Charaterization of Sugar Binding by Osteoclast Inhibitory Lectin*

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Osteoclast inhibitory lectin (OCIL) is a membrane-bound C-type lectin that blocks osteoclast differentiation and, via binding to its cognate receptor NKRPlD, inhibits natural killer cell-mediated cytotoxicity. OCIL is a member of the natural killer cell receptor C-type lectin group that includes CD69 and NKRPlD. We investigated carbohydrate binding of soluble recombinant human and mouse OCIL in enzyme-linked immunosorbent assay-based assays. OCIL bound immobilized high molecular weight sulfated glycosaminoglycans, including fucoidan, λ-carrageenan, and dextran sulfate, but not unsulfated dextran or sialated hyaluronic acid. Carbohydrate binding was Ca2+-independent. Binding of immobilized low molecular weight glycosaminoglycans, including chondroitin sulfate (A, B, and C forms) and heparin, was not observed. However, the soluble forms of these low molecular weight glycosaminoglycans competed for OCIL binding of immobilized fucoidan (as did soluble fucoidan, dextran sulfate, and λ-carrageenan), indicating that OCIL does recognize these carbohydrates. Inhibition constants for chondroitin sulfate A and heparin binding were 380 and 5 nM, respectively. Immobilized and soluble monosaccharides did not bind OCIL. The presence of saturating levels of fucoidan, dextran sulfate, and λ-carrageenan did not affect OCIL inhibition of osteoclast formation. The fucoidan-binding lectins Ulex europaeus agglutinin I and Anguilla anguilla agglutinin did not block osteoclast formation or affect the inhibitory action of OCIL. Although the osteoclast inhibitory action of OCIL is independent of sugar recognition, we have found that OCIL, a lectin widely distributed, but notably localized in bone, skin, and other connective tissues, binds a range of physiologically important glycosaminoglycans, and this property may modulate OCIL actions upon other cells.

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§ The abbreviations used are: OCIL, osteoclast inhibitory lectin; mOCIL, murine OCIL; hOCIL, human OCIL; NK, natural killer; GAG, glycosaminoglycan; ELISA, enzyme-linked immunosorbent assay; ABTS, 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; UEA-I, U. europaeus agglutinin I; AAA, A. anguilla agglutinin; OCILrP1 and murine OCILrP2 (also called CLR-g) (6), and one human homolog has also been identified (2). We have previously shown that the soluble recombinant C-type lectin domains of each of these molecules inhibit osteoclast formation with similar potency (1, 2, 6).

The OCIL family belongs to the natural killer (NK) cell receptor group of the C-type lectin superfamily, sharing ~36% amino acid identity with CD69 in the extracellular domain (6). The OCIL family members have been mapped to the NK cell receptor gene complex on mouse chromosome 6 (3), where other ccr genes and genes for the Ly49 subfamily, CD94, the NKRPl subfamily, and CD69 are located. Recently, murine OCIL has been demonstrated to bind NKRPlD, resulting in inhibition of NK cell killing of target cells (4). Whereas OCILrP2 shares 74% amino acid identity with OCIL in the extracellular domain (6), this molecule recognizes the NK cell-activating receptor NKRPlF, and not NKRPlD (4).

Lectins are nonenzymatic sugar-binding proteins that bind specific carbohydrate moieties on cell surfaces, the extracellular matrix, and secreted glycoproteins, and such C-type lectin carbohydrate binding can play an important role in cellular functions (7, 8). Alignment of the protein sequences of murine OCIL with human CD69 and rat mannose-binding protein A is shown in Fig. 1. Mannose-binding protein A contains residues conserved in classical C-type lectins that have been shown through biochemical and structural studies to coordinate the binding of Ca2+ and to confer calcium dependence to sugar recognition (7). However, members of the NK cell receptor group of C-type lectins, including CD69, murine OCIL (mOCIL), and human OCIL (hOCIL), do not contain these conserved residues. Surface plasmon resonance and crystallographic investigations of the NK cell receptor Ly49A bound to its bacterially expressed major histocompatibility complex I ligand have suggested no requirement for carbohydrate recognition (9, 10). These studies did, however, indicate two distinct sites at which Ly49A can bind its ligand, one of which is in close proximity to a conserved major histocompatibility complex I glycosylation site. In some cellular systems, sugar binding may inhibit protein-ligand interactions and subsequent cellular responses. For example, the binding of transfected Ly49C-expressing cells to BSA is inhibited by the lectin. This suggests that sugar binding may block receptor-ligand interactions.

