A Novel Role of IL-15 in the Development of Osteoclasts: Inability to Replace Its Activity with IL-2

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IL-15 shares many activities with IL-2 on stimulating lymphocytes, hematopoietic progenitor cells, and macrophages. However, the role of IL-15 in osteoclastogenesis has not been elucidated. The recent finding of abundant IL-15 in rheumatoid arthritis synovial fluids suggested a possible role for this cytokine in the pathological destruction of bone and prompted us to determine whether IL-15 stimulates osteoclast formation. IL-15 stimulated the formation of multinucleated osteoclast-like cells in rat bone marrow cultures. In stroma-free cultures, IL-15 increased the number of mononuclear preosteoclast-like cells in the early stage of osteoclast formation. The stimulation was observed even after treatment with IL-15 for only 24 or 48 h of culture. Moreover, low IL-15 concentration (0.1 ng/ml) strongly increased the level of calcitonin receptor mRNA of mononuclear preosteoclast-like cells. Although IL-15 is known as a potent stimulator of TNF-α, its activity was not abolished by addition of anti-TNF-α Ab. Interestingly, IL-2 and IL-7, which utilize some IL-15R components, had no effect on osteoclast differentiation, but pretreatment with IL-2 or IL-7 of bone marrow cells before the addition of IL-15 inhibited the enhancing activity of IL-15. In summary, IL-15 has a novel activity to stimulate the differentiation of osteoclast progenitors into preosteoclasts, which cannot be replaced by IL-2 but may use components in common with IL-2R to mediate its effects. The Journal of Immunology, 1999, 162: 2754–2760.

Interleukin 15 was originally discovered as a cytokine with IL-2-like activity from the supernatant of simian kidney epithelial cell line CV-1/EBNA (1) and human adult T cell leukemia cell line HuT-102 (2, 3). IL-15 shares most of the biological activities of IL-2 on several types of lymphocytes, including the proliferation and activation of T, NK, and B cells, since IL-15 and IL-2 bind common receptor components β and γc, to elicit signals in these cells (1–5). However, differences have been found in the range of possible target cells and functions between IL-15 and IL-2. Besides β and γc, IL-15 binds with high affinity to a unique component, IL-15Rα, which is distinct from IL-2Rα (6). Compared with the mRNA expression of IL-2Rα, that of IL-15Rα showed a much wider cellular distribution, suggesting that IL-15 has the ability to exert various effects on many types of cells. For NK cell differentiation, it is thought that IL-2 is not required but IL-15 is (7). In addition, Tagaya et al. (8) recently found that IL-15 could stimulate the proliferation of mast cells in the absence of IL-2Rα, β, and IL-15Rα using a novel unique receptor system. However, little is known about those functions of IL-15 not shared by IL-2.

Osteoclasts are bone-resorptive cells that have a crucial role in physiological bone remodeling (9) and also function in the local bone destruction that occurs in association with chronic inflammatory diseases (10). Diseases such as rheumatoid arthritis have been associated with an accumulation of proinflammatory cytokines such as IL-1, IL-6, TNF-α, and IL-8 (11–17), some of which likely mediate local bone destruction by stimulating osteoclasts or osteoclast differentiation. In fact, IL-6 was found to stimulate osteoclastogenesis in an in vitro study (18). Recently, McInnes et al. (19) reported the presence of high concentrations of IL-15 in rheumatoid arthritis fluid, suggesting that IL-15 plays a role in pathological bone destruction.

In the present study, we investigated the possible role of IL-15 in osteoclast differentiation. Osteoclasts are hematopoietic in origin, and these multinucleated cells are formed by fusion of mononuclear preosteoclasts (20). We have recently developed two types of rat bone marrow culture systems in which the process of osteoclast development could be separated. In one of these systems, multinucleated osteoclast-like cells (MNCs) are formed (21), while in the other, mononuclear preosteoclast-like cells (POCs) are formed from stroma-free cultures in the presence of conditioned medium of osteoblastic cells (22). The POCs have several osteoclast characteristics including the expression of tartrate-resistant acid phosphatase (TRAP), calcitonin receptor (CTR), and Kat1, a rat osteoclast-specific Ag (23). Here we show that IL-15 has the novel activity of enhancing the osteoclastogenesis by stimulating the formation of preosteoclasts, an activity not shared by IL-2.

