We have cloned and expressed murine osteoclast inhibitory lectin (mOCIL), a 207-amino acid type II transmembrane C-type lectin. In osteoclast formation assays of primary murine calvarial osteoblasts with bone marrow cells, antisense oligonucleotides for mOCIL increased tartrate-resistant acid phosphatase-positive mononuclear cell formation by 3–5-fold, whereas control oligonucleotides had no effect. The extracellular domain of mOCIL, expressed as a recombinant protein in *Escherichia coli*, dose-dependently inhibited multinucleate osteoclast formation in murine osteoblast and spleen cell co-cultures as well as in spleen cell cultures treated with RANKL and macrophage colony-stimulating factor. Furthermore, mOCIL acted directly on macrophage/monocyte cells as evidenced by its inhibitory action on adherent spleen cell cultures, which were depleted of stromal and lymphocytic cells. mOCIL completely inhibited osteoclast formation during the proliferative phase of osteoclast formation and resulted in 70% inhibition during the differentiation phase. Osteoblast OCIL mRNA expression was enhanced by parathyroid hormone, calcitriol, interleukin-1α, and -11, and retinoic acid. In rodent tissues, Northern blotting, *in situ* hybridization, and immunohistochemistry demonstrated OCIL expression in osteoblasts and chondrocytes as well as in a variety of extraskeletal tissues. The overlapping tissue distribution of OCIL mRNA and protein with that of RANKL strongly suggests an interaction between these molecules in the skeleton and in extraskeletal tissues.

The interactions of the recently discovered proteins RANKL (Receptor Activator of NF-κB ligand), osteoprotegerin (OPG),

and RANK, the cognate receptor for RANKL, along with M-CSF are crucial to the formation of osteoclasts. Osteoclasts, multinucleate cells responsible for bone resorption, are derived from hematopoietic stem cells that differentiate along the macrophage/monocyte lineage (1, 2). Direct contact between osteoclasts or stromal cells with mononuclear precursors of osteoclasts is required for their differentiation into mature, functional, multinucleate osteoclasts (3). Osteoblasts/stromal cells express a membrane-bound protein termed RANKL, which stimulates the differentiation and formation of multinucleate osteoclasts from mononuclear precursors when it binds to its receptor, RANK (4). RANKL is also known as TRANCE (5, 6), ODF (7), or OPGL (8, 9). Recombinant protein corresponding to the extracellular domain of RANKL stimulates the formation of active, bone-resorbing osteoclasts from hematopoietic cells derived from spleen even in the absence of stromal cells (10). RANKL expression is stimulated by bone-resorbing factors such as parathyroid hormone, PGE2, 1,25-dihydroxyvitamin D3, and interleukin-1β and -11 (7, 11, 12). In addition to the stimulation of osteoclast differentiation, RANKL also enhances the activity of mature osteoclasts (13), inhibits osteoclast apoptosis (14), and enhances osteoclast survival (15).

OPG is a soluble member of the tumor necrosis factor receptor family that is secreted by osteoblastic stromal cells. It acts as a decoy receptor for RANKL, antagonizing its biological actions by preventing it from binding to and activating its receptor, RANK (16). Overexpression of OPG in transgenic mice results in severe osteopetrosis, with impaired formation of marrow cavity and profound depletion of osteoclasts. Furthermore, OPG blocks ovarectomy-associated bone loss in the rat. In addition to inhibition of osteoclast formation, OPG also inhibits resorption pit formation by mature osteoclasts and antagonizes the induction of bone resorption by 1,25-dihydroxyvitamin D3, parathyroid hormone, PGE2, and IL-1α as well as RANKL (14, 17–19). Other inhibitors of osteoclast formation have been identified in recent years. They include IL-4, IL-10, IL-18, interferon-γ, and GM-CSF (20, 21) as well as legumain and Sca, which were cloned and isolated from a human osteoclast cDNA expression library. Legumain (22) is a member of the mammalian cysteine protease family, the asparaginyl endopeptidases (23), whereas Sca is a glycosylphosphatidylinositol-linked osteoclast inhibitory factor (24).

