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The Role of Osteoclast-Associated Receptor in Osteoimmunology

Katharina Nemeth,* Michael Schoppet,† Nadia Al-Fakhri,* Susann Helas,‡ Rolf Jessberger,* Lorenz C. Hofbauer,*† and Claudia Goettsch‡

The term osteoimmunology is coined for molecular and cellular cross talk between the skeletal and immune system. Immunomodulatory signals have long been implicated as key regulators of bone metabolism. Recently, osteoclast-associated receptor (OSCAR), an IgG-like receptor, has been identified as an important osteoimmunological mediator. OSCAR expression in bone is highly conserved across different species, and the molecule is an important costimulatory receptor for osteoclast differentiation through activation of NFATc1. In humans, OSCAR is expressed by macrophages, monocytes, and monocyte-derived dendritic cells and modulates the response of the innate and adaptive immune systems by promoting cell activation and maturation, Ag presentation, and proinflammatory circuits. Human studies indicate that OSCAR may contribute to the pathogenesis and severity of osteoporosis and rheumatoid arthritis. In this paper, we review the structure-function relationship, expression pattern, and physiological role of OSCAR in osteoimmunology and summarize its potential implications for human diseases. The Journal of Immunology, 2011, 186: 13–18.

Bone represents a vital organ that protects the brain, the heart and lungs, and the bone marrow. It is also a major storage site for calcium, phosphorus, and proteins, which are released upon osteoclastic resorption. In addition, bone is an endocrine organ that produces hormones and serves as a target organ for paracrine and endocrine factors that control calcium and phosphate homeostasis. The skeleton is also a complex and dynamic tissue that, in addition to its biomechanical properties, harbors stem cells and committed precursor cells of the hematopoietic and immune system (1, 2). Bone mass is determined by the coordinated actions of bone-resorbing osteoclasts and bone-forming osteoblasts. Osteoclasts are derived from macrophage/monocytic precursor cells and are phenotypically closely related to macrophages and dendritic cells (DCs). Cells of the osteoclastic and osteoblastic lineages communicate through cell-to-cell contact and paracrine factors in a bidirectional fashion. Osteoclast precursors express cell surface receptors, including the GM-CSF receptor c-fms, receptor activator of NF-κB (RANK), and receptors for costimulatory molecules such as osteoclast-associated receptor (OSCAR) (3). RANKL is essential for the differentiation, activation, activity, and survival of osteoclasts (4). The equilibrium and coupling of bone resorption with bone formation requires fine-tuning mechanisms. Osteoimmunology evaluates the mutual effects of the skeletal and the immune system at the molecular and cellular level in both health and disease. Numerous immunomodulatory signals are concurrently involved in the regulation of bone metabolism and vice versa bone-derived proteins, such as osteopontin, may have immunomodulatory effects (5).

With the emerging field of osteoimmunology, it is clear that both the innate and the adaptive immune system are major determinants of bone remodeling (6). Within the bone/bone marrow microenvironment, T and B lymphocytes as well as mast cells and macrophages provide important signals that affect the coordinated activity of osteoblasts, osteocytes, and osteoclasts through various pathways (7). Regulatory processes become apparent when immune cells are activated during acute estrogen withdrawal in postmenopausal bone loss, during autoimmune diseases such as rheumatoid arthritis, or in response to oral pathogens in periodontitis (8). Such internal or external stimuli often create a proinflammatory cytokine milieu that includes RANKL, TNF-α, IL-1 and -6, M-CSF, and others. This altered cytokine microenvironment initiates and maintains systemic and/or local bone loss. The discovery of RANKL, its receptor RANK, and the soluble receptor antagonist osteoprotegerin (