Materials and Methods

**Materials**

Male Sprague Dawley rats age 5–6 wk were purchased from SEAC Yoshitomi (Fukuoka, Japan). 1α,25-Dihydroxyvitamin D3 (1α,25(OH)2D3) was purchased from Biomol (Plymouth Meeting, PA). Human rIL-15, mouse anti-human IL-15 mAb (M111), and human rIL-7 were purchased from Genzyme (Cambrige, MA), and rat rIL-2 was purchased from Serotec.

Abbreviations used in this paper: MNCs, multinucleated osteoclast-like cells; POCs, mononuclear preosteoclast-like cells; CTR, calcitonin receptor; 1α,25(OH)2D3, 1α,25-dihydroxyvitamin D3; sCT, salmon calcitonin; TRAP, tartrate-resistant acid phosphatase; hROSCM, heat-treated conditioned medium derived from rat osteoblastic cell line ROS17/2.8; GADPH, glyceraldehyde-3-phosphate dehydrogenase; JAK, Janus kinase; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor.
POCs, except in the experiment of Fig. 1 marrow cells were used, nonadherent bone marrow cells were used to form colonies of stromal cells after 14 days of culture. Because there was no significant difference in the osteoclastogenesis-enhancing activity of TRAP-positive cells with more than three nuclei were counted.

Whole bone marrow cell cultures

For the formation of POCs, adherent stromal cells were eliminated from bone marrow cells using a Sephadex G-10 column, and nonadherent bone marrow cells were cultured in the presence of $10^{-8}$ M 1α,25(OH)$_2$D$_3$ and 10% (v/v) heat-treated conditioned medium derived from rat osteoblastic cell line ROS17/2.8 (htROSCM), as described by Kukita et al. (21). Various concentrations of IL-15 were added to the cultures. After 3 days of culture, the cells were fixed and then stained for TRAP, a marker enzyme for osteoclasts, using a commercial kit.

Stroma-free bone marrow cell cultures

For the formation of MNCs, bone marrow cells isolated from tibias and femurs of rats were cultured in α-MEM containing 15% FCS in the presence of $10^{-8}$ M 1α,25(OH)$_2$D$_3$ and 10% htROSCM as described by Kukita et al. (22). Cells (2 x $10^7$) were cultured in 96-well culture plates (Falcon, Lincoln Park, NJ) in the presence or absence of various concentrations of cytokines and mAb for the indicated times. After 4 days of culture, the cells were fixed and stained with TRAP or with the osteoclast-specific mAb Kat1 (23). After the staining, the presence of CTR in some cultures was confirmed by autoradiography. In some experiments, nonadherent bone marrow cells were further purified on a Sephadex G-10 column once more to eliminate stromal cells completely. These purified nonadherent bone marrow cells did not form colonies of stromal cells after 14 days of culture. Because there was no significant difference in the osteoclastogenesis-enhancing activity of IL-15 when purified nonadherent bone marrow cells or nonadherent bone marrow cells were used, nonadherent bone marrow cells were used to form POCs, except in the experiment of Fig. 1B.

Immunocytochemistry

Immunostaining with Kat1 was performed as described (23). Briefly, the cells were incubated with mAb Kat1 for 45 min and fixed with 2% paraformaldehyde for 20 min at room temperature. After blocking with 3% goat serum for 60 min, the cells were incubated with biotinylated anti-mouse IgM as the second Ab for 30 min and then stained using a Vectastain ABC-AP kit (Vector, Burlingame, CA) according to the manufacturer’s instructions.

Preparation of rat primary osteoblasts

Rat primary osteoblasts were isolated by sequential digestion from the calvariae of Sprague Dawley rats, according to the method of Takahashi et al. (24). The cells were cultured in α-MEM containing 10% FCS in 100-mm culture dishes at 3 $\times$ 10$^4$ cells/dish. After 4 days of culture, the cells were trypsinized and used as primary osteoblasts.

