MCP-1 Is Induced by Receptor Activator of Nuclear Factor-κB Ligand, Promotes Human Osteoclast Fusion, and Rescues Granulocyte Macrophage Colony-stimulating Factor Suppression of Osteoclast Formation*

Received for publication, November 10, 2004, and in revised form, February 14, 2005
Published, JBC Papers in Press, February 17, 2005, DOI 10.1074/jbc.M412713200

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Human osteoclast formation from monocyte precursors under the action of receptor activator of nuclear factor-κB ligand (RANKL) was suppressed by granulocyte macrophage colony-stimulating factor (GM-CSF), with down-regulation of critical osteoclast-related nuclear factors. GM-CSF in the presence of RANKL and macrophage colony-stimulating factor resulted in mononuclear cells that were negative for tartrate-resistant acid phosphatase (TRAP) and negative for bone resorption. CD1a, a dendritic cell marker, was expressed in GM-CSF, RANKL, and macrophage colony-stimulating factor-treated cells and absent in osteoclasts. Microarray showed that the CC chemokine, monocyte chemotactic protein 1 (MCP-1), was profoundly repressed by GM-CSF. Addition of MCP-1 reversed GM-CSF suppression of osteoclast formation, recovering the bone resorption phenotype. MCP-1 and chemokine RANTES (regulated on activation normal T cell expressed and secreted) permitted formation of TRAP-positive multinuclear cells in the absence of RANKL. However, these cells were negative for bone resorption. In the presence of RANKL, MCP-1 significantly increased the number of TRAP-positive multinuclear bone-resorbing osteoclasts (p = 0.008). When RANKL signaling through NFATc1 was blocked with cyclosporin A, both MCP-1 and RANTES expression was down-regulated. Furthermore, addition of MCP-1 and RANTES reversed the effects of cyclosporin A and recovered the TRAP-positive multinuclear cell phenotype. Our model suggests that RANKL-induced chemokines are involved in osteoclast differentiation at the stage of multinucleation of osteoclast precursors and provides a rationale for increased osteoclast activity in inflammatory conditions where chemokines are abundant.

Osteoclasts are bone-resorbing cells that differentiate from hematopoietic precursors of the monocyte/macrophage lineage (1). Osteoclasts are multinuclear giant cells that stain positive for tartrate-resistant acid phosphatase (TRAP). Receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) are necessary signals for osteoclast differentiation (2). RANKL is present on the surface of stromal cells and osteoblasts (3). RANKL interacts with receptor activator of nuclear factor-κB on osteoclast precursors, resulting in a cascade of gene expression controlled by transcription factors including nuclear factor-κB and NFATc1 (4). Authentic human osteoclasts that have high bone-resorbing activity are made in vitro from peripheral blood mononuclear cells (PBMCs) by culturing with M-CSF and recombinant RANKL (M+R treatment).

Granulocyte macrophage colony-stimulating factor (GM-CSF) is a cytokine produced by T cells following activation and by most myeloid lineage cells, such as macrophages and granulocytes (5). The effect of GM-CSF on osteoclast formation is controversial: both inhibition (6–9) and stimulation (10) are reported. Short-term treatment with GM-CSF potentiated osteoclast differentiation, whereas long-term exposure suppressed osteoclast differentiation (11). We previously showed that the GM-CSF receptor-α was induced during osteoclast differentiation (9), providing a basis for paracrine signaling from GM-CSF-producing cells to osteoclasts.

Chemokines are small cytokines known to be involved in immune response and in development of several cell types (12). Chemokines are classified into two main subfamilies, CC and CXC, according to the location of the first two of the four cysteine residues (13). Many ligands within the CC chemokine superfamily are capable of sharing receptors (12). Monocyte chemotactic protein 1 (MCP-1) is a CC chemokine commonly found at the site of tooth eruption, rheumatoid arthritic bone degradation, and bacterially induced bone loss (14). MCP-1 is expressed by mature osteoclasts, and its expression is regulated by nuclear factor-κB (15, 16). In this report we show that GM-CSF suppresses the formation of TRAP-positive multinuclear osteoclasts by RANKL and M-CSF. Gene expression studies show that GM-CSF treatment causes potent down-regulation of MCP-1. Addition of exogenous MCP-1 reversed the GM-CSF-mediated suppression of osteoclast formation, permitting the recovery of authentic multinuclear bone-resorbing osteoclasts.