In pursuit of a factor that regulates the formation of osteoclasts, we have identified an osteoblast-derived membrane protein with structural homology to the C-type lectin family that limits osteoclast formation. The full-length cDNA of osteoclast inhibitory lectin (OCIL) predicts OCIL to be a 207-amino acid type II membrane protein with a 143-amino acid extracellular domain, a 21-amino acid transmembrane domain, and a 43-amino acid cytoplasmic domain.
EXPERIMENTAL PROCEDURES

Materials—Rat UMR 201 cells, rat UMR 106 cells, and primary mouse calvarial osteoblasts were routinely grown in α-minimal essential medium containing 10% fetal bovine serum. Incubation was carried out at 37 °C in a humidified atmosphere equilibrated with 5% CO₂ in air. Dr. S. Rodan (Merck Research Laboratories, West Point, PA) generously provided a rat ROS 17/2.8 cDNA library. M-CSF was a gift from Genentech Institute (Cockeysville, MA). Recombinant murine Rankl (CLONTECH) was obtained from Preprotech Inc. (Canton, MA). α-32PIdCTP was purchased from PerkinElmer Life Sciences. All other reagents were of analytic grade and were obtained from standard suppliers. The mouse cDNA library was purchased from Stratagene (La Jolla, CA).

cDNA Cloning from Rat and Mouse cDNA Libraries—We hypothesized that mature osteoblasts might express osteoclastogenic inhibitors, based on observations that mature osteoblasts have limited potential to support osteoclast formation (11). The preosteoblastic cell line UMR 201 was differentiated with 1 μM retinoic acid to enable the expression of a more mature osteoblast phenotype (25, 26), and mRNA profiles were compared between untreated and retinoic acid-treated cells by differential display-polymerase chain reaction as previously described (27).

Briefly, total RNA was isolated from untreated and retinoic acid-treated preosteoblastic UMR 201 cells using guanidine thiocyanate (28). First strand cDNA was synthesized from 2 μg of total RNA by incubation for 1 h at 42 °C with 15 units of avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) following T₅ΨA, T₅VC, or T₇ΨG priming. The primers used in the PCR were 5′-ATG CTG GCC ACG TAG ACA CAA C-3′ and either T₅ΨA, T₅VC, or T₇ΨG (CLONTECH, CA). The PCR conditions utilized a touch-up PCR protocol with denaturation for 5 min and 35 cycles of 94 °C for 1 min, 37 °C for 1 min, and 72 °C for 1 min, followed by 35 cycles of 94 °C 1 min, 49 °C for 1 min, and 72 °C for 1 min. For these experiments, the Expand High Fidelity PCR system (Roche Molecular Biochemicals, Mannheim, Germany) was used in a PerkinElmer Life Sciences 480 thermal cycler. A 321-bp PCR product was obtained. To extend its sequence, anchored PCR was used to screen a rat ROS 17/2.8 cDNA library. A 25-bp antisense primer that was complementary to a sequence in the 321-bp fragment, 5′-TGA GTG TTT TGC TGT GTC CAC TTC CAA C-3′, was used with either the αgt11 forward primer (5′-GGT GGC GAC GAC TCC TGG AGC C-3′) or the αgt11 reverse primer (5′-ACC AGA AGA ACA ACT GGT AAT G-3′) (CLONTECH) to amplify further nucleotide sequences. Cycling parameters were 94 °C for 5 min and then 80 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 2 min, followed by a final extension step of 72 °C for 10 min. A 402-bp fragment, designated rOCIL402, was obtained with the αgt11 reverse primer as the anchored primer. The 402-bp fragment was labeled with α-32PIdCTP using a random primer labeling kit (Roche Molecular Biochemicals) to screen a mouse liver cDNA library at 65 °C in hybridization buffer containing 4× SSPE (SSPE = 0.15 M NaCl, 0.01 M NaH₂PO₄, and 0.001 M EDTA), 5× Denhardt’s solution, and 0.5% SDS for 24 h. Filters were washed in 2× SSC at 65 °C for 15 min, 2× SSC with 0.1% SDS at 65 °C for 30 min, and finally 0.1× SSC at 65 °C for 10 min. A 1206-bp cDNA termed mOCIL was obtained.

The full-length rat OCIL cDNA was obtained from total RNA isolated from human parathyroid hormone-(1–34)-treated rat osteoblast-like UMR 106 cells. A 3′-rapid amplification of cDNA ends strategy was used to obtain the 3′-end of rat OCIL using the 3′-adaptor primer 5′-GGC CAC GCG TCG ACT AGT TTG TTT TTT TTT TTT T-3′ (CLONTECH) for the reverse transcription, followed by amplification with universal adaptor primer 5′-GGC CAC GCG TCG ACT AGT-3′ and the sense primer 5′-GAA ACA TCC CCC TGG ATC ATC-3′, which was complementary to sequences within rOCIL402. A 5′-rapid amplification of cDNA ends strategy was used to obtain the 3′-end of the rat OCIL cDNA using the SMART RACE cDNA amplification kit (CLONTECH). The antisense primer 5′-CCT AGT GGT TTC TGG CTA CTT CCA AGG G-3′ was complementary to sequences within rOCIL402. The full-length rat OCIL cDNA sequence is 1628 bp.