Coculture and dentine resorption assay

Bone resorption assays were performed according to the method of Hata et al. (25). Nonadherent bone marrow cells (1 x $10^5$ in 500 μl of α-MEM containing 15% FCS) seeded into 24-well culture plates in the presence of 10% htROSCM with or without 100 ng/ml IL-15 for 5 days. Dog primary osteoblasts (1 x $10^5$ cells/well) were added to each well. After 4 days of culture, cells were detached from the culture plates with 0.05% trypsin and 0.02% EDTA in PBS, followed by replating onto human dentine. The cells were then incubated in α-MEM containing 15% FCS for 7 days. After the end of culture, the resorption pits were examined with a JEOL JSM-5200LV scanning electron microscope as described previously (26).

$^{125}$I-labeled sCT binding assay

CTR were detected by autoradiography using $^{125}$I-labeled sCT as described (22). Briefly, the cells were rinsed once with α-MEM containing 0.1% BSA and incubated with $1\mu$Ci/ml $^{125}$I-labeled sCT (74 TBq/mmol) at room temperature for 2 h in the absence or presence of excess sCT (1 μg/ml). After the cells were rinsed three times with α-MEM containing 0.1% BSA, they were fixed with 0.1 M cacodylate buffer (pH 7.3) containing 2% formaldehyde and 2% glutaraldehyde for 10 min. The cells were then washed with α-MEM containing 0.1% BSA and were stained for TRAP. Subsequently, the bottoms of the wells were cut out from the culture plate, dipped in NR-M2 emulsion, and air dried. After exposure for 2–3 wk at 4°C, autoradiographs were developed with Konica X and fixed with Konicafix.

Detection of CTR mRNA by RT-PCR

Nonadherent bone marrow cells (1.1 x $10^7$) were cultured in 60-mm tissue culture dishes (Falcon) (5.7 ml/dish) in the presence of $10^{-8}$ M 1α,25(OH)$_2$D$_3$, 10% (v/v) htROSCM, and various concentrations of IL-15 for 4 days. Total RNA was extracted by using a commercial kit (Isogen, Nippon Gene, Toyama, Japan), and single-stranded cDNA was synthesized by using a RT-PCR kit (Takara, Japan). The cDNA was amplified using specific primers for rat CTR mRNA as described (27). A 518-bp (C1a isoform) or 629-bp (C1b isoform) fragment of CTR cDNA was amplified using primers (5'-AAGAACATCGT/CTTCT/C/G/T/ACTTA-3', nucleotides 625–644, and 5'-ACAAACATGGA/TGC/TGT/CAGCGAGG GCAC-3', nucleotides 1288–1253). The PCR products were separated on a 1% agarose gel, transferred to a nylon membrane (Gene Screen, NEN Research Products, Boston, MA), and then detected with $^{32}$P-labeled CTR cDNA as a probe by Southern hybridization. The probe used was HindIII fragment (2 kb) of rat CTR (C1a) cDNA (generously provided by Dr. P. Sexton, St. Vincent’s Institute of Medical Research, Fitzroy, Australia) and labeled using a random primer DNA labeling kit (Nippon Gene). Hybridization was performed under stringent conditions at 60°C. For the internal control for RNA quantity, the same cDNA was amplified using primers specific for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. A 414-bp fragment of GAPDH cDNA was amplified using primers (5'-CATGGAAGGCTGGGCTC-3', nucleotides 306–325, and 5'-AAGCAGATACATTGCGTAG-3', nucleotides 701–720) as described (28). PCR products were separated on a 1% agarose gel and stained with ethidium bromide.