EXPERIMENTAL PROCEDURES

Preparation and Culture of Human Monocytes—Human PBMCs were isolated by Ficoll-Paque (Amersham Biosciences) density gradient centrifugation as previously described (9). PBMCs were plated at 10°/ml. Human PBMCs were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 100 units/ml penicillin/streptomycin. After 3 days of culture, cells were collected and either used for preparation of monocytes or rested in the same medium for additional 2 days. Monocytes were isolated by Ficoll-Paque density gradient centrifugation as previously described (9). PBMCs were plated at 10°/ml. PBMCs were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 100 units/ml penicillin/streptomycin. After 3 days of culture, cells were collected and either used for preparation of monocytes or rested in the same medium for additional 2 days. Monocytes were isolated by Ficoll-Paque density gradient centrifugation as previously described (9). PBMCs were plated at 10°/ml. PBMCs were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 100 units/ml penicillin/streptomycin. After 3 days of culture, cells were collected and either used for preparation of monocytes or rested in the same medium for additional 2 days. Monocytes were isolated by Ficoll-Paque density gradient centrifugation as previously described (9).
cells/cm² and non-adherent cells removed by washing in normal saline. Cells were cultured in minimal essential medium (supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin (Invitrogen), 5% CO₂ supplemented with 25 ng/ml M-CSF and 20 ng/ml soluble RANKL to induce osteoclast formation. GM-CSF was used at concentrations from 0.1 to 25 ng/ml. MCP-1 and RANTES were used at 25 ng/ml. Neutralizing antibody directed against MCP-1 was used at 4 μg/ml (Serotec). GM-CSF, RANKL, M-CSF, MCP-1, RANTES, and neutralizing anti-MCP-1 antibody were purchased from Peprotech (Rocky Hill, NJ). All data are based on a minimum of three replicate experiments performed independently on different occasions, unless otherwise stated. All cultures were for 21 days. After 21 days, PBMC cultures were fixed in acetone, citrate, and formaldehyde solution and stained for TRAP using a leucocyte acid phosphatase staining kit (Sigma). TRAP-positive cells that had three or more nuclei were considered multinuclear. Bone resorption assays were performed on dentine slices in 96-well plates as previously described (11). Dentine slices were sputter-coated with gold and observed by scanning electron microscopy.

Flow Cytometry Analysis of CD1a Expression—Cells cultured on Bio-Coat collagen I plates (BD Biosciences) for 21 days were dissociated using cell dissociation buffer (Invitrogen), incubated with fluorescein isothiocyanate-conjugated human CD1a antibody (Chemicon, Temecula, CA) for 45 min on ice, and washed with phosphate-buffered saline prior to flow cytometry (FACS Calibur; BD Biosciences). The unstained cells were gated out and data acquisition and analysis were done using CellQuest software (BD Biosciences).

RNA Studies—At 21 days, cultures were lysed using 4 M guanidine isothiocyanate, 1% lauryl sarcosine, and total RNA pelleted through a 5.7 M cesium chloride, 100 mM EDTA cushion by ultracentrifugation in a Beckman SW41 rotor at 27,000 rpm for 16 h (17). Total RNA was converted into cDNA using ImProm-II reverse transcriptase (RT; Promega) and oligo(dT) primer. Quantitative PCRs were performed and analyzed using SYBR Green I Supermix (Bio-Rad) in a Bio-Rad i-Cycler (9). Primers and conditions for quantitative PCR assays were as described previously (9, 18) except for MCP-1 assays that used primers 5'-TCGCGAGCTATAAGAGAATCA-3' and 5'-TGTTCAAGTCTTCGGAAGTTTG-3. Gene arrays containing 19,000 duplicate spotted cDNA representing human genes were hybridized and analyzed according to the manufacturer’s protocols (University of Ontario Cancer Center).

Statistical Analysis—Analysis of variance with Fisher’s post hoc t-test was used to determine significance of effects. Data are presented as mean values ± S.E.

RESULTS
Phenotype of Cells Treated with GM-CSF in the Presence of RANKL and M-CSF—We clarified the effect of continuous exposure of human PBMC to GM-CSF on osteoclast differentiation mediated by M+R treatment (Fig. 1). The appearance of TRAP-positive multinuclear cells was suppressed dose dependently by GM-CSF, with osteoclast differentiation suppressed up to 97% by exposure to 25 ng/ml GM-CSF (Fig. 1A). Furthermore, delayed addition of GM-CSF had similar suppressive effects (Fig. 1B). Whereas normal osteoclasts differentiated using M+R treatment were TRAP-positive and multinuclear with potent bone resorption (Fig. 1C), cultures treated with maximal doses of GM-CSF and M+R treatment were TRAP-negative, mononuclear, and completely negative for bone resorption (Fig. 1C).

