Isolation Kit[®], Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). All procedures were performed according to the manufacturers' instructions. More than 92% of the isolated cells were found to be CD14+, as determined by flow cytometry.

qPCR Analysis

Cells were lysed, and total RNA was isolated using a NucleoSpin[®] RNA kit (Takara-Bio Japan). Total RNA (200 ng) was then reverse transcribed to cDNA using a PrimeScript[™] RT reagent kit with gDNA Eraser (Takara-Bio, Japan). qPCR was then performed using Brilliant III ultrafast SYBR Green qPCR master mix (Agilent Technologies, CA) with the CFX connect real-time PCR detection system (Bio-Rad Laboratories, Inc.). PCR amplification was performed for 40 cycles at 95 °C for 5 s and 60 °C for 10 s. The primers used in this study are shown in Table 1. Gene expression was normalized to β -actin for each sample.

ELISA for Activin A and CXCL10

Activin A and CXCL10 concentrations in culture supernatant were measured using a sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

In vitro Osteoclastogenesis

Peripheral blood monocytes (PBMs) were cultured in a 96-well tissue culture plate at a concentration of 2×10^4 cells/well. Cultures were maintained in 200 μ L of α -minimal essential medium containing 10% fetal bovine serum for 7–10 days with M-CSF (BioLegend) and RANKL (Wako Pure Chemical Industries) in the presence or absence of activin A (Wako Pure Chemical Industries). The cells were pretreated with M-CSF and/or activin A for 24 h,

Table 1. Primers used for real-time PCR

Gene	Sense	Antisense
<i>Activin A</i>	5'-GAGAATTTGCTGAAGAGGAG-3'	5'-CAAAAGTTGTTGTGATTGCC-3'
<i>IL-1β</i>	5'-CTAAACAGATGAAGTGCTCC-3'	5'-GGTCATTCTCCTGGAAGG-3'
<i>TNFα</i>	5'-AGGCAGTCAGATCATCTTC-3'	5'-TTATCTCTCAGCTCCACG-3'
<i>IL-6</i>	5'-GCAGAAAAAGGCAAAGAATC-3'	5'-CTACATTTGCCGAAGAGC-3'
<i>IL-10</i>	5'-GCCTTTAATAAGCTCCAAGAG-3'	5'-ATCTTCATTGTTCATGTAGGC-3'
<i>IL-12A</i>	5'-AAGACCTCTTTTATGATGGC-3'	5'-CATTTCATGGTCTTGAAGTCC-3'
<i>IL-23A</i>	5'-AGATAAATCTACCACCCAG-3'	5'-CACATGTCAGTCAGTATTGG-3'
<i>CXCL5</i>	5'-ATTTGTCTTGATCCAGAAGC-3'	5'-TCAGTTTTCCTTGTTTCCAC-3'
<i>CXCL8</i>	5'-GTTTTTGAAGAGGGCTGAG-3'	5'-TTTGCTTGAAGTTTCACTGG-3'
<i>CXCL9</i>	5'-AGGTCAGCCAAAAGAAAAAG-3'	5'-TGAAGTGGTCTCTTATGTAGTC-3'
<i>CXCL10</i>	5'-AAAGCAGTTAGCAAGGAAAG-3'	5'-TCATTGGTCACCTTTTATGTG-3'
<i>CCL2</i>	5'-AGACTAACCCAGAAACATCC-3'	5'-ATTGATTGCATGTGGCTG-3'
<i>CCL3</i>	5'-TCTCTGCAACCAGTTCTC-3'	5'-AATTCTGTGGAATCTGCC-3'
<i>CCL5</i>	5'-AAGTCTCTAGGTTCTGAGC-3'	5'-TTTTATGGTTGCATTGAGAAC-3'
<i>CCL20</i>	5'-TATATTGTGCGTCTCCTCAG-3'	5'-GCTATGTCCAATTCCATTCC-3'
<i>β-Actin</i>	5'-GACGACATGGAGAAAATCTG-3'	5'-ATGATCTGGGTTCATCTTCTC-3'

followed by the addition of RANKL to the culture medium. Every 3 days, half of the culture medium was replaced with fresh medium containing cytokines and chemicals.