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pressing COS cells to major histocompatibility complex I ligand-expressing cells is blocked by soluble dextran sulfate, \( \lambda \)-carrageenan, and fucoidan (11). Ly49A-mediated cell adhesion is also inhibited by fucoidan (12). Treatment of H-2D\(d \) and MHC class I+ target cells with the deglycosylase tunicamycin also prevents binding of Ly49A\(^a \) to target cells, blocking the inhibitory action of Ly49A and allowing target cell lysis (12). CD69 has also been reported to recognize fucoidan (13) and because the cross-linking of CD69 by monoclonal antibodies elicits intracellular signals, it is possible that clustering or cross-linking of CD69 and other C-type lectins at the cell surface may result in physiological responses. Therefore, identification of the sugar binding specificities of OCIL is important to characterize its function, particularly in bone and other tissues rich in glycosaminoglycans (GAGs). In this study, we have determined the carbohydrate binding specificities of mOCIL and hOCIL using soluble recombinant forms of these proteins in an enzyme-linked immunosorbent assay (ELISA)-based assay. Because many (but not all) C-type lectins contain conserved residues for binding calcium (7, 12), we investigated the calcium dependence of sugar binding by OCIL. We also investigated whether binding of the sugar moieties identified may play a role in mediating the inhibitory action of OCIL on osteoclast formation.

**EXPERIMENTAL PROCEDURES**

**Materials**—(+)-Glucose and D-maltose were purchased from Merck (Kilsyth, Australia). 2-Deoxy-\( \beta \)-glucose, \( N \)-acetylglucosamine, \( N \)-acetylgalactosamine, \( \beta \)-mannose, \( \beta \)-glucose, 3-O-methyl-\( \alpha \)-gluco- pyranose, \( \alpha \)-fructose, \( \alpha \)-fucose, D-fucose, L-galactose, \( \alpha \)-galactose, \( \beta \)-galactose-6-sulfate, and high molecular weight carbohydrates (chondroitin sulfate A (bovine trachea)) chondroitin sulfate B (porcine intestinal mucosa), chondroitin sulfate C (shark cartilage), heparin (porcine intestinal mucosa), heparin sulfates (porcine intestinal mucosa), keratan sulfate (bovine cornea), fucoidan (\( F \)ucus vesiculosus), \( \lambda \)-carrageenan type IV (Irish moss), and hyaluronic acid (human placenta) were purchased from Sigma. Heparin sulfate and the chondroitin sulfates were >90% pure, except for chondroitin sulfate A, which was 70% pure, with the other 30% being made up of chondroitin sulfate C. At least two independent lots of each carbohydrate were examined in binding assays. Chemical reagents including sodium metaperiodate on ice for 1 h to yield reactive aldehyde groups. The reaction was stopped by dilution with 3 volumes of 0.2 M carbonate buffer (pH 9.6). Sugars were then further diluted to the desired concentration in carbonate buffer, and 200 \( \mu \)l was added per well to Covalink™ ELISA plates (Nunc, New York, NY) and incubated overnight at 4 °C. Unbound sugar was removed, and the wells were washed with carbonate buffer. Sugars were then covalently linked to free amine groups present on the well surface with 0.3% sodium cyanoborohydride in 200 mM NaCl and 20 mM NaHPO\(_4\) for 1.5 h at room temperature. The wells were washed three times with 0.1% BSA in 0.05% Tween 20 (binding buffer). 200 \( \mu \)M of dextran, dextran sulfate, \( \lambda \)-carrageenan, or hyaluronic acid was coupled to wells. The wells were then washed twice with wash buffer and blocked again with 2% BSA in wash buffer (200 \( \mu \)l) for 1 h at room temperature. The wells were then washed twice with wash buffer and blocked again with 2% BSA in wash buffer for 20 min at room temperature. Horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (DakoCytomation, Glostrup, Denmark) diluted 1000-fold in wash buffer containing 0.1% BSA was then added to the wells for 1 h at room temperature. The wells were washed three more times. 200 \( \mu \)l of buffer composed of 25 mM citric acid and 25 mM sodium citrate (pH 5.5) containing 0.3% hydrogen peroxide and the substrate ABTS (1 mg/ml) was added per well and incubated for 20 min at room temperature. The color reactions were measured at 405 nm absorbance. To determine whether binding by mOCIL to the GAGs is calcium-dependent, 5 \( \mu \)M EDTA or 5 mM CaCl\(_2\) was included in the binding buffer in which mOCIL or GST was diluted prior to adding to the wells.