Osteoclast Formation Assays—Osteoclast formation was assessed either by culturing bone marrow cells with calvarial osteoblasts or by culturing mouse spleen cells with soluble Rankl and M-CSF. Mouse cell cultures were established as described previously (29). Bone marrow cells from long bones of 6–9-week-old male mice (C57Bl/6j) were co-cultured with calvarial osteoblasts obtained from newborn mice in the absence or presence of 10 ng 1,25-dihydroxyvitamin D₃ and 10 μM PGE₂. The expression of mOCIL was inhibited by concurrently treating the co-cultures with antisense oligonucleotides added with medium changes on days 0 and 3 of a 7-day co-culture. The following phosphorothioate oligodeoxynucleotides for mOCIL were used at 5 μM with the co-cultures: 5′-GAG TGT TGT CTG TCC ACT TCC-3′, complementary to the extracellular domain, and 5′-GAG AGA GAA GCC TTT GTG AC-3′, complementary to the intracellular domain. Scrambled phosphorothioate oligonucleotides with the same base composition as the antisense oligonucleotides for these experiments were 5′-GCG TCG CTG TAT GTC TGC TAT-3′ for the extracellular domain and 5′-AGT CGT GCG TAG TGC GAA TGA-3′ for the intracellular domain. After 7 days, cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) using an acid phosphatase leucocyte commercial kit (Sigma). TRAP-positive multinucleate cells were further characterized as osteoclasts by the presence of receptors, demonstrated by immunohistochemistry (30).

Mouse spleen cells were obtained from adult mice and cultured for 7 days in culture medium containing 10% fetal bovine serum, 25 ng/ml M-CSF, and 50 ng/ml soluble RANKL in the absence or presence of maltose-binding protein (MBP) vector control or recombinant MBP-mOCIL fusion protein at various concentrations. To obtain cultures of adherent spleen cells that were depleted of non-adherent erythrocytes and lymphocytes, spleen cell suspensions were added to 10-mm diameter culture wells (2× 10⁵ cells/well) containing 6-mm diameter glass coverslips. The cells were allowed to settle and attach for 40–60 min at 37 °C before the coverslips were removed and rinsed vigorously in α-minimal essential medium containing 10% fetal bovine serum. Coverslips were then placed on fresh 10-mm diameter wells containing 200 μl of α-minimal essential medium and 10% fetal bovine serum. Treatments were added on day 0 and with the medium change on day 7.

Expression of Recombinant mOCIL—A DNA fragment encoding the extracellular domain (residues 76–207) of mOCIL was obtained by PCR and cloned into the EcoRI and HindIII sites of pMAL-c2 (New England Biolabs Inc., Beverly, MA), creating a gene fusion with the MBP-encoding maltE gene. PCR was performed using mOCIL cDNA as a template. The reaction used a sense primer representing nucleotides 285–303 of mOCIL, encoding amino acids 76–81 (TYAAP), with an EcoRI site (5′-TCA GAA TTA ACC TAT CCT GGT TCG CCG C-3′) and an antisense primer representing nucleotides 711 to 690 of mOCIL after the termination codon with a HindIII site (5′-GTT TAA GTA TCA TCA GCG TAA AAA GGC TUT CCT GG-3′). The PCR product was digested with EcoRI and HindIII and cloned into pMAL-c2. E. coli BL21 cells were transformed with this construct, and the fusion protein was induced with 0.3 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h at 37 °C. The MBP-OCIL fusion protein was isolated from the soluble bacterial fraction by affinity chromatography as outlined in the manufacturer’s instructions. The eluant fractions were subjected to SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The fusion protein at various concentrations. To obtain cultures of mouse calvarial osteoblasts with primers mOCIL-12 (5′-GGG ACC GAC GAC TCC TGG AGC C-3′) and mOCIL-17 (5′-TGG AAA CTC AGC TCC TCA GCT CTG-3′), representing nucleotides 34–57) and mOCIL-1 (5′-GGC TCC TGG TAT GTC TGC TAT-3′), representing nucleotides 285–303 of mOCIL, encoding from human parathyroid hormone-(1–34)-treated rat osteoblast-like cells by reverse transcription-PCR. Mouse bone marrow was cultured for 10 days in the presence of 10 ng 1,25-
RESULTS

OCIL—The full-length mOCIL cDNA was 1206 bp in length. The open reading frame encodes a putative 207-amino acid peptide whose structure has characteristics typical of a type II membrane protein, with a predicted 143-amino acid extracellular domain, a 21-amino acid transmembrane domain, and a 43-amino acid cytoplasmic domain. The extracellular domain has 5 cysteine residues and three predicted N-linked glycosylation sites at residues 74, 100, and 158. A myristoylation motif is also predicted in the intracellular domain (Fig. 1).

