

IL-23 promotes osteoclastogenesis in osteoblast-osteoclast co-culture system

Y.K. Kang¹ and M.C. Zhang²

¹Department of Clinical Laboratory, People's Hospital of Zhengzhou, Zhengzhou, Henan Province, China

²Department of Pathology, The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan Province, China

Corresponding author: Y.K. Kang
E-mail: yunkaikang@163.com

Genet. Mol. Res. 13 (2): 4673-4679 (2014)

Received July 23, 2013

Accepted December 12, 2013

Published June 18, 2014

DOI <http://dx.doi.org/10.4238/2014.June.18.10>

ABSTRACT. The aim of this study was to determine the effect of IL-23 on the activity and proliferation of osteoclasts (OC) in co-culture with osteoblasts (OB). OB and OC were individually separated from the skull and femoral bone of a SD rat. OB-OC co-culture with IL-23 added was designed as the experimental group, while the OB-OC co-culture without IL-23 was the control group. In the experimental group, five different concentrations of IL-23 were added, and the cells were then cultured for 24, 48 and 72 h. For each concentration at these three time points, cell proliferation, tartrate-resistant acid phosphatase (TRAP) activity and the lacunae in the bone slices were evaluated, compared with control group at the same time points. Compared to the control group, proliferation and TRAP activity of OC were significantly increased at 24, 48 and 72 h with addition of 0.5 to 10 ng/mL IL-23 ($P < 0.05$). In addition, a dose- and time-dependent correlation between the effect of IL-23 and osteoclastogenesis was noticed through the comparison. Moreover, the area of lacunar resorption in each experimental group was significantly larger than in the control group (P

< 0.05). In conclusion, IL-23 promotes the proliferation, TRAP activity and bone resorption of OC in OB-OC co-culture.

Key words: Osteoclasts; Osteoblast co-culture system; Interleukin-23; Tartrate-resistant acid phosphatase

INTRODUCTION

Osteoporosis is a serious health issue in human society. Since osteoclastogenesis is abnormally induced, it renders the osteoporosis patient susceptible to bone volume reduction and spontaneous bone fracture. Excessive osteoclastogenesis is the main pathophysiologic mechanism of osteoporosis. Many cytokines, variant cell types, and endocrine functions are involved in the complicated process of osteoclastogenesis.

Osteoclasts (OC) and osteoblasts (OB) are two critical cell types in osteoclastogenesis. OB takes charge of bone accumulation and bone formation; meanwhile, OC plays a pivotal role in bone resorption and reconstruction. It is assumed that there is a dynamic balance between OC and OB activities, leading to normal bone development. Osteoporosis typically reflects that bone resorption exceeds bone formation.

Recently, the function of several interleukins in OC-OB activities have been highlighted. For example, IL-1 β has been shown to improve the adhesion between OC and bone micro-architecture and would thereby promote bone resorption (Tsutsumimoto et al., 1999). IL-6, another important immune regulator, has been shown to be involved in enhancing the growth and activities of osteoclasts. Haden et al. (2000) studied the effect of age on a few serum, including IL-6, and radiological parameters. In addition, IL-4 and IL-13 have been reported to suppress osteoclastogenesis and bone resorption (Ura et al., 2000). Moreover, IL-18 may regulate the differentiation of OB by alerting the function of T cells (Dai et al., 2004).

Interestingly, as a clinically novel member of the IL-12 family (Boniface et al., 2008), IL-23 indirectly regulates the secretion of IL-6 and IL-8 by targeting RANKL of Th17 cells, leading to chronic inflammation and bone deformation (Sato et al., 2006; Paradowska et al., 2007). Chen et al. (2010) hypothesized that IL-23 could stimulate osteoclast differentiation and bone formation. It has been demonstrated that lipopolysaccharide induces OB to secrete IL-23 (Chen et al., 2011), indicating that IL-23 also plays a regulatory role in the balance of bone formation and resorption. However, the pathologic mechanism is still unclear.

To clarify the relation between IL-23 and OC activities in bone formation-resorption balance, we determined how IL-23 affected cell proliferation, tartrate-resistant acid phosphatase (TRAP) activity and bone resorption using an OB-OC co-culture system. OB-OC co-culture had been previously well established, and all three major parameters, cell proliferation, TRAP activity and area of lacunar resorption, were deeply investigated in this study.