Tartrate-Resistant Acid Phosphatase Staining

The cells were fixed, and tartrate-resistant acid phosphatase (TRACP) staining was performed using an acid phosphatase, leukocyte (TRACP) staining kit (Sigma-Aldrich Co.) according to the manufacturer's instructions. Cells that stained dark red were considered as differentiated osteoclast-like cells, and cells with ≥ 3 nuclei were designated as multinucleated cells. TRACP-positive, multinucleated cells were counted on 96-well plates with a light microscope (EVOS[®] XL Core Cell Imaging System, Thermo Fisher Scientific, Japan).

In vitro Assays for Osteoclast Resorption

To measure bone-resorptive function, PBMs were cultured at a density of 2×10^4 cells/well on an Osteo plate (Corning, New York, NY, USA). The Osteo plate comprises a surface made of an inorganic crystalline calcium phosphate coating that mimics living bone material for 7–10 days. After culturing, the adherent cells were lysed by osmotic shock and stained with 5% silver nitrate (Sigma-Aldrich) in PBS to visualize the resorption area under an EVOS microscope using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Western Blotting

PBMs cultured with RANKL and M-CSF with or without activin A were washed with PBS, and protein was extracted using a radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 8.0, 150 mM sodium chloride, 0.5 w/v% sodium deoxycholate, 0.1 w/v% SDS, and 1.0 w/v% NP-40 substitute; Wako Pure Chemical Industries). Total cell lysates were obtained by centrifugation at 12,000 rpm for 10 min at 4°C. Protein levels were determined, and equal protein levels were adjusted by the Pierce[™] BCA protein assay (Thermo Fisher Scientific) according to the manufacturer's in-

structions. Samples were loaded on 10% SDS-polyacrylamide gels (Wako Pure Chemical Industries) and transferred to polyvinylidene fluoride (PVDF) membranes (Novex). Next, the membranes were washed with TBS-T (25 mM Tris-HCl, pH 7.4, 0.1 w/v% Tween-20, 137 mM NaCl, 2.68 mM KCl; Wako Pure Chemical Industries) and blocked with 4% skim milk powder (Wako Pure Chemical Industries) for 1–2 h at room temperature. Following blocking, the membranes were incubated with primary antibodies against NFATc1 (BioLegend), cathepsin K (Abcam plc, Cambridge, UK), and β -actin (Wako Pure Chemical Industries) overnight at 4°C, and then incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Tokyo, Japan) for 1 h at room temperature. The membranes were washed several times with TBS-T between the blocking and incubation steps at room temperature. The protein signals were developed with an enhanced chemiluminescence reagent (ImmunoStar[®] LD; Wako Pure Chemical Industries) using an Image Quant[™] LAS 500 (GE Healthcare) according to the manufacturer's instructions.

Statistical Analysis

All statistical analyses were performed using Student's *t* test. A value of $p < 0.05$ was considered to be statistically significant. The 95% confidence intervals were calculated using GraphPad Prism software.

Results

Synergistic Increase in Activin A Expression in Fresh-RSC Stimulated with TGF- β_1 and other Inflammatory Cytokines

Several studies have demonstrated that expression of activin A in human synovial fibroblasts is induced by inflammatory cytokines such as IL-1 β , TNF α , and TGF- β_1

Fig. 1. Effect of proinflammatory cytokines (concentration: 50 ng/mL, respectively) on the production of activin A in freshly prepared rheumatoid synovial cells (fresh-RSC). **a, b** Fresh-RSC were cultured with IL-1 β , TNF α , IL-6 with IL-6R α , IL-17A, and TGF- β_1 either alone or in combination for 48 h. **c** In the dose-dependent experiments, fresh-RSC were cultured with the indicated cytokines in the presence of increasing TGF- β_1 doses for 48 h. Activin

A levels in the culture supernatant were determined by ELISA. **d** Fresh-RSC were cultured in the presence of the indicated cytokines either alone or in combination for 24 h. Gene expression of *activin A* was measured by quantitative PCR analysis. The data were normalized to those of β -actin. Results represent the mean $\pm$ SD ($n = 3$).