Effect of mOCIL Antisense Oligonucleotides on Osteoclast Formation—Primary calvarial osteoblasts were co-cultured with mouse bone marrow cells to generate osteoclasts. After 7 days, there was a 3–5-fold increase in the number of mononuclear TRAP-positive cells in the co-cultures treated with antisense oligonucleotides for the intracellular and extracellular domains of mOCIL compared with co-cultures performed in the absence of oligonucleotides or in the presence of OCIL scrambled oligonucleotides of the same base composition as the antisense oligonucleotides (Fig. 3A). Under these experimental conditions, multinuclear osteoclasts are generally not formed unless stimulated by 1,25-dihydroxyvitamin D3 and PGE2. A small number of multinuclear osteoclasts were observed in co-cultures treated with OCIL sense oligonucleotides for 7 days (eight ± five/well), but none were observed in control co-cultures that were unstimulated or in those treated with OCIL sense or scrambled oligonucleotides. These experiments were performed three times, and representative results are shown. When the co-cultures were stimulated with 10 nM 1,25-dihydroxyvitamin D3 and 10 nM PGE2, multinuclear TRAP-positive osteoclasts were formed after 7 days. Treatment with 5 μM OCIL antisense oligonucleotide complementary to sequence in the extracellular domain resulted in a 7-fold increase in the number of multinuclear osteoclasts (Fig. 3B).

Both mononuclear and multinuclear TRAP-positive cells were further characterized as osteoclasts by the presence of calcitonin receptors demonstrated by immunostaining, using a rabbit polyclonal antibody specific for the C-terminal intracellular domain of the mouse and rat Clalpha calcitonin receptor (30) (data not shown). Co-cultures treated with sense or scrambled oligonucleotides did not alter osteoclast formation, and osteoclast formation was compared with mRNA expression of OPG. A set of sense and antisense primers were used as reported (11) with nucleotide sequences represented by OPG-7 (5′-TGA GTG TGA GGA AGG CGG TTA C-3′, nucleotides 405–426) and OPG-3 (5′-TTT CTT GTC TCT TGA ATC TCA-3′, nucleotides 1021–1040), respectively. The PCR was run at 94 °C for 5 min and then for 35 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min, followed by a final extension step of 72 °C for 10 min. Southern blot analysis was carried out as reported (33). 20 μl of each PCR mixture was run on a 2% agarose gel and transferred to nylon membranes, and products were authenticated by probing with an internal antisense strand oligonucleotide complementary to mOCIL, labeled with digoxigenin-dUTP using a 3′-tailing kit (Roche Molecular Biochemicals). Hybridization was carried out with 2 pmol labeled oligonucleotides in buffer containing 5× SSC, 0.2% SDS, 0.1% sarcosine, and 100 ng/ml poly(A) at 55 °C for 14 h. Detection was by chemiluminescence using CDP (Roche Molecular Biochemicals) according to the manufacturer’s instructions.

The time course of OCIL mRNA expression during osteoclast formation was compared with mRNA expression of OPG. A set of sense and antisense primers were used as reported (11) with nucleotide sequences represented by OPG-7 (5′-TGA GTG TGA GGA AGG CGG TTA C-3′, nucleotides 405–426) and OPG-3 (5′-TTT CTT GTC TCT TGA ATC TCA-3′, nucleotides 1021–1040), respectively. The PCR was run at 94 °C for 5 min and then for 35 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min, followed by a final extension step of 72 °C for 10 min. Hybridization was carried out using the digoxigenin-labeled internal sense oligonucleotide OPG-1 (5′-ACC AAA GTG AAT GCC GAG-3′) under the same conditions described above. To ensure equal starting quantities of RNA in each sample, the reverse-transcribed material was also amplified using oligonucleotide primers specific for rat GAPDH as described (33).

Tissue Distribution of OCIL Using Northern Blot Analysis—Total RNA was extracted from adult mouse tissues for the detection of OCIL mRNA expression using Northern blot analysis as described above. Relative mRNA levels were normalized for loading variability by comparison with 18 S RNA levels in the same filters.

In Situ Hybridization and Immunohistochemistry—Skeletal and extraskeletal tissues were obtained from C57BL/6J male mice. Long bones from the femurs and tibiae of embryo (15 days of gestation), newborn (day 1), and adult (week 5 adult only) mice, and calvariae from newborn (day 1) and adult (week 5) mice were removed and dissected free of tissue. For the study of extraskeletal tissue distribution, the following tissues were obtained from embryonic (15 days of gestation), newborn (day 1), and adult (week 8) mice: brain, kidney, lung, heart, liver, small intestine, skin, skeletal muscle, and spleen (week 5 adult only).