The data presented are the means of two to five independent experiments in which values were obtained from duplicate wells. The data presented were corrected for background levels when recombinant GST fusion proteins were incubated in wells blocked with BSA alone (no sugar present), unless otherwise specified.

**Carbohydrate Binding Assays**—Sugars were diluted in 100 mM sodium acetate (pH 5.5) to 2 mg/ml and mixed with 20 \( \mu \)l of high molecular weight carbohydrate. The data presented are the means of two to five independent experiments in which values were obtained from duplicate wells. The data presented were corrected for background levels when recombinant GST fusion proteins were incubated in wells blocked with BSA alone (no sugar present), unless otherwise specified.
were co-incubated with mOCIL (10 μg/ml) in binding buffer for 2 h at room temperature prior to the addition of 200 μl/well, resulting in a 10-fold excess of competing soluble sugar compared with immobilized GAG. Detection of binding was carried out as described under “Carbohydrate Binding Assays.”

Soluble low molecular weight (LMW) GAGs were used to compete for binding of mOCIL to immobilized fucoidan (30 pmol/well). mOCIL (10 μg/ml) was co-incubated at room temperature in binding buffer with LMW GAGs (0–50 μM). 200 μl was then added per well. Detection of binding was carried out as described under “Carbohydrate Binding Assays.”

Osteoclast Formation Assays—Mouse bone marrow cells were prepared by flushing the marrow cavities of femora and tibiae from adult C57BL/6j mice with phosphate-buffered saline. The mouse bone marrow cells were centrifuged and resuspended in α-minimal essential medium containing 10% fetal bovine serum (CSL Ltd.) with penicillin and streptomycin. Cells were added at 10^5 cells/well to 10-mm diameter plastic tissue culture wells and stimulated with 25 ng/ml human macrophage colony-stimulating factor (M-CSF) (R&D Systems, Minneapolis, MN) and 100 ng/ml RANKL. GST, mOCIL, UEA-1, AAA, or soluble GAG (dextran, dextran sulfate, fucoidan, or λ-carrageenan) was added at the start of the culture period (day 0) to determine direct effects of these reagents on subsequent osteoclast formation. To investigate whether binding of any of the GAGs to mOCIL could mediate mOCIL action, GAGs were co-incubated at 10 nm with mOCIL for at least 2 h at room temperature prior to addition to cultures on day 0. The culture medium and mediators were removed and replenished on day 3. After 7 days, the cells were fixed and histochemically stained for tartrate-resistant acid phosphatase using an acid phosphatase kit as described previously (16). Tartrate-resistant acid phosphatase-positive multinucleated cells with more than three nuclei were counted as osteoclasts. Similar experiments were carried out with UEA-1 or AAA in the presence of mOCIL.

Statistical Analysis—Statistical analysis was carried out using either a paired t test or analysis of variance as indicated.

RESULTS

Carbohydrate Binding Specificity of mOCIL and hOCIL—CD69 has been shown previously to bind the HMW sulfated GAG fucoidan using an ELISA-based assay (13). Because of the sequence identity between CD69 and OCIL, we analyzed binding of mOCIL and hOCIL to fucoidan as well as other HMW sulfated GAGs, including λ-carrageenan and dextran sulfate, when these sugars were immobilized to the surface of Covalink™ ELISA plates. Both mOCIL and hOCIL showed significant binding to these three GAGs (Fig. 2). The binding of λ-carrageenan was ~3.5-fold greater than that of dextran sulfate and 2.5-fold greater than that of fucoidan. No significant binding was observed for unsulfated dextran of a similar molecular weight, indicating that the presence of the sulfate groups may be important for carbohydrate recognition by OCIL. In addition, neither mOCIL nor hOCIL bound hyaluronic acid, an anionic sugar with sialic acid residues rather than sulfate groups, suggesting that the interaction with the sulfated GAGs is not due to nonspecific anionic charge.