To further characterize the phenotype of cells treated with GM-CSF in the presence of RANKL and M-CSF, the expression of a series of osteoclast-related genes was examined (Fig. 2A). Gene expression profiles were compared between osteoclasts (M+R treatment, 25 ng/ml M-CSF and 20 ng/ml RANKL), macrophage-like cells (M-CSF-alone treatment, 25 ng/ml), and
osteoclast-related genes. Graph shows GM-CSF receptor-
macrophages and was further up-regulated in GMR treatment
GM-CSF receptor-
ml) with RANKL and M-CSF (GMR treatment) as above. The
cells derived from continuous treatment with GM-CSF (25 ng/
ml) with RANKL and M-CSF (GMR treatment) as above. The
GM-CSF receptor-α was induced in osteoclasts relative to
macrophages and was further up-regulated in GMR treatment
(Fig. 2A). The up-regulation of GM-CSF receptor-α by RANKL
provides an explanation why delayed addition of GM-CSF is
still potent at suppressing osteoclast differentiation, because
cells may be sensitized to inhibition by increased receptor. We
previously established a series of nuclear factors, including
NFATc1, that are regulated strongly by RANKL during oste-
clast differentiation (9). These nuclear factors show pro-
duced differential patterns of expression in the three treat-
ment groups (Fig. 2A). In particular, the RANKL-mediated
induction of NFATc1 is suppressed by GM-CSF in GMR-
treated cells relative to M+R treatment with M+R treatment.

GM-CSF Suppresses RANKL-mediated Induction of
MCP-1—Because GMR treatment suppressed NFATc1, we
hypothesized that GM-CSF in the presence of RANKL and M-CSF
inhibits the induction of key factors in osteoclast differentia-
tion. Such factors would appear suppressed by GM treatment
compared with M+R treatment. A large number of genes dem-
strated significant differential expression in microarray
analysis (data not shown), although only a few are discussed.
The expression of various genes was consistent with suppres-
sion of the osteoclast phenotype by GM-CSF: cathepsin D,
vacuolar H+ ATPase proton pump (ATP6C), NFATc1, and the
osteoclast protease cathepsin K were repressed (data not
shown). The GM-CSF receptor-α was up-regulated, in keeping
with the real-time PCR analysis (Fig. 2A). Of 19,000 genes
assayed, the gene most repressed by GMR treatment in com-
parison to M+R treatment was MCP-1, a CC chemokine not
associated previously with osteoclast differentiation. To verify
the regulation of MCP-1 by GM-CSF, new cultures were estab-
lished on three separate occasions, differentiated over 21 days
under comparable conditions with M-CSF alone, M+R, and
GMR treatment (Fig. 2D). MCP-1 mRNA content, measured by
quantitative real-time PCR, was $17.1 \pm 0.1$ (copies/ng total
RNA) in M+R cultures compared with $0.24 \pm 0.06$ in GMR-
treated cultures, meaning that GM-CSF treatment results in a
72-fold decrease in MCP-1 mRNA ($p = 5 \times 10^{-3}$). Further-
more, MCP-1 was induced by RANKL during osteoclast differen-
tiation (15-fold) compared with macrophage cultures (M-CSF-
alone treatment). These data confirm that GM-CSF represses
MCP-1 expression in the GMR treatment compared with M+R
and also show that MCP-1 is induced in osteoclasts relative to
macrophage-like cells. Another CC chemokine, RANTES, is
induced during human (9) and mouse (15, 16) osteoclast differ-
entiation. RANTES induction by RANKL was also suppressed
by GM-CSF (Fig. 2D).