Isolation and culture of macrophages and measurement of the activity of rat TNF-α

Peritoneal macrophages were obtained from Lewis rats by peritoneal lavage with cold PBS 3 days after i.p. injection of 4.05% (w/v) thioglycolate.
IL-15-stimulated culture in which a number of dense grains were
seen over the cells. No grains were seen over the cells when an
excess amount of unlabeled sCT was added to the cells (Fig. 3B).
These results demonstrate that IL-15 strongly enhances the forma-
tion of POCs, which fulfill several osteoclastic characteristics in
the presence of hROS. However, in the absence of hROS, IL-
15 alone had no inducing activity on POCs (data not shown).

Treatment of cultures for forming POCs with IL-15 increases
the resorbing activity of MNCs formed after coculture with
osteoblasts
We further examined whether the POCs treated with IL-15 has
increased their ability to form bone-resorbing cells. Nonadherent

FIGURE 3. Demonstration of CTR expressed in the POCs induced by
IL-15. Nonadherent bone marrow cells were cultured in the presence of
hROS and 1α,25(OH)2D3 for 4 days with or without IL-15 (100 ng/ml) and anti-IL-15 mAb (200
μg/ml). The number of CTR-positive cells was assessed by CT autoradiography. Each
value represents the mean ± SEM of quadruplicate cultures. Data were analyzed by
Student’s t test. **, p < 0.01 compared without IL-15; §§, P < 0.01 compared with
IL-15-treated cultures.

Table 1. Effect of IL-15 on the formation of CTR-positive cells and the
blocking effect of neutralizing anti-IL-15 Ab on the response by IL-15

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of CTR-Positive Cells/Well</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>17.0 ± 1.8</td>
</tr>
<tr>
<td>IL-15</td>
<td>48.8 ± 2.1**</td>
</tr>
<tr>
<td>IL-15 + anti-IL-15 Ab</td>
<td>21.8 ± 2.4§§</td>
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*Nonadherent bone marrow cells were cultured in the presence of hROS and 1α,25(OH)2D3 for 4 days with or without IL-15 (100 ng/ml) and anti-IL-15 mAb (200
μg/ml). The number of CTR-positive cells was assessed by CT autoradiography. Each
value represents the mean ± SEM of quadruplicate cultures. Data were analyzed by
Student’s t test. **, p < 0.01 compared without IL-15; §§, P < 0.01 compared with
IL-15-treated cultures.

Materials and Methods

Effect of IL-15 on the formation of MNC and POC in rat bone
marrow cultures
IL-15 enhanced the formation of TRAP-positive MNCs in whole
bone marrow cultures after 3 days of culture. Significant stimula-
tion (1.8-fold that of control) was seen only in the cultures treated
with high concentrations (100 ng/ml) of IL-15 (Fig. 1A), and the
stimulation was not detected after longer than 3 days of culture
data not shown). We next examined the effect of IL-15 on the
formation of POCs induced by hROS. IL-15 increased the
number of POCs which were positive for TRAP, a marker of oste-
oclast, in a dose-dependent manner (Fig. 1B). Because in this
experiment we used purified bone marrow cells that were com-
pletely depleted of stromal cells, the result demonstrates the en-
hancing effect that IL-15 exerts in the absence of stromal cells.
Significant stimulation was observed at a concentration as low as
12.5 ng/ml of IL-15. As shown in Fig. 2, we also found that IL-15
(100 ng/ml) markedly enhanced the formation of POCs that were
positive for Kat1, another marker for rat osteoclasts (23), and this
response was neutralized by the addition of graded concentrations
of anti-human IL-15 mAb dose dependently. CTR is another
reliable marker for osteoclasts. We next examined whether IL-15
increases the number of POCs that express CTR. IL-15 (100 ng/
ml) significantly enhanced the formation of CTR-positive POCs.
Addition of anti-human IL-15 mAb (200 μg/ml) completely neu-
tralized the stimulatory formation of CTR-positive POCs by IL-15
(Table 1). Fig. 3A demonstrates CTR-positive POCs formed in the
IL-15-stimulated culture in which a number of dense grains were