Sugar moieties that exist in the bone environment such as heparin, heparin sulfate, and keratan sulfate as well as the chondroitin sulfates (A, B, and C) were also analyzed for binding by OCIL. Neither mOCIL nor hOCIL showed significant binding to any of these LMW GAGs when immobilized on the solid surface of ELISA wells (30 pmol/well) compared with the GST control (Fig. 2). No binding was observed when the amount of GAG immobilized was increased to 250 pmol/well (1.25 μM) (data not shown). GST, the recombinant tag for hOCIL and mOCIL, showed no binding to GAGs (Fig. 2).

A range of monosaccharides were also analyzed for binding by mOCIL, including maltose, D(+)-glucose, 2-deoxy-D-glucose, N-acetylglalactosamine, N-acetylgalactosamine, D(-)-mannose, 3-O-methyl-D-glucopyranose, D(-)-fructose, L(-)-fucose, D-fucose, L-galactose, D(+)-galactose, and D-galactose 6-sulfate. No significant binding was observed (data not shown).

Dose-dependent Binding of mOCIL to HMW GAGs—Dose-dependent binding of mOCIL to the HMW GAGs dextran sulfate, fucoidan, and λ-carrageenan was noted when up to 30 pmol of sugar was immobilized per well (Fig. 3A). Binding of λ-carrageenan was greatest, with higher binding achieved at 10 pmol relative to 30 pmol for either dextran sulfate or fucoidan (Fig. 3A). Unsulfated dextran was used as a negative control.

When increasing amounts of fucoidan were immobilized per well, the addition of 50 pmol of mOCIL/well showed saturation in the range of 30–60 pmol of fucoidan/well (Fig. 3B). Alternatively, when wells were coupled with 30 pmol of fucoidan/well, the addition of increasing amounts mOCIL showed saturation at 50 pmol of mOCIL (Fig. 3C). This is equivalent to saturation at 1.52 μg of mOCIL/pmol of fucoidan.

Calcium-independent Binding of mOCIL to Glicosaminoglycans—To determine whether binding by mOCIL to the GAGs is calcium-dependent, 5 mM CaCl₂ or 5 mM EDTA was included in the binding buffer in which recombinant mOCIL or GST was diluted prior to addition to wells for carbohydrate binding. 5 mM CaCl₂ was chosen to achieve saturating amounts of Ca²⁺ based on previous reports of Ca²⁺ affinities of calcium-dependent C-type lectins in the range of 0.1–1.0 mM (17–19). Even in the presence of 5 mM CaCl₂, mOCIL showed no significant increase in the binding capacity for either the HMW or LMW GAGs (Fig. 4). This shows that the mOCIL binding of these HMW sugars is calcium-independent. We did note, however, a decrease in the binding of mOCIL to λ-carrageenan (Fig. 4) and a slight increase in binding to λ-carrageenan in the presence of 5 mM EDTA. The presence of CaCl₂ did not enable mOCIL to bind monosaccharides (data not shown).
Competition of Soluble and Immobilized Sugars for mOCIL Binding—To determine whether the capacity of OCIL to bind sugars is influenced if the sugar is soluble rather than immobilized, soluble HMW GAGs preincubated with mOCIL at a 10-fold excess (300 pmol/well) were used to compete with immobilized sugars. The mOCIL/soluble HMW sugar mixture was then added to ELISA wells containing the immobilized GAGs. In the absence of competing soluble sugars, mOCIL showed the same profile for binding to immobilized dextran sulfate, fucoidan, and \( \lambda \)-carrageenan (Fig. 5A) as noted previously in Fig. 2. No competition of mOCIL binding to immobilized fucoidan, dextran sulfate, or \( \lambda \)-carrageenan was observed upon co-incubation of mOCIL with soluble hyaluronic acid, a HMW sugar that mOCIL did not recognize in an immobilized form (Fig. 5A). mOCIL binding to immobilized dextran sulfate, fucoidan, or \( \lambda \)-carrageenan was observed upon co-incubation of mOCIL with soluble hyaluronic acid, a HMW sugar that mOCIL did not recognize in an immobilized form (Fig. 5A). mOCIL binding to immobilized dextran sulfate, fucoidan, or \( \lambda \)-carrageenan was observed upon co-incubation of mOCIL with soluble hyaluronic acid, a HMW sugar that mOCIL did not recognize in an immobilized form (Fig. 5A).