Chemokine Effects on Osteoclasts—We reasoned that exoge-
nous MCP-1 would affect osteoclast differentiation. Adding
MCP-1 to the standard M+R treatment protocol resulted in
33% more osteoclasts (329 ± 26 n = 14 versus 252 ± 13 n = 19,
respectively; $p = 0.008$) (Fig. 3A) that were TRAP-positive
and positive for bone resorption (Fig. 3B). Unexpectedly, MCP-1
treatment with M-CSF in the absence of exogenous RANKL
resulted in TRAP-positive, multinucleated giant cells (Fig. 3B).
Although these cells had the appearance of osteoclasts, they
were unable to form resorption pits on dentine, suggesting that
MCP-1 and M-CSF treatment results in an intermediate phe-
notype on the path to osteoclasts, permitting monocyte fusion
but not further differentiation. Like MCP-1, RANTES treat-
ment with M-CSF resulted in multinuclear TRAP-positive cells
that were unable to degrade bone (Fig. 3D). Unlike MCP-1,
exogenous RANTES had no effect on osteoclast number or bone

![Image](./image.png)
FIG. 3. Effect of chemokines MCP-1 and RANTES on cellular phenotypes with and without RANKL. A, in the presence of M-CSF and chemokines, TRAP-positive multinuclear cells were formed. Both MCP-1 and RANTES (at 25 ng/ml each) significantly increased TRAP-positive multinuclear cells compared with M-CSF-alone-treated cells. *, significant difference compared with M-CSF- and MCP-1-treated PBMCs, where $p < 0.05$. #, significant difference compared with M-CSF- and RANTES-treated PBMCs, where $p < 0.01$. B, in the presence of RANKL and M-CSF (M+R), continuous exposure to MCP-1 (25 ng/ml) results in TRAP-positive multinuclear cells (upper left panel) that are able to resorb bone (upper right panel). In the presence of M-CSF alone, MCP-1 treatment results in TRAP-positive multinuclear cells (lower left panel) that are negative for bone resorption (lower right panel). C, in the presence of M+R and MCP-1, a significant increase in TRAP-positive multinuclear cells was observed. Neutralizing antibody directed against MCP-1 reversed the effect of MCP-1; adding antibody to M+R plus MCP-1 treatment significantly decreased the number of TRAP-positive multinuclear cells. *, significant difference compared with M+R-treated PBMCs, where $p < 0.05$. #, significant difference compared with M+R plus MCP-1-treated PBMCs, where $p < 0.05$. #, significant difference compared with M+R plus RANTES-treated PBMCs, where $p < 0.05$. D, RANTES (25 ng/ml) has a similar phenotype to MCP-1. In the presence of RANKL and M-CSF (M+R), continuous exposure to RANTES results in TRAP-positive multinuclear osteoclasts (upper left panel) that are positive for bone resorption (upper right panel). PBMCs treated with RANTES and M-CSF show TRAP-positive multinuclear cells (lower left panel) that are negative for bone resorption (lower right panel). Numbers of independent experiments are shown in panels A and C. Bar, 100 μm. E, quantitative real-time PCR analysis of TRAP and cathepsin K expression in cultures treated with RANKL and M-CSF (black columns) and M-CSF and MCP-1 (gray columns). Data from four independent experiments.
resorption in the presence of RANKL (Fig. 3, C and D). MCP-1-mediated effects were sensitive to specific neutralizing anti-MCP-1 antibody, preventing the MCP-1-mediated enhancement of osteoclast formation observed in M+R/M+/H11001R-treated cultures and inhibiting the formation of multinuclear cells in cultures treated with MCP-1 and M-CSF (Fig. 3, A and C). Neutralizing anti-MCP-1 antibody significantly reduced the number of osteoclasts in standard M+/H11001R cultures (p < 0.01) (Fig. 3C). Control antibody had no effect on osteoclast number (data not shown.

Treatment with MCP-1 and M-CSF resulted in multinuclear cells that had the appearance of osteoclasts but were negative for bone resorption (Fig. 3B). The levels of expression of two osteoclast-related genes, TRAP and cathepsin K, were compared by quantitative PCR in cultures treated with MCP-1 and M-CSF compared with standard M+R treatment (Fig. 3E). The mRNA content of TRAP was virtually identical in the multinuclear TRAP-positive non-bone-resorbing cells from MCP-1- and M-CSF-treated cells compared with authentic osteoclasts. In contrast, cathepsin K mRNA content was substantially lower in MCP-1- and M-CSF-treated cells. This may provide a rational for the inability of these osteoclast-like TRAP-positive multinuclear cells to degrade bone: cathepsin K and possibly other essential osteoclast-related genes are not appropriately induced in MCP-1-treated cells.