Fig. 2. Effect of activin A on the expression of inflammatory cytokines and chemokines with or without TNF α . **a** Freshly prepared rheumatoid synovial cells (fresh-RSC) were cultured with activin A (50 ng/mL) and/or TNF α (50 ng/mL) for 24 h. Gene expression levels of inflammatory cytokines (*IL-1 β* , *TNF α* , *IL-6*, *IL-10*, *IL-12A*, and *IL-23A*) and chemokines (*CXCL5*, *CXCL8*, *CXCL9*, *CCL2*, *CCL3*, *CCL5*, and *CCL20*) were assessed and normalized to β -actin by quantitative PCR (qPCR) analysis. **b** Fresh-RSCs were cultured with TNF α and/or activin A for 24 h. *CXCL10* gene ex-

pression was determined by qPCR analysis. Data represent 6 experiments conducted using synovial samples obtained from 4 patients with RA. * $p < 0.05$. **c** Fresh-RSC were cultured with increasing doses of activin A in the presence of TNF α (50 ng/mL) for 48 h. After the culture, CXCL10 levels in cultured supernatants were determined using an ELISA kit (R&D). Data represent the mean $\pm$ SD from triplicate experiments. * $p < 0.05$, *** $p < 0.001$, vs. TNF α .

Fig. 3. Analysis of the effect of activin A on RANKL-induced osteoclast formation from human peripheral monocytes. **a, b** TRACP staining: human peripheral monocytes were cultured with M-CSF (50 ng/mL) in the presence of increasing doses of activin A for 24 h, followed by the addition of RANKL (20 ng/mL). After 5–7 days, the cells were stained with TRACP. **c** Bone resorption activity: human peripheral monocytes were cultured with M-CSF (50 ng/

mL) with or without activin A (20 ng/mL) for 24 h, followed by the addition of RANKL (20 ng/mL) on Osteo Assay Surface 96-well plates for 7–10 days. After culturing the cells, the adherent cells were lysed by osmotic shock and stained with 5% silver nitrate (Sigma-Aldrich) in PBS to quantify the resorption area using an EVOS microscope with ImageJ software. Data represent the mean $\pm$ SD from triplicate experiments. ** $p < 0.01$, *** $p < 0.001$.

[10]. However, the culture conditions and sources of fibroblasts and FLS varied among different studies, which might have complicated the interpretation of results. Therefore, we first examined the effect of proinflammatory cytokines on the expression of activin A in fresh-RSC from 3 patients with RA. The expression of an *activin A* gene was quantified by qPCR, and protein concentrations in the cultured media were determined by ELISA. As shown in Figure 1a–d, the induction of activin A was

minimal in response to treatment with individual cytokines, including IL-1 β , TNF α , IL-6, IL-17A, and TGF- β ₁, which were previously reported to induce activin A. In contrast, the combination of cytokines synergistically enhanced both the protein and mRNA expression levels of activin A (Fig. 1a, d). Notably, TGF- β ₁ potentially enhanced the production of activin A in combination with IL-1 β , TNF α , IL-6, and IL-17A (Fig. 1b). In our dose-response experiment, the effect of TGF- β ₁ reached a plateau at a

Fig. 4. Peripheral blood monocytes were cultured at a cell density of 2×10^5 cells/well in a 24-well culture plate in the presence of M-CSF (50 ng/mL) with or without activin A (20 ng/mL) for 24 h, followed by the addition of RANKL (50 ng/mL). Following culture for 72 h, protein expression of NFATc1 and cathepsin K in the treated cells was determined by Western blotting. β -Actin protein levels were determined as a loading control.

concentration of 1 ng/mL (Fig. 1c). These data suggest that TGF- β_1 is the primary cytokine involved in activin A induction.