Tissue specimens were fixed immediately by immersion in 4% paraformaldehyde in diethyl ester pyrocarbonate/phosphate-buffered saline and maintained overnight at 4 °C (34). Long bones obtained from the 7-day-old and 7-week-old mice were decalcified with 15% EDTA in 0.5% paraformaldehyde/phosphate-buffered saline solution (pH 8.0) for 4 and 10 days, respectively, at 4 °C. The decalcifying solution was changed daily. All tissue specimens were processed and embedded in paraffin under sterile conditions. In situ hybridization using the 713-bp mOCIL antisense and sense riboprobes was carried out as described previously (34, 35).

For immunohistochemistry, the peptide H-Cys-Met-Ala-Gln-Glu-Ala-Gln-Leu-Ala-Arg-Phc-Asp-Asn-Gln-Asp-Glu-Leu-Asn-Phe-OH, located in the extracellular domain of OCIL, was synthesized and used to immunize rabbits following standard protocols (36). The standard peroxidase-labeled streptavidin/biotin detection method was used according to the manufacturer’s instructions (Dako Corp., Carpinteria, CA) with minor modifications. The dilution of the antiserum used was optimized in preliminary experiments. Incubation of tissue sections with a 1:100 dilution of the primary antiserum was carried out overnight at 4 °C in a humidified chamber. Peroxidase activity was detected with 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and 0.15% H2O2. Slides were counterstained with hematoxylin, dehydrated, and mounted with a coverslip. To confirm specificity of immunostaining, the primary antiserum was substituted with preimmune rabbit serum at the same dilution.

Conclusion—Statistical Analysis—Statistics were performed using Student’s t test and expressed as means ± S.E.
The effect of MBP-OCIL fusion protein on osteoclast formation was determined by the addition of MBP vector control alone or increasing concentrations of MBP-OCIL fusion protein to adherent adult mouse spleen cells treated with 50 ng/ml soluble RANKL and 25 ng/ml M-CSF for 7 days in the presence of 10 nM PGE2. Multinucleate TRAP-positive osteoclasts (TRAP+ MNC) were formed after 7 days. Mononucleate and multinucleate TRAP-positive cells express calcitonin receptors (data not shown). Data are representative of experiments performed three times. Bars represent means ± S.E. (n = 3 for each treatment). *, p < 0.001 versus control. A/S, antisense.

numbers were equivalent to similar co-cultures in which oligonucleotides were not added.

**Effect of Recombinant mOCIL on Osteoclast Formation**—The increase in osteoclast formation when OCIL expression was inhibited by antisense oligonucleotides suggested that OCIL acts to inhibit osteoclast formation. This was tested by assessing osteoclast formation in the presence of the extracellular domain of mOCIL expressed as a soluble recombinant protein.

The effect of MBP-OCIL fusion protein on osteoclast formation by adherent adult mouse spleen cells treated with 50 ng/ml soluble RANKL and 25 ng/ml M-CSF for 7 days is shown in Fig. 4. Using this osteoclast generation system, potential effects of OCIL on osteoclast formation can be divided into two distinct phases: proliferation (days 0–3) and differentiation (days 4–7) (42). The effects of MBP-OCIL fusion protein on the two phases of osteoclast development were examined with adult murine adherent spleen cells. MBP-OCIL fusion protein was effective in inhibiting osteoclast formation predominantly during the proliferative phase (days 0–3), with a smaller effect (70% inhibition) during the differentiation phase (days 4–7) (Fig. 5).

**Expression of OCIL and OPG mRNAs during Osteoclast Formation in Mouse Bone Marrow Culture**—In this system, multinucleate osteoclast formation was observed after day 5 and was associated with an increase in expression of mRNA for the IL-11 receptor as well as the calcitonin receptor (43). OCIL mRNA was constitutively expressed in fresh bone marrow cells. Upon stimulation by 1,25-dihydroxyvitamin D3, a time-dependent decrease in OCIL mRNA relative to GAPDH mRNA occurred. By day 3, OCIL mRNA was 13% of control levels, decreasing even further by day 8. Like OCIL, OPG mRNA was constitutively expressed in fresh bone marrow cells. However, unlike OCIL, OPG mRNA expression increased 2-fold by day 4 in cultures treated with 1,25-dihydroxyvitamin D3; and by day 8, it had increased 4-fold (Fig. 6).