MATERIAL AND METHODS

Experimental animals

Newborn SD rats at 1 and 5 days were used in this experiment. Animals were purchased from the Experimental Animal Center, Henan Province [Protocol No. SCXK (YU) 2005-0001]. This study was carried out in strict accordance with the recommendations in the

Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol had been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the People's Hospital of Zhengzhou.

Isolation and culture of rat osteoblasts

According to the literature (Wu et al., 2004; Chen et al., 2008), on the first day after birth, the rat skull was isolated in a superclean hood. The skull was gently washed three times with PBS. Soft tissue of the skull was cut into small pieces, and 0.25% trypsin was added for digestion. The tissue was digested at 37°C for 30 min, followed by another 1-h digestion with 0.1% collagenase type α (Gibco, Grand Island, NY, USA). Single cells were obtained by filtering the cell suspension with a 40- μ m cell strainer. The cell suspension was centrifuged at 1000 rpm for 10 min. The supernatant was then aspirated and 5 mL DMEM culture medium (Invitrogen, Carlsbad, CA, USA) containing 15% fetal bovine serum (FBS) (Sijiqing Inc, Hangzhou, China) and 1% 100X Pen/Strep were added. After gently mixing the cell pellets several times, the cells were cultured in the incubator until the formation of a cell monolayer. Cells were passaged after treating with 0.25% trypsin, and the third passage was used for experiments.

Isolation and culture of rat osteoclasts

According to the literature (Chen et al., 2008), on the fifth day after birth, the rat femoral bone was isolated in a superclean hood and gently washed three times with PBS. Muscle and soft tissue were scraped off with scissors. Bone was dissected in 5 mL DMEM culture medium containing 15% FBS. Bone marrow cells were collected in DMEM medium. The debris was sorted out by sedimentation at room temperature for 10 min. Single cells were obtained by filtering the cell suspension through a 40- μ m cell strainer. The cell suspension was centrifuged at 1000 rpm for 10 min. The supernatant was aspirated and the cell pellet suspended in 5 mL DMEM culture medium containing 15% FBS and 1% antibiotic solution. After gently mixing the cell pellets several times, the cells were cultured in the incubator until the formation of a cell monolayer. Cells were grown for 12 h after treating with 0.25% trypsin, and the attached cells used for experiments.

OB-OC co-culture

We prepared the culture plate according to the literature (Fu et al., 2006). Two consecutive wells were connected by drilling a 2-mm hole at the well bottom. A 0.45- μ m filter was installed in this OB-OC well connection. First, OB were seeded in the OB-wells, and 4 h later, OC seeded in the OC well. The supernatant, but not the cells, could exchange between these two wells. The experimental cells were collected with similar cell cycles and then grown in no-serum medium.

Experimental groups

OB-OC co-culture cells were separated into two groups: IL-23 (Sigma, San Francisco, CA, USA) and control groups. In the IL-23 group, 5 subgroups were made by adding IL-23 at concentrations of 0.5, 1, 2.5, 5 and 10 ng/mL. Each subgroup contained 6 wells. Cells were collected at 24, 48 and 72 h. In the control group, DMSO was added with the same conditions.

MTT method for determining proliferation rate of osteoclasts

D-Hanks solution was used to wash the cells at 24, 48 and 72 h, and no-serum culture medium with 62.5 μg MTT was then added to each well. Cells were cultured at 37°C for 4 h. Supernatant medium was discarded and fresh medium was added with 400 μL DMSO for each well. The plate was gently shaken and OD at 570 nm was measured for each group.

Diazonium salt method for detecting TRAP activities of osteoclasts

D-Hanks solution was used to wash the cells at 24, 48 and 72 h, and no-serum culture medium was then added. TRAP activity was measured according to the protocol of the diazonium salt method kit (Jiancheng Inc., Nanjing, China).