Inhibition of TNF α -Induced CXCL10 Expression by Activin A

To clarify the role of activin A in RA inflammation, we next screened the expression of inflammatory cytokines (IL-1 β , TNF α , IL-6, IL-10, IL-12A, and IL-23A) and chemokines (CXCL5, CXCL8, CXCL9, CXCL10, CCL2, CCL3, CCL5, and CCL20) using qPCR analysis. As shown in Figure 2a, activin A did not stimulate RSC to induce these inflammatory cytokines. On the other hand, activin A significantly inhibited the TNF α -induced expression of CXCL10, a chemokine involved in chemoattraction of T cells and monocytes at both the protein and the mRNA level (Fig. 2b, c). This activin A-induced inhibition was consistently observed in fresh-RSC from 5 patients with RA (Fig. 2b). Dose-response analysis revealed that significant suppression was observed at the concentration of 1 ng/mL of activin A (Fig. 2c).

Inhibitory Effect of Activin A on RANKL-Induced Osteoclast Generation in Human Peripheral Monocytes

Although it has been shown that osteoclasts play an important role in joint destruction in RA, the role of activin A in osteoclastogenesis remains unclear. Therefore, we next evaluated the effect of activin A on RANKL-induced osteoclast formation from human monocytes. The resulting osteoclasts were identified with TRACP staining (Fig. 3a, b), and their bone resorption activity was assessed using Osteo plates (Fig. 3c). These data show that RANKL treatment induced osteoclast generation from PBMs, while activin A potentially inhibited RANKL-mediated osteoclast generation. Notably, activin A-induced inhibition occurred in a dose-dependent manner (Fig. 3b). In addition, as shown in Figure 4, activin A potentially inhibited the expression of RANKL-induced expression of NFATc1 (the critical signal molecule) and cathepsin K (a marker of osteoclastic differentiation).

Discussion

In the current study, we demonstrated that various inflammatory cytokines have either additive or synergistic effects on the expression of activin A in RSCs, and that TGF- β_1 is extremely important for the induction of activin A expression. Expression of activin A by FLS is stimulated by inflammatory cytokines such as TNF α , IL-1 β , and TGF- β_1 , and IL-1 β appears to be the strongest inducer of activin A secretion in FLS [10]. In contrast, our data showed that stimulation of these inflammatory cytokines alone resulted in minimal induction of activin A, which may be attributed to cell-type specific differences. Notably, fresh-RSC used in this study also contained CD14+ macrophages. To the best of our knowledge, this is the first study to report a strong synergistic relationship between TGF- β_1 and inflammatory cytokines such as IL-1 β , TNF α , and IL-6 in RSCs.

TGF- β_1 , which is expressed in the rheumatoid synovium [24], is a major T-cell cytokine released from regulatory T cells [25, 26]. A previous study reported potent induction of activin A secretion from human monocytes and bone marrow stromal fibroblasts by cognate interaction with activated T cells [27], suggesting the importance of T-cell cytokines for the induction of activin A. These data suggest that the induction of activin A in rheumatoid synovium may be related to the interaction of infiltrating T cells with synoviocytes via TGF- β_1 .

We reported for the first time that activin A suppressed TNF α -induced CXCL10 expression in rheumatoid synovium. In contrast, activin A did not affect the gene expression of inflammatory cytokines and chemokines. Consistent with the results of the current study, a number of previous studies have reported the anti-inflammatory actions of activin A, such as activin A-induced inhibition of IL-1 β expression, IL-6 activity [20], and matrix metalloproteinase-3 expression [17] in human tissues. In contrast, activin A has been reported to exert inflammatory actions by directing macrophage polarization toward the acquisition of an M1 proinflammatory phenotype [16]. In addition, CD14 $^{+}$ macrophages from RA synovial fluid exhibited an activin A-dependent proinflammatory profile [28]. These reports show the complexity of activin A actions in the inflammatory process. Further studies are needed to highlight the action of activin A in mediating inflammation in RA.