Soluble LMW GAGs were similarly preincubated with mOCIL at a 300-fold excess (50 nmol/well) compared with immobilized fucoidan, and mOCIL binding to the immobilized fucoidan was analyzed. Chondroitin sulfates A and B and heparin were each able to block binding of mOCIL to fucoidan by >90%, whereas chondroitin sulfate C blocked binding of mOCIL by ~40% (Fig. 5B).

Competition curves relative to immobilized fucoidan were also determined for chondroitin sulfates A and C and heparin (Fig. 5C). This enabled us to determine the inhibition constants \( (K_i) \) for each of these sugars. Heparin was the most effective competitor, with \( K_i = 5 \text{ nm} \), whereas the \( K_i \) for chondroitin sulfate A was 380 \text{ nm}. The inhibition constant could not be calculated for chondroitin sulfate C, for which only ~30% competition of mOCIL binding to fucoidan was achieved at the highest dose used.

Effect of HMW Sugars and UEA-I and AAA Lectins on mOCIL Inhibition of Osteoclast Formation in Vitro—The involvement of carbohydrate recognition in the action of mOCIL in inhibiting osteoclast formation was assessed using GAGs with epitopes recognized by mOCIL. First, the direct effects of the GAGs were investigated. Bone marrow cells were stimulated with recombinant RANKL and M-CSF for 7 days, resulting in ~200 tartrate-resistant acid phosphatase-positive multinucleated cells/well (Fig. 6). At concentrations of 5, 25,
and 50 nM, dextran had no significant effect on osteoclast formation. Dextran sulfate reduced the number of tartrate-resistant acid phosphatase-positive multinucleated cells at 25 and 50 nM, whereas \( \alpha \)-carrageenan and fucoidan reduced osteoclast formation at all doses used (Fig. 6A). mOCIL dose-dependently inhibited osteoclast formation (Fig. 6B) as described previously (1). Because HMW GAGs bound mOCIL in solution, 10 nM \( \alpha \)-carrageenan, fucoidan, dextran sulfate, or dextran was co-incubated with mOCIL prior to addition to RANKL/M-CSF-stimulated bone marrow cultures. None of these GAGs were able to affect the inhibitory action of mOCIL, even though the GAG concentrations were in excess of the binding capacity of 500 ng/ml mOCIL (Fig. 6B).

Because the non-mammalian fucose-binding lectins UEA-I and AAA are able to bind terminal \( \alpha \)-L-fucose residues and fucoidan, we also investigated whether these lectins are able to block the inhibitory action of mOCIL on osteoclast formation. Neither UEA-I nor AAA had any significant direct effect on osteoclast formation when present at doses up to 0.3 \( \mu \)M (Fig. 7A). The addition of 0.3 \( \mu \)M UEA-I or AAA in the presence of mOCIL in the osteoclast assays also showed that neither of these lectins was able to ablate the action of OCIL in inhibiting osteoclast formation (Fig. 7B).
DISCUSSION

We have reported the binding of OCIL to several carbohydrate moieties, consistent with its homology to C-type lectins of the NK cell receptor group. Furthermore, these carbohydrates include GAGs that are found in connective tissues such as skin and bone where OCIL is localized (1), suggesting possible roles for OCIL in extracellular matrix recognition, adhesion, and cellular interactions.

mOCIL and hOCIL recognized the HMW sulfated GAGs dextran sulfate, fucoidan, and λ-carrageenan in a dose-dependent manner when those sugars were present in an immobilized form. However, unsulfated dextran (which has a similar molecular weight as the sulfated form) was not bound by OCIL, suggesting that sulfate groups are important for OCIL in sugar recognition. In addition, mOCIL and hOCIL did not bind hyaluronic acid, an anionic sugar with sialic acid residues rather than sulfate groups, indicating that OCIL interaction with the sulfated GAGs is not simply due to anionic charge. Similar studies with soluble recombinant proteins of other murine OCIL family members, OCILrP1 and OCILrP2 (6), indicated a similar binding profile compared with mOCIL and hOCIL, consistent with their high sequence identity (data not shown). The recognition of HMW sulfated GAGs is also consistent with their high sequence identity of the NK cell receptor group as a whole. However, it must be borne in mind that the complex branched structure of GAGs makes exact characterization of lectin binding specificities difficult for purposes of comparison, and impurities that are difficult to eliminate from GAGs may further complicate this.