Evaluate the area of lacunar resorption in co-culture system

OB-OC cells were co-cultured in the presence of dentin for 7 days according to Zhang et al. (2006). The dentin was then taken out and fixed in 2.5% glutaraldehyde for 7 min. Ultrasonic wash with 0.25% ammonium hydroxide was used to clean the surface 3 times. The dentin was dehydrated with alcohol and air-dried. A 1% toluidine blue solution was added, followed by incubation for 3-4 min at room temperature. After washing three times with ddH₂O, the area of lacunar resorption was measured under a microscope (Olympus, Tokyo, Japan).

Statistical analysis

SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data are reported as means \pm SD. Statistical significance was determined by the Student *t*-test. $P < 0.05$ was considered to be statistically significant.

RESULTS

IL-23 regulated the proliferation of osteoclasts in OB-OC co-culture system

In the OB-OC co-culture system, the OC proliferation first increased and then decreased. OC proliferation peaked at 48 h. At all concentrations of IL-23, from 0.5 to 10 ng/mL, OC showed an increased proliferation rate. Interestingly, this increased proliferation appeared to be dose- and time-dependent. The difference relative to the control groups was statistically significant ($N = 6$, $P < 0.05$) (Figure 1).

IL-23 improved the TRAP activities of osteoclasts in co-culture system

In the co-culture without IL-23 added, TRAP activity peaked and then decreased with time. However, by adding IL-23 at concentrations from 0.5 to 10 ng/mL, it was significantly enhanced at 24, 48 and 72 h compared to the control group ($P < 0.05$). In addition, the treatment for 72 h showed the strongest effect (Figure 2).

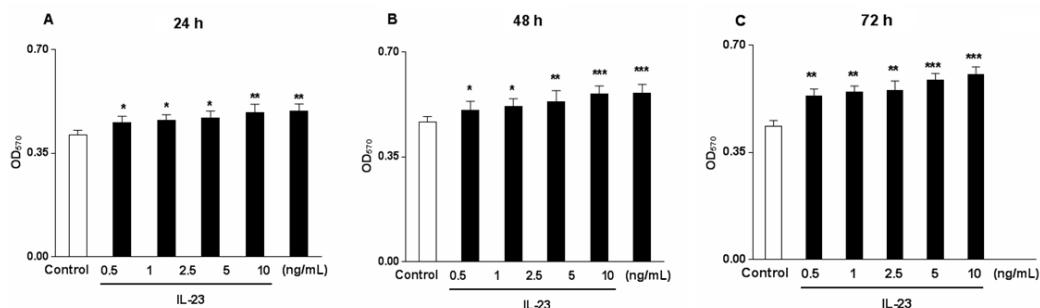


Figure 1. Effects of IL-23 on the proliferation of osteoclasts in co-culture system. Compared to control group, *P < 0.05, **P < 0.01, ***P < 0.001.

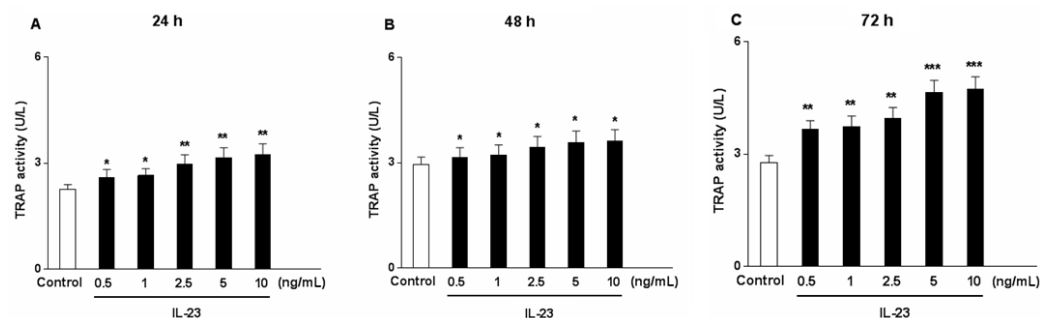


Figure 2. Effects of IL-23 on the TRAP activity in co-culture system. Compared to control group, *P < 0.05, **P < 0.01, ***P < 0.001.

IL-23 increased the area of lacunar resorption in co-culture system

Under the microscope, lacunar resorption could be seen in each dentin specimen. Photographs of this lacunar resorption were taken for each group. IL-23 significantly increased the area of lacunar resorption. Furthermore, the longer the IL-23 treatment, the larger the areas were (Figure 3).