Results

Effect of IL-15 on the formation of MNC and POC in rat bone
marrow cultures

Broth (Eiken Chemical, Tokyo, Japan) (5 ml/animal). The cells were
washed, resuspended in α-MEM containing 10% FCS, and seeded at 1.2 ×
106 in 1 ml/well of 24-well plates. They were incubated for 16 h to allow
macrophages to adhere, and then nonadherent cells were removed by as-
piration. The adherent cells were used as peritoneal macrophages and stim-
ulated by LPS (1 ng/ml) (Difco, Detroit, MI) for 2 h. The supernatant was
collected and stored at −80°C. The activity of rat TNF-α in supernatants
was assessed by its cytotoxic effect on murine L-929 cells as described
(29). Briefly, L-929 cells were seeded in 96-well plates and cultured in
DMEM containing 5% FCS as monolayer. Actinomycin D (500 ng/ml)
(Sigma, St. Louis, MO) was added with macrophage supernatants, which
were pretreated without or with various concentrations of anti-murine
TNF-α Ab and then incubated for 24 h. The cytotoxic effect was then
assessed by staining with amido black, and the absorbance at 540 nm was
read.
bone marrow cells treated with IL-15 (100 ng/ml) were cocultured
with primary osteoblasts to form MNCs, followed by replating
onto dentine slices. As shown in Fig. 4, typical resorption pits were
observed on dentine slices. The number of resorption pits of MNCs formed by treatment with IL-15 was
greater than that of the MNCs formed without IL-15; magnification, \( \times 150 \). Bar = 50 \( \mu m \).

**FIGURE 4.** Demonstration of resorption pits on dentine slices. Nonadherent bone marrow cells were cultured with htROSCM and 1\(\alpha\),25(OH)\(_2\)D\(_3\) in the presence (A) or absence (B) of 100 ng/ml IL-15 for 5 days. Then rat primary osteoblasts were added to each well. After 4 days of coculture, cells were detached from the culture plates, followed by replating onto human dentine slices, and were cultured for 7 days. After removal of cells, the dentine slices were processed for scanning electron microscopy. Typical resorption pits were observed on dentine slices. The number of resorption pits of MNCs formed by treatment with IL-15 was greater than that of the MNCs formed without IL-15; magnification, \( \times 150 \). Bar = 50 \( \mu m \).

The resorption area was larger when the culture was treated with IL-15 (Fig. 4A). Fig. 5 shows a quantitative datum of the dentine resorption using dentine slices with equal diameters. The resorption area of the formed MNCs was increased by treatment of the culture to form POCs with IL-15 before coculture with osteoblasts.

**FIGURE 5.** Comparison of dentine-resorbing activity between MNCs formed in culture with IL-15 and those formed in culture without IL-15. Nonadherent bone marrow cells were cultured in the presence of htROSCM and 1\(\alpha\),25(OH)\(_2\)D\(_3\) with or without 100 ng/ml for 5 days. Then rat primary osteoblasts were added to each well. After 4 days of coculture, cells were replaced onto dentine slices, and were cultured for 7 days. Total resorption area were measured in each dentine. Each bar represents the mean ± SEM of quadruplicate cultures, \( **, p<0.01 \) compared with the culture without IL-15.

**Effect of IL-15 on the various stages of POC formation**

We next examined whether the induction of POCs is required for IL-15 to enhance activity or whether pretreatment with IL-15 promotes the formation of POCs by htROSCM. As shown in Fig. 7A, when the bone marrow cells were pretreated with htROSCM, the subsequent treatment with IL-15 markedly increased the formation of TRAP-positive mononuclear cells. Conversely, as shown in Fig. 7B, when the bone marrow cells were pretreated with IL-15 before the addition of htROSCM, the pretreatment with IL-15 did not have an effect on the formation of TRAP-positive mononuclear cells. These results suggest that IL-15 amplifies the number of POCs induced by htROSCM, but IL-15 does not exert its osteoclastogenesis-enhancing activity until POC formation has initiated.

We next focused on the action of IL-15 on the differentiation stage of POC formation. As shown in Fig. 8, treatment of the cells with IL-15 (100 ng/ml) for only the first or second day of culture markedly stimulated the formation of TRAP-positive mononuclear cells. In contrast, when IL-15 was present in the culture on only the third or fourth day, no significant stimulation was observed. These findings show that the enhancing activity of IL-15 is due to its ability to stimulate the early stage but not the late stage of POC formation.