**MCP-1 Rescues Suppression of Osteoclast Differentiation**—MCP-1 acts as an enhancer of osteoclast differentiation and was strongly repressed by GM-CSF. We hypothesized that the lack of MCP-1 may be influential in GM-CSF-mediated suppression of osteoclast differentiation from PBMCs. This hypothesis was tested by addition of exogenous MCP-1 under conditions of maximal suppression of osteoclast differentiation by GM-CSF (25 ng/ml). The addition of MCP-1 (at 25 ng/ml) to the GMR treatment protocol dramatically increased the formation of TRAP-positive multinucleate cells (p = 3.8 x 10^{-8}; Fig. 4A). Although the multinuclear cells derived from MCP-1-treated GMR cultures were generally smaller than osteoclasts derived from control M+R-treated cultures (47 ± 4 and 148 ± 22 μm, respectively; average longest axis, p = 2.2 x 10^{-5}), they were almost twice as abundant and were positive for bone resorption (Fig. 4B). Although RANTES overcame the block in multinucleation imposed by GM-CSF, resulting in a similar number of TRAP-positive multinucleate cells as in M+R controls, the cells were negative for bone resorption (Fig. 4B).
If MCP-1 and RANTES are involved in cell fusion events, we reasoned that these chemokines should be able to rescue multinuclearity from blockade of osteoclast formation by other chemicals, such as cyclosporin A, a blocker of NFATc1 activation by calcineurin. We showed in human (9), and others in mouse (16, 19, 21), that cyclosporin A inhibits the formation of multinuclear cells in cultures treated with RANKL and M-CSF. Cell cultures exposed to M+R treatment and maximally repressive concentrations of cyclosporin A (1 μg/ml) showed virtually no multinuclear cells (Fig. 4C). In addition, the differentiation of human osteoclasts on dentine and bone resorption activity is potently inhibited by cyclosporin A at 1 μg/ml (data not shown). In marked contrast, MCP-1 or RANTES addition showed a dramatic recovery of TRAP-positive multinuclear cells in cultures treated with cyclosporin A and M+R (Fig. 4C). These cells have the appearance of osteoclasts but are negative for bone resorption (Fig. 4D). Under cyclosporin A blockade, the RANKL-mediated induction of MCP-1 was repressed completely, consistent with nuclear factor-κB or NFATc1 being responsible for the induction of MCP-1 during osteoclast differentiation (Fig. 4E). RANTES induction was repressed similarly.

**DISCUSSION**

Cell fate is controlled by relative concentrations of cytokines, integrated over their respective signaling pathways. Without RANKL, M-CSF treatment of monocytes results in macrophage-like cells; therefore, RANKL overrides the macrophage differentiation pathway to form osteoclasts in the presence of RANKL and M-CSF. Likewise, GM-CSF and RANKL represent two competing differentiation signals, RANKL to osteoclasts and GM-CSF to dendritic-like cells. RANKL-dependent up-regulation of GM-CSF receptorα might provide a negative feedback signal, sensitizing cells to the effect of GM-CSF found in the bone marrow milieu and thus aiding in regulation of osteoclast number. In the presence of RANKL and M-CSF, GM-CSF dominates cell fate and osteoclast differentiation is suppressed, with concomitant suppression of osteoclast-related genes.

MCP-1 was the most potently down-regulated gene in microarray analysis of the inhibitory effect of GM-CSF. Furthermore, exogenous MCP-1 was able to recover authentic osteoclasts from GM-CSF inhibition of osteoclast differentiation. In the presence of sufficient MCP-1, the balance of cell fate is changed back toward the RANKL signal, countervailing the GM-CSF repressive signal. We conclude that the absence of MCP-1 in GMR-treated cultures is a key deficit in osteoclast differentiation. Once this deficit is overcome, by adding exogenous MCP-1, osteoclast differentiation can proceed in the presence of RANKL. The smaller size of the osteoclasts formed under these conditions may reflect a further inhibitory effect of GM-CSF on osteoclasts, even after rescue by MCP-1, because the up-regulation of the GM-CSF receptor by RANKL was unaffected by the presence of GM-CSF.

To date, MCP-1 has not been implicated in osteoclast function, although data exist on its role in recruitment of monocytes during tooth eruption (14). Our data on the effects of MCP-1 and RANTES on osteoclast differentiation may explain why inflammatory diseases that feature increased chemokine activity, such as rheumatoid arthritis, are associated with increased osteoclast activity leading to bone degradation. We demonstrated previously that CCR2, the receptor for MCP-1, is also induced by RANKL, providing evidence for an autocrine cycle involving MCP-1. Furthermore, both MCP-1 and RANTES treatment resulted in multinucleated cells in the absence of RANKL, suggesting that chemokines are sufficient for fusion events.