**Regulation of OCIL mRNA Expression in Rat Osteoblasts**—OCIL mRNA levels were examined by Northern blot analyses in primary mouse calvarial osteoblast cells and the rat osteoblast-like cell line UMR 106 following treatment with osteotropic factors for 24 h (Fig. 7). OCIL mRNA expression was up-regulated by IL-1α, IL-11, and 1,25-dihydroxyvitamin D3 in primary mouse calvarial cells and by retinoic acid as well as parathyroid hormone-(1–34) in UMR 106 cells.

**Tissue Distribution of OCIL mRNA in Adult Mouse**—Total RNA was extracted from adult (week 7) mice to determine the expression of OCIL mRNA in extraskeletal tissues by Northern...
blot analysis. OCIL mRNA was expressed by all tissues examined, with the highest expression in kidney, liver, gut, heart, and skeletal muscle (Fig. 8).

In Situ Hybridization and Immunohistochemistry—Skeletal and extraskeletal tissues were obtained from C57BL/6J male mice to study the spatial distribution of OCIL mRNA and protein by in situ hybridization and immunohistochemistry, respectively. Long bones from the femurs and tibiae were obtained from embryonic, newborn, and adult mice, and calvarial bones were obtained from newborn and adult mice. In long bones, immunohistochemistry showed strong expression of OCIL protein in osteoblasts, chondrocytes in the growth plate, and skeletal muscle overlying the bone (Fig. 9A). In situ hybridization similarly showed strong expression of OCIL mRNA in osteoblasts (Fig. 9C).

For the study of extraskeletal tissue distribution, the following tissues were obtained from embryonic (15 days of gestation), newborn (day 1), and adult (week 8) mice: brain, kidney, liver, heart, lung, spleen, intestine, skin, skeletal muscle, and spleen (week 5 adult only). The results of in situ hybridization and immunohistochemistry for OCIL expression are tabulated in Table I.

DISCUSSION

We report the cloning and characterization of an inhibitor of osteoclast formation (mOCIL) that belongs to the C-type lectin family. The structure of mOCIL is reminiscent of a type II
transmembrane protein, with a carboxyl-terminal extracellular domain and a short amino-terminal cytoplasmic domain.

Lectins are nonenzymatic sugar-binding proteins that bind with considerable specificity to complex carbohydrate structures found on cell surfaces and in the extracellular matrix and secreted glycoproteins. They are involved in numerous cellular processes such as host-pathogen interactions, targeting of proteins within cells, and cell-cell interactions (40, 44). The calcium-dependent (C-type) lectin family includes cell adhesion molecules like selectins, which target leukocytes to lymphoid tissues and sites of inflammation (45, 46); mannose-binding proteins that function in antibody-independent host defense against pathogens (47, 48); and lecticans, a family of chondroitin sulfate proteoglycans including aggrecan, versican, neurocan, and brevin (49, 50). These proteins contain a C-type carbohydrate recognition domain attached to other domains responsible for the physiological functions of the molecule (51). Protein sequences containing C-type lectin domains are classified into seven different categories according to sequence homology and/or the overall modular architecture of the protein (52). The C-type lectin domain of mOCIL has a 36% homology to the C-lectin domain of human CD69. CD69 is the earliest leukocyte activation antigen and is expressed mainly by activated T, B, and natural killer cells. Studies in mice deficient in CD69 through targeted gene deletion suggested that CD69 plays a role in B cell development, with otherwise normal hematopoietic lineage development.
C-type Lectin Inhibition of Osteoclast Formation

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REFERENCES

1. Martin, T. J., Ng, K. W., and Suda, T. (1998) Endocrinol. Metab. Clin. North Am. 18, 833–858

2. Suda, T., Takahashi, N., and Martin, T. J. (1992) Endocr. Rev. 13, 66–80

3. Suda, T., Takahashi, N., and Martin, T. J. (1995) in Endocrine Reviews. Monographs (Bikle, D. D., and Negro-vilar, A., eds) Vol. 4, pp. 266–270

4. Hsu, H., Lacey, D. L., Dunstan, C. R., Solovyev, I., Colombero, A., Elliott, R., Chen, L., Black, T., Scully, S., Capparelli, C., Morony, S., Shimamoto, G., Bass, M. B., and Boyle, W. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3540–3545

5. Anderson, D. M., Maraskovsky, E., Billingsley, W. C., Tomatsko, M. E., Roux, E. R., Teepe, M. C., Dubose, R. F., Cosman, D., and Galibert, L. (1997) Nature 387, 170–179

6. Wong, B. R., Ruo, J., Aron, J., Robinson, E., Orlinick, J., Chao, M., Kalashnikov, S., Cayani, E., Bartlett, F. S., III, Frankel, W. N., Lacey, D. L., Lee, S. Y., and有机会次