**FIGURE 6.** Demonstration of CTR mRNA expression in POCs treated with graded concentrations of IL-15. Nonadherent bone marrow cells were cultured in the presence of htROSCM and 1\(\alpha\),25(OH)\(_2\)D\(_3\) with 0 ng/ml (lanes 1), 0.1 ng/ml (lanes 2), 1 ng/ml (lanes 3), and 10 ng/ml (lanes 4) of IL-15 for 4 days. Total RNA was reverse transcribed, and cDNA was amplified by 30 cycles of PCR for CTR and GAPDH mRNA using the specific primers described in Materials and Methods. The PCR products of CTR were transferred to nylon membrane and hybridized with \( ^{32}P \) labeled DNA fragment of rat CTR cDNA. For GAPDH mRNA analysis, the PCR products were stained with ethidium bromide.

**Low concentration of IL-15 augments CTR mRNA expression of POCs**

To investigate whether the stimulatory activity of IL-15 is correlated with the expression of CTR mRNA, the level of CTR mRNA expression was analyzed in the cultures treated with graded concentrations of IL-15. The expression of CTR mRNA was markedly increased by the treatment of a low concentration (0.1 ng/ml) of IL-15 at maximal level and was not increased by the addition of higher concentrations of IL-15 (1 and 10 ng/ml) (Fig. 6). In this experiment, the detected band of CTR mRNA corresponded to the C1a isoform.
TNF-α and PG are not involved in osteoclastogenesis-enhancing activity of IL-15

To establish that the enhancing activity of IL-15 on POC formation is not a secondary effect, we examined the possibility that TNF-α and PG, which stimulate bone resorption, were involved in POC formation stimulated by IL-15. Neutralizing Ab against murine TNF-α abolished the cytotoxic activity of rat TNF-α on L929 cells (Fig. 9A), but it did not abolish POC formation stimulated by IL-15 even at high concentration (100 ng/ml) (Fig. 9B). In addition, we found that 10^{-6} M and 10^{-8} M indomethacin, an inhibitor of the synthesis of PG, had no suppressive effect on POC formation enhanced by IL-15 (data not shown). These findings suggest that the effect of IL-15 is not mediated by TNF-α and PG.

Discussion

In the present study, we have shown that IL-15 has a novel activity to enhance osteoclast differentiation, whereas IL-2, which shares
The activity of IL-15 enhanced the formation of POCs, which occurs at the early stage of osteoclastogenesis, rather than the formation of MNCs. IL-15 increased the number of POCs with osteoclast phenotypes including TRAP, CTR, and Kat1, a unique osteoclast Ag that we recently isolated (23). In addition, IL-15 markedly increased the level of CTR mRNA. These results demonstrate that IL-15 is an enhancer of osteoclastogenesis.

IL-15Rα mRNA is expressed in various types of cells including macrophages and bone marrow stromal cell lines (6). In addition, several cytokines are known to stimulate osteoclastogenesis by mediating stromal cells (30–32). The question was raised whether the enhancing activity of IL-15 on POC formation might be mediated by other cytokines secondarily induced. However, we obtained several results that were contrary to this prediction: 1) IL-15 exerted its activity in culture completely free of stromal cells and macrophages. This result indicates that the activity of IL-15 is not mediated by these cells; 2) although IL-15 is known to strongly stimulate TNF-α from NK cells or macrophages (4, 33, 34), we confirmed with anti-TNF-α Ab that the effect of IL-15 is not mediated by TNF-α produced in the cultures; 3) some of the stimulation activity on osteoclast generation is dependent on PG synthesis (35, 36). We also found that the enhancing activity of IL-15 does not require synthesis of PG; 4) without htrROSCM, IL-15 has no effects on the development of cells with osteoclast phenotypes. IL-15 may interact with some factors in htrROSCM. Especially, macrophage CSF (M-CSF) is one of important factors for osteoclast differentiation. However, because htrROSCM is heated medium, we have found that the activity of M-CSF in this conditioned medium was destroyed (21). We also found that IL-15 did not induce POCs with the combination of M-CSF, IL-3, and stem-cell factor (SCF) (our unpublished results), suggesting that the effect of IL-15 is not mediated by these cytokines. Taken together, it is likely that the enhanced POC formation induced by IL-15 is not attributable to other cytokines but is caused by direct action.