MCP-1 overcomes GM-CSF-mediated repression of osteoclast differentiation, permitting the cells to pass through multinucleation to authentic bone-resorbing osteoclasts. Cyclosporin A and GM-CSF similarly inhibit both osteoclast differentiation and MCP-1 expression; GM-CSF represses NFATc1 induction, whereas cyclosporin A inhibits activation of NFATc1 by blocking calcineurin. Under continuous chemical blockade of NFATc1 by cyclosporin A, a recovery of bone re-
RANTES treatment of cyclosporin A blockaded M
motion by RANKL was similarly repressed by cyclosporin A, and resorption in cyclosporin A-inhibited cultures. RANTES induc-
sorption in GM-CSF-inhibited cultures but did not recover bone resorption in cyclosporin A-inhibited cultures. RANTES induc-
treatment of cyclosporin A blockaded M+R-treated cells changed the cell phenotype from TRAP-positive mononu-
clear to TRAP-positive multinuclear cells.

Both MCP-1 and RANTES recover the multinuclear pheno-
type in cyclosporin A blockade of osteoclast differentiation but do not recover bone resorption, providing evidence that chemok-
ines are involved in cell fusion events during osteoclast differen-
tiation. In our experiments, although MCP-1 and RAN-
TES were associated with cell fusion events, the presence of RANKL was necessary for bone resorption, as treatment with either MCP-1 or RANTES and M-CSF leads to multinuclear cells but not bone resorption. Taken together, these data sug-
gest that RANKL induction of MCP-1 and RANTES is an im-
portant component of osteoclast differentiation, providing an au-
tocrine signal acting on the osteoclast and a paracrine signal that acts on osteoclast precursors to accelerate osteoclast dif-
ferentiation by promoting fusion.

The expression of osteoclast marker genes in cells treated with MCP-1 and M-CSF suggests that some osteoclast charac-
teristics can be acquired independently of RANKL. Because RANKL induces the MCP-1 receptor (CCR2), RANKL in-
duction of MCP-1 sets up both autocrine (affecting the osteoclast-
producing MCP-1) and paracrine pathways (affecting cells des-
tined to fuse with the RANKL-stimulated osteoclast). We present a model for chemokine action during osteoclast differen-
tiation (Fig. 5). Osteoclasts form by fusion of RANK+ mono-
nuclear precursors after contact with a cell expressing RANKL. Intimate cell-cell contact is necessary in vitro for RANKL sig-
naling, a process mimicked in vitro with soluble recombinant RANKL. An osteoclast precursor cell in contact with a RANKL-
presenting cell (Fig. 5) will receive the RANKL signal and initiate a cascade of gene expression that includes the produc-
tion of MCP-1, RANTES, and possibly other chemokines. MCP-1 and RANTES are chemotactic signals for monocytes, resulting in migration to the source of production of the che-

mokine. The data show that either MCP-1 or RANTES can cause cell fusion in monocytes treated with M-CSF. We propose that cell fusion is a key event in the next step of osteoclast differen-
tiation (Fig. 5) where monocyte-like cells, that have not yet seen RANKL are attracted by chemokines to the site of cou-
ppling of the RANK+ precursor and the RANKL-presenting cell. Chemokine-mediated fusion increases the size of the oste-
oclast and also transfers the RANKL signal to the additional nuclei that are now in the multinucleated cell. We show that fusion can occur in the absence of RANKL but that bone re-
sorption depends on RANKL. If the chemokine signal is strong enough, such monocyte cell fusion could occur prior to contact with the RANKL-influenced osteoclast precursor. Such TRAP-
positive “prefused” cells would still require the RANKL signal to develop into an authentic osteoclast capable of bone resorp-
tion because they have a deficit in cathepsin K expression.

In conclusion, MCP-1 is induced by RANKL during oste-
oclast differentiation and is sensitive to cyclosporin A and repressed by GM-CSF but is able to overcome GM-CSF repression of osteoclast differentiation. Furthermore, chemokines stimulate the formation of fused cells in the absence of RANKL and overcome the block in fusion caused by cyclosporin A. These data indicate that chemokines play a crucial role in osteoclast function.

Acknowledgment—We thank G. Nicholson for the kind gift of bone chips.

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