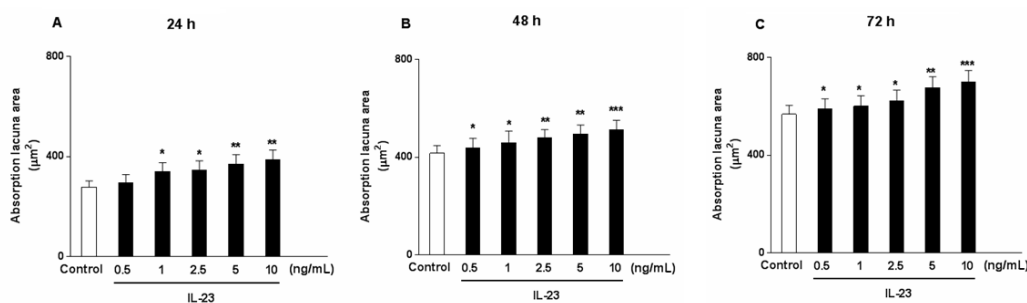


Figure 3. Effects of IL-23 on the resorption lacuna area in co-culture system. Compared to control group, *P < 0.05, **P < 0.01, ***P < 0.001.

DISCUSSION

Bone development and bone metabolism are determined by the dynamic balance between the OB and OC. OB secretes the bone matrix and induces bone mineralization. OC is responsible for osteolysis and dissolution of the bone matrix via bone resorption, leading to lacuna formation and bone reconstruction (Fu et al., 2006; Zhang et al., 2006). Recently, it was demonstrated that the functions of OB and OC showed correlative dependence (Choi et al., 2012; Yamashita et al., 2012). OB could regulate OC maturation and activation by secreting interleukin and other cytokines (Quan et al., 2012). However, the mechanism is still unclear. The regulation functions of interleukins between OB and OC were well realized.

IL-23, which is a new member of the interleukin family, may be involved in the differentiation process of OC. Chen et al. (2011) found that by adding lipopolysaccharide, OB can also secrete IL-23. Moreover, IL-23 could induce the secretion of IL-6 and IL-8 by stimulating RANKL in Th17 cells, leading to bone erosion (Cua et al., 2003; Hunter, 2005; Yago et al., 2007). This impairment could be involved in the derangement of receptor for activation of nuclear factor kappa B (NF- κ B) (RANK), RANK ligand (RANKL), and osteoprotegerin (Iwakura and Ishigame, 2006). As mentioned in the above studies, OB could secrete IL-23, which could promote osteoclast differentiation and bone formation. Chen et al. (2010) also found that IL-23 could also increase lacunar resorption in mouse OC culture. However, it is still not clear whether IL-23 can regulate the proliferation and activation of OC in OB-OC co-culture.

All values of MTT, TRAP activity and lacunar resorption were increased when IL-23 was added to the OB-OC co-culture. Moreover, the peak of the activation curve appeared at 48 h, and then declined. It could have been due to the apoptosis of OC, or the repressive effect of OC derived from OB, which is to maintain an appropriate concentration of IL-23. However, the values of the experimental group were all significantly higher compared to the control group at any test time. In addition to the TRAP activities, a dose-dependent curve was evident, indicating that IL-23 increased OC proliferation, suppressed OC apoptosis and enhanced TRAP activity. Furthermore, this phenotype could be improved with IL-23 treatment. Enhanced resorption was also correlated to the duration of treatment. Compared to the control group, all concentrations of IL-23 from 0.5 to 10 ng/mL had the same effect ($P < 0.05$).

In conclusion, this study provides strong evidence about how IL-23 regulates the proliferation and activation of OC. It could relate to increased cell proliferation and enhanced TRAP activity. Since OB can also secrete IL-23, there is also a possibility that bone resorption was enhanced by OB activity. However, the exact mechanism of IL-23 regulation in osteoclastogenesis still needs to be further studied.