Surprisingly, IL-15 exerts its strong activity by stimulating cells at the very early stage (only the first 24 or 48 h) of culture. It has previously been reported that IL-15 has an ability to induce differentiation of NK cells from CD34+ hematopoietic progenitor cells (7). These results suggest that some populations of immature hematopoietic cells are capable of responding to IL-15 and mediate differentiation toward osteoclastogenesis. In addition, we found that IL-15 alone did not have any osteoclastogenesis activity; however, when bone marrow cells were pretreated with htrROSCM, the subsequent treatment of these cells with IL-15 markedly enhanced the formation of POCs. These results raise the possibility that the enhancing activity of IL-15 is mediated by the receptor expressed in the osteoclast progenitor cells and preosteoclasts. Very interestingly, an IL-15 concentration as low as 0.1 ng/ml markedly increased the expression level for CTR mRNA, suggesting that the receptor system mediating osteoclastogenesis acts through the high affinity receptor. Such low concentration of IL-15 stimulates the proliferation of T cells (1) in which high affinity IL-15 receptor complex is induced.

Since mRNA of IL-15Rα and that of IL-2Rα are coexpressed in lymphocytes, IL-2 shares many stimulative activities with IL-15 in these cells. However, the cellular distribution of IL-15Rα message is known to be different from that of IL-2Rα. The inability of IL-2 to stimulate osteoclast differentiation suggests that some populations of hematopoietic cells destined to differentiate into osteoclasts express mRNA for IL-15Rα but not for IL-2Rα. However, certain other IL-2R components seem to be involved in the IL-15 receptor system in osteoclastogenesis. IL-2 shares receptor components β and γc with IL-15, while IL-7, a stimulator of B cell and macrophage differentiation (37, 38), shares a γc chain with IL-15 and IL-2. Although neither IL-2 nor IL-7 stimulated POC formation, the treatment of bone marrow cells with IL-7 or IL-2 before the treatment with IL-15 partially abolished the enhancing effect of IL-15. This result suggests that the IL-15 receptor system in osteoclastogenesis involves the common component, γc. The pretreatment of bone marrow cells with IL-7 or IL-2 probably down-regulates available γc molecules and inhibits the formation of POCs.

Recently, a receptor designated as IL-15RX was found in mast cells (8). The IL-15RX system in mast cells does not involve IL-2Rα, β, γc or IL-15Rα, and the signal is transduced by activating JAK-2, unlike the JAK-1/3 used in lymphocytes. In our preliminary experiments, we found that the enhancement of POC formation by IL-15 was not abolished by the treatment with the inhibitor of JAK-2 (our unpublished result). This result suggests that the IL-15 receptor system in osteoclast progenitor cells is different from those of mast cells. However, further studies are necessary to elucidate the mechanism of signal transduction of IL-15 in POC formation.

This study provides the first evidence of different biological activities between IL-15 and IL-2 in osteoclast development. It would be of interest to investigate what components of the IL-15 receptor system are used in osteoclast progenitor cells. Previous studies related to the wide distribution of mRNA for IL-15Rα and IL-15 suggested that IL-15 had pleiotropic functions in a variety of cells. Our findings demonstrate one such activity of IL-15 in osteoclastogenesis and suggest a possible role for IL-15 in the pathological loss of bone in inflammatory diseases.

Acknowledgments

We thank Dr. P. Sexton (St. Vincent’s Institute of Medical Research) for donating the rat CTR cDNA and Dr. K. Ohki for helpful suggestions regarding the assay of the activity of TNF-α.

References

IL-15 ENHANCES OSTEOCLASTOGENESIS