CXCL10 binds the receptor CXCR3 on T cells and Toll-like receptor 4 on monocytes and macrophages and upregulates immune responses through the activation and recruitment of T cells and monocytes [29–31]. CXCL10 has been detected in sera, synovial fluid, and tissues in RA patients [32, 33]. Recently, CXCL10 has been reported to recruit inflammatory cells and be involved in bone erosion. For example, CXCL10 has been shown to recruit activated T cells which mediate osteoclastogenesis and recruit monocytes, which function as osteoclast precursors, to the inflamed joints [34]. Furthermore, in vivo treatment with antibodies against CXCL10 or its receptor attenuated bone destruction in mice with collagen-induced or adjuvant arthritis [35, 36]. From these data, it is likely that activin A-induced inhibition of CXCL10 may lead to the prevention of bone destruction in RA.

Accumulation of activated osteoclasts at the inflamed joints plays a critical role in joint destruction in RA patients. Here, we demonstrated that activin A directly inhibited RANKL-induced osteoclast generation from human monocytes. Activin A also inhibited the protein expression of NFATc1 and cathepsin K. However, conflicting reports on the action of activin A on osteoclastogenesis have been published in the past. Fuller et al. [8] reported that activin A powerfully synergized with RANKL to induce osteoclast-like cells from murine bone marrow precursors, and osteoclast formation was prevented by exposure to soluble type IIA activin A receptor. Murase et al. [21] reported that the phosphorylation of p44/42 MAP kinase, p38 MAP kinase, and Smad2 is involved in activin A-enhanced osteoclast differentiation. In addition, activin A has been shown to enhance

RANK expression in osteoclast precursors [22]. In contrast, Fowler et al. [23] reported that activin A completely inhibited RANKL-stimulated osteoclast motility, differentiation, and bone resorption in stroma-free murine bone marrow macrophage cultures. Recently, Schoenmaker et al. [37] reported that activin A inhibits osteoclast formation stimulated with periodontal ligament fibroblasts from patients with fibrodysplasia ossificans progressiva, which is a genetic disease characterized by a mutation in the activin receptor type 1 (*ACVR1*) gene that results in enhanced responsiveness of the receptor to activin A. Moreover, consistent with our results, activin A inhibited RANKL-induced osteoclastogenesis in monocultures of CD14 $^{+}$ monocytes [37]. The discrepancy between the reported effects of activin A on osteoclastogenesis may be due to differences between species or culture systems.

For example, mouse bone marrow cells are used for osteoclastogenesis in murine experiments, but the possibility of contamination of adherent cells other than osteoclast precursor cells cannot be completely excluded. These cells may have modified the effects of activin A. On the other hand, activin A generally suppressed osteoclast differentiation in experiments using human monocytes, suggesting that the conflicting effects of activin A may be due to differences in species. Additional studies are needed to further clarify the mechanism of activin A action.

In summary, activin A production from fresh-RSC was markedly enhanced by a synergistic effect between TGF- β_1 and inflammatory cytokines. Activin A inhibited TNF α -induced CXCL10, an important chemoattractant for pathogenic-activated T cells and monocytes of osteoclast precursors, but it did not affect the expression of inflammatory cytokines and chemokines. In addition, activin A directly inhibited osteoclast formation in RA patients. These results suggest that activin A exerts an anti-inflammatory effect at inflamed joints to prevent joint destruction. Although additional studies are required to further elucidate this protective mechanism, our findings provide new insights into the role of activin A in protecting against joint destruction in RA patients. These findings may help to develop appropriate therapies for RA patients in the future.

Acknowledgments

The authors especially thank Dr. Yasunori Shimaoka (Hama-waki Orthopedic Hospital) for rheumatoid synovial specimens.

Statement of Ethics

The study was approved by the Institutional Research and Ethics Committee of Hiroshima University (E-668-1), and written informed consent was obtained from all participants (healthy donors and patients with RA).

Disclosure Statement

The authors have no conflict of interest to declare.

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Funding Sources

This work was supported by JSPS KAKENHI (grant No. 19K08908 to E.S., 19K18499 to S.M. and 19K07940 to S.H).

Author Contributions

E.S., S.H., S.M., and T.K.: conception and design of the study; E.S., T.K., K.O., T.T., K.Y., H.K., and Y.Y.: performing all experiments; E.S. and T.K.: drafting of the articles; E.S., S.H., S.M., and T.K.: critical revision of the articles for important intellectual content.

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