7. Yasuda, H., Shimura, K., Namagawa, Y., Yamaguchi, K., Kinosaki, M., Mochimin, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Murinaga, T., Higashio, K., Udagawa, N., Takahashi, N., and Martin, T. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3597–3602

8. Lacey, D. L., Timms, E., Tan, H. L., Lacey, G., Kelley, M. J., Sarsero, I., Wang, L., Xin, X. Z., Elliott, R., Chiu, L., Black, T., Scully, S., Capparelli, C., Morony, S., Shimamoto, G., Bass, M. B., and Boyle, W. J. (1998) J. Cell Biol. 143, 66–80

9. Takahashi, N., Udagawa, N., and Suda, T. (1999) Biochem. Biophys. Res. Commun. 256, 449–455

10. Quinn, J. M., Elliott, J., Gillespie, M. T., and Martin, T. J. (1998) Endocrinology 139, 4424–4427

11. Horwood, N. J., Elliott, J., Martin, T. J., and Gillespie, M. T. (1998) Endocrinology 139, 4743–4746

12. Hofbauer, L. C., Lacey, D. L., Dunstan, C. R., Spelsberg, T. C., Riggs, B. L., and Khosla, S. (1999) Bone (Flensburg) 25, 255–259

13. Burgess, T. L., Qian, Y., Kaufman, S., Ring, B. D., Van, G., Capparelli, C., Kelley, M., Hsu, H., Boyle, W. J., Dunstan, C. R., Hu, S., and Lacey, D. L. (1999) J. Cell Biol. 145, 527–538
14. Fuller, K., Wong, B., Fox, S., Choi, Y., and Chambers, T. J. (1998) J. Exp. Med. 188, 997–1001

15. Udagawa, N., Takahashi, N., Jimi, E., Matsuzaki, K., Tsurukai, T., Ishik, K., Nakagawa, N., Yasuda, H., Goto, M., Tsuda, E., Higashio, K., Gillespie, M. T., Martin, T. J., and Suda, T. (1999) Bone (Elmsford, N.Y.) 25, 517–523

16. Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M. S., Luyth, E., Nguyen, H. Q., Wouden, S., Bennett, L., Bune, T., Shimamoto, G., Del Rose, M., Elliott, R., Colombo, A., Tan, H. L., Trail, G., Sullivan, J., Davy, E., Bucay, N., Renshaw-Gegg, L., Hughes, T. M., Hill, D., Pattison, W., Campbell, F., Sander, S., Van, G., Tarpley, J., Derby, P., Lee, R., and Boyle, W. J. (1997) Cell 89, 369–319

17. Tsukii, K., Shima, N., Mochizuki, S., Yamaguchi, K., Kinossaki, M., Yano, K., Shihata, O., Udagawa, N., Yasuda, H., Suda, T., and Higashio, K. (1998) Biochem. Biophys. Res. Commun. 246, 337–341

18. Kwon, B. S., Wang, S., Udagawa, N., Hardas, V., Lee, Z. H., Kim, K. K., Oh, K. O., Greene, J., Li, Y., Su, J., Gonet, R., Aggarwal, B. B., and Ni, J. (1998) FASEB J. 12, 845–854

19. Hakeda, Y., Kohayashi, Y., Yamaguchi, K., Yasuda, H., Tsuda, R., Higashio, K., Miyata, T., and Kumeedge, M. (1998) Biochem. Biophys. Res. Commun. 251, 796–801

20. Martin, T. J., Romas, K., and Mousli, M. T. (1998) Crit. Rev. Eukaryotic Gene Expression 8, 107–123

21. Horwood, N. J., Udagawa, N., Elliott, J., Grail, D., Okamura, H., Kurimoto, M., Dunn, A. R., Martin, T., and Gillespie, M. T. (1998) J. Clin. Invest. 101, 585–603

22. Choi, S. J., Reddy, S. V., Devlin, R. D., Meneta, C., Chung, H., Boyle, W. J., and Roodman, G. D. (1999) J. Biol. Chem. 274, 27747–27753

23. Chen, J. M., Dando, P. M., Rawlings, N. D., Brown, M. A., Young, N. E., Stevens, R. A., Hewitt, E., Watts, C., and Barrett, A. J. (1997) J. Biol. Chem. 272, 8090–8098

24. Choi, S. J., Devlin, R. D., Meneta, C., Chung, H., Roodman, G. D., and Reddy, S. V. (1998) J. Clin. Invest. 102, 1360–1368

25. Ng, K. W., Gummer, P. R., Michelangeli, V. P., Bateman, J. F., Mascara, T., Cole, W. G., and Martin, T. J. (1988) J. Bone Miner. Res. 3, 53–61