Like other NK cell receptors, but unlike classical C-type lectins, OCIL recognition of the HMW sugars was not dependent on calcium. Again, this was suggested by the amino acid sequence of OCIL and other family members that lack conserved residues critical for calcium binding found in classical C-type lectins that are required for subsequent carbohydrate recognition (7). Examples of calcium dependence are found among the collectins, which have two calcium-binding sites. The crystal structure of a well characterized collectin, mannos-binding protein, complexed with a high mannose oligosaccharide shows binding through direct coordination of the site 2 calcium ion (20). However, selectins contain only one conserved calcium-binding site and bind glycoprotein oligosaccharides that contain core fucose trisaccharides with negatively charged sulfates (7). Although we have not investigated the calcium ion affinities of OCIL, the lack of critical calcium-binding residues in members of the NK cell receptor group suggests a mechanism of carbohydrate binding by these molecules different from that of the collectins and selectins. Furthermore, we found that the addition of calcium decreased binding of mOCIL to λ-carrageenan, perhaps due to sequestration of calcium ions by the anionic sugars, thus affecting OCIL binding. Such effects may be of importance in calcium-rich tissues such as bone.

In addition to mOCIL recognition of HMW GAGs, the same GAGs were recognized in soluble form. This was demonstrated by the ability of soluble dextran sulfate, fucoidan, and λ-carrageenan to block mOCIL binding to immobilized dextran sulfate, fucoidan, and λ-carrageenan in turn. The data indicate that recognition of these three HMW sugars involves a common binding site (or sites) present in the OCIL C-type lectin domain. Using this approach, we then investigated OCIL recognition of LMW GAGs. Whereas mOCIL did not bind immobilized LMW GAGs or monosaccharides, the soluble LMW GAGs chondroitin sulfate (A, B, and C forms) and heparin (but not monosaccharides) were able to block mOCIL binding to immobilized fucoidan. This confirmed that OCIL does indeed bind these physiologically important GAGs that are present in the bone microenvironment, presumably by a mechanism similar to that used by the HMW GAGs. Inhibition constants were measured in the nanomolar range for chondroitin sulfate A and heparin competition for mOCIL binding to immobilized fucoidan. The reason for lack of recognition of immobilized LMW (but not HMW) sugars by mOCIL is unclear, but may indicate that the mode of presentation of these GAGs is critical for OCIL recognition. Coupling of LMW GAGs to amine groups on the solid surface of the ELISA plate could have occluded critical OCIL recognition sites or disturbed some important conformational aspect of the sugars. However, the complex branched structure of these carbohydrates makes this difficult to investigate. Nevertheless, our data underline the importance of determining the binding capacity of sugars in soluble forms when profiling the carbohydrate specificity of a lectin.

We have determined that bacterially expressed OCIL as well as mammalian cell- and baculovirus-expressed OCIL are able to inhibit osteoclastogenesis in vitro (1, 2, 6). We therefore investigated whether sugar binding is required for this anti-osteoclastogenic action of OCIL, as there are several ways in which carbohydrate recognition could be involved. For example, OCIL may bind a sugar moiety or membrane-bound proteoglycan to exert its effects on osteoclast progenitors. Alternatively, binding of OCIL to a soluble extracellular sugar may result in a conformational change important for cellular action.

We found that the presence of large amounts of soluble GAGs to which OCIL binds does not affect its inhibitory action on osteoclast progenitors. Unless OCIL possesses other sugar binding specificities that employ different binding sites, the data indicate that sugar recognition at the cell surface is unlikely to be important for this action of OCIL. Consistent with this, the addition of other lectins that recognize fucoidan to the osteoclastogenesis assay neither affected osteoclast formation nor ablated the inhibitory action of mOCIL. However, we did note that the addition of high concentrations of dextran sulfate, fucoidan, and λ-carrageenan reduced osteoclast formation. It is possible that complex carbohydrate cross-linking of surface lectins may cause intracellular responses, as has been shown for monoclonal antibody cross-linking of CD69 (14). Indeed, one lectin expressed by bone marrow macrophages (the cell population in bone marrow that gives rise to osteoclasts) is OCIL (1). However, further investigation is required to determine the cellular effects of cross-linking endogenous membrane-bound OCIL.

Because carbohydrate binding does not interfere with the anti-osteoclastogenic actions of soluble OCIL, this suggests that a site independent of that for carbohydrate recognition on OCIL interacts with a receptor on myeloid mononcytic cells to inhibit osteoclast formation. NKRP1D is currently the only known protein receptor (4) for mOCIL. We have found mRNA expression of NKRP1D by bone marrow macrophages, although it has not yet been determined whether this molecule mediates OCIL inhibition of osteoclast formation. NK cell killing of target cells is inhibited by OCIL binding to NKRP1D (4). This points to a much wider range of functions for OCIL than merely inhibiting osteoclast formation, as does the wide tissue distribution of OCIL (1), which although not ubiquitous, is similar to that of RANKL (21). It remains to be seen whether novel functions of OCIL that are influenced by carbohydrate binding will be found.

Like OCIL, NKRP1D is a type II transmembrane molecule that binds to the NK cell receptor group of the C-type lectin
superfamily (4). Among other NKRPl family members, NKRPlA (with high sequence identity to NKRPlD and others in the NKRPl family in the extracellular domain) has been reported to bind various sugars, including sulfated fucose-containing Lea and Leb oligosaccharides as well as the HMW and LMW sugars that OCIL recognizes (8). The significance of OCIL recognition of similar sulfated sugars is unclear, but suggests that sugar recognition may play a role in the interaction of each of these molecules to enable NK cell mediated cytotoxicity, i.e., these sugars may play a modulatory role for their interaction. The presence of these receptors on osteoclast progenitors and their possible role in the bone environment are currently under investigation.

Several effects of sugars on bone have been documented, most notably heparin administration, which causes osteopenia. Fuller et al. (22) showed that heparin and other GAGs increase bone resorption by osteoclasts disaggregated from neonatal rat long bones. Their results suggest that the ability of GAGs to enhance resorption is dependent on the degree of sulfation and their size; fucoidan and dextran sulfate have the highest pro-resorptive activity, followed by a HMW heparin sulfate, whereas LMW heparin and chondroitin sulfates A, B, and C were without effect. Unlike the HMW sugars that OCIL recognizes (which are not found in mammalian cells), the LMW sugars that OCIL binds are found in abundance within the bone environment of mammalian tissues. Chondroitin sulfates are sulfated linear polysaccharides comprising alternate uronic and N-acetyl-D-galactosamine residues. They are the predominant carbohydrate in proteoglycans produced by chondrocytes and monocytes/macrophages and are abundant in the extracellular matrix, accelerating the mineralization process and bone repair (23). Calcification and bone maturation are associated with a decrease in proteoglycan components of the matrix (24). Physiologically, the chondroitin sulfates are bound to a core protein, which leads to aggregates of monomers with high molecular weights (23). This, along with the observations that OCIL has a higher binding capacity for the HMW sulfated GAGs than for the LMW sulfated GAGs and that the mOCIL lectin domain has a common binding site for recognition of both the LMW and HMW sugars, suggests that binding of the chondroitin sulfates by mOCIL may play an important physiological role in bone metabolism by possible interaction with these HMW aggregate sugars. Furthermore, mOCIL binds chondroitin sulfate A with higher affinity compared with chondroitin sulfate C, which is significantly increased in proliferative tissue in contact with the mineralization zone during calcification (25). It has been reported that bone morphogenic proteins bind heparin and that sulfated polysaccharides, including heparin, heparin sulfate, and dextran sulfate (but not desulfated heparin) enhance the osteoblast differentiation induced by homodimers and heterodimers of bone morphogenetic proteins (26). Therefore, although mOCIL has no requirement for carbohydrate recognition in inhibiting osteoclastogenesis, the possibility that OCIL could require sugar recognition in other bone-specific roles cannot be excluded.

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