REFERENCES

- Boniface K, Blom B, Liu YJ and de Waal MR (2008). From interleukin-23 to T-helper 17 cells: human T-helper cell differentiation revisited. *Immunol. Rev.* 226: 132-146.
- Chen CH, Fang TH, Zhou LL and Zhou XP (2008). Effect of Qingluo Tongbi granule-containing serum on osteoblasts and osteoclasts. *Chin. Trad. Pat. Med.* 30: 35-38.
- Chen L, Wei XQ, Bronwen E and Daniel A (2010). The regulation effect of IL-23 on osteoclast differentiation and function. *Beijing J. Stomatol.* 18: 307-310.
- Chen L, Wei XQ, Rachel JW and Alastair JS (2011). The *in vitro* study of LPS-induced IL-23 expression in murine osteoblast. *Beijing J. Stomatol.* 19: 125-127.
- Choi C, Lee H, Lim H, Park S, et al. (2012). Effect of *Rubus coreanus* extracts on diabetic osteoporosis by simultaneous

- regulation of osteoblasts and osteoclasts. *Menopause* 19: 1043-1051.
- Cua DJ, Sherlock J, Chen Y, Murphy CA, et al. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421: 744-748.
- Dai SM, Nishioka K and Yudoh K (2004). Interleukin (IL) 18 stimulates osteoclast formation through synovial T cells in rheumatoid arthritis: comparison with IL1 β and tumour necrosis factor α . *Ann. Rheum. Dis.* 63: 1379-1386.
- Fu SP, Zhang RH and Xu LQ (2006). Effect of yigu capsule drug-containing serum on proliferation, ALP activity and expression of interleukin-11 mRNA in rat osteoblasts in the co-culture. *Chin. J. Pathophysiol.* 22: 1171-1173.
- Haden ST, Glowacki J, Hurwitz S, Rosen C, et al. (2000). Effects of age on serum dehydroepiandrosterone sulfate, IGF-I, and IL-6 levels in women. *Calcif. Tissue Int.* 66: 414-418.
- Hunter CA (2005). New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nat. Rev. Immunol.* 5: 521-531.
- Iwakura Y and Ishigame H (2006). The IL-23/IL-17 axis in inflammation. *J. Clin. Invest.* 116: 1218-1222.
- Paradowska A, Maśliński W, Grzybowska-Kowalczyk A and Łacki J (2007). The function of interleukin 17 in the pathogenesis of rheumatoid arthritis. *Arch. Immunol. Ther. Exp.* 55: 329-334.
- Quan J, Zhou C, Johnson NW, Francis G, et al. (2012). Molecular pathways involved in crosstalk between cancer cells, osteoblasts and osteoclasts in the invasion of bone by oral squamous cell carcinoma. *Pathology* 44: 221-227.
- Sato K, Suematsu A, Okamoto K, Yamaguchi A, et al. (2006). Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J. Exp. Med.* 203: 2673-2682.
- Tsutsumimoto T, Kawasaki S, Ebara S and Takaoka K (1999). TNF-A and IL-1 B suppress N-cadherin expression in MC3T3-E1 cells. *J. Bone Miner. Res.* 14: 1751-1760.
- Ura K, Morimoto I, Watanabe K, Saito K, et al. (2000). Interleukin (IL)-4 and IL-13 inhibit the differentiation of murine osteoblastic MC3T3-E1 cells. *Endocr. J.* 47: 293-302.
- Wu PF, Zhong DB, Qu WJ and Wang M (2004). Isolation and culture of osteoblast by modified enzyme digestion on mouse. *J. Gansu Agr. Univ.* 39: 512-515.
- Yago T, Nanke Y, Kawamoto M, Furuya T, et al. (2007). IL-23 induces human osteoclastogenesis via IL-17 *in vitro*, and anti-IL-23 antibody attenuates collagen-induced arthritis in rats. *Arthritis Res. Ther.* 9: R96.
- Yamashita T, Takahashi N and Udagawa N (2012). New roles of osteoblasts involved in osteoclast differentiation. *World J. Orthop.* 3: 175-181.
- Zhang RH, Xu LQ, Fu SP and Yang L (2006). Effects of benefiting-bone capsule contained serum on activity and apoptosis of osteoclast in osteoblast and osteoclast co-culture system. *Chin. J. Pathophysiol.* 22: 1001-1004.