26. Zhao, H., Hummonds, R. G. J., Findlay, D. M., Fuller, P. J., Martin, T. J., and Ng, K. W. (1991) J. Bone Miner. Res. 6, 767–777

27. Trainanes, K., Findlay, D. M., Martin, T. J., and Gillespie, M. T. (1995) J. Biol. Chem. 270, 20891–20894

28. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159

29. Takahashi, N., Akatsu, T., Udagawa, N., Sasaki, T., Yamaguchi, A., Moseley, T. J., Martin, T. J., and Suda, T. (1988) Endocrinology 123, 2600–2602

30. Quinn, J. M., Morfis, M., Lam, M. H., Elliott, J., Kartogianis, V., Williams, E. D., Gillespie, M. T., Martin, T. J., and Sexton, P. M. (1999) Bone (Elmsford, N.Y.) 25, 1–8

31. Hu, Y., S. Zhou, H., Kartogianis, V., Eisman, J. A., Martin, T. J., and Ng, K. W. (1998) Mol. Endocrinol. 12, 1721–1732

32. Igegame, M., Rakopoupolis, M., Zhou, X., Hossamis, S., Martin, T. J., Moseley, J. M., and Findlay, D. M. (1995) J. Bone Miner. Res. 10, 59–65

33. Zhou, H., Manji, S. S., Findlay, D. M., Martin, T. J., Heath, J. K., and Ng, K. W (1994) J. Biol. Chem. 269, 22343–22349

34. Kartogianis, V., Moseley, J., McKeilve, B., Chou, S. T., Harde, D. K., Ng, K. W, Martin, T. J., and Zhou, H. (1997) Bone (Elmsford, N.Y.) 21, 385–391

35. Kartogianis, V., Udagawa, N., Ng, K. W., Martin, T. J., Moseley, J. M., and Zhou, H. (1998) Bone (Elmsford, N.Y.) 22, 189–194

36. Danks, J. A., Ebeling, P. R., Hayman, J., Chou, S. T., Moseley, J. M., Dunlop, J., Kemp, B. E., and Martin, T. J. (1989) J. Bone Miner. Res. 4, 273–278

37. Lopezbreva, M., Santis, A. G., Fernandeuzrua, E., Blacher, R., Esch, S., Sanchezmateos, P., and Sanchezmadrid, F. (1995) J. Exp. Med. 176, 537–547

38. Marazo, R., Jirillo, E., Vanitsma, J., Mauel, J., and Corradin, S. B. (1997) J. Leukoc. Biol. 62, 156–159

39. Sharron, N., and L. (1995) Essays Biochem. 30, 59–75

40. Gubi, H. J. (1997) Eur. J. Biochem. 243, 543–576

41. Roodman, G. D. (1999) J. Clin. Invest. 103, 1025–1032

42. Hofbauer, L. C., Dunstan, C. R., Spelsberg, T. C., Riggs, B. L., and Khosla, S. (1989) Endocrinology 125, 2312–2320

43. Brandstrom, H., Jonsson, K. B., Ohlsson, C., Vidal, O., Ljunghall, S., and Ljunggren, O. (1998) Biochem. Biophys. Res. Commun. 247, 338–341

44. Hofbauer, L. C., Rodon, J., Lacey, D. L., Dunstan, C. R., Spelsberg, T. C., and Khosla, S. (1999) Endocrinology 140, 4382–4389

45. Ravina, B. A., Moseley, J. M., Martin, T. J., and Pennington, J. M. (2000) Cell 103, 41–50

46. Pan, R., Yoshida, H., Sarosi, I., Tan, H. L., Timms, E., Capparelli, C., Morrey, S., Oliveira-dos-Santos, A. J., Van, G., He, A., Khoo, W., Wakeham, A., Dunstan, C. R., Lacey, D. L., Mak, T. W., Byeon, W. J., and Pennington, J. M. (1999) Nat. Cell Biol. 3, 315–323

47. Miyata, T., and Kumegawa, M. (1998) J. Clin. Invest. 104, 1101–1112

48. Hofbauer, L. C., della Porta, M., Lacey, D. L., Dunstan, C. R., Spelsberg, T. C., and Khosla, S. (1999) J. Clin. Invest. 103, 1395–1403

49. Hofbauer, L. C., Delporte, C., Boulanger, C., and Sanchez-Madrid, F. (1993) J. Exp. Med. 178, 537–547

50. Hofbauer, L. C., Cerny, B. A., and Sanchez-Madrid, F. (1993) J. Exp. Med. 178, 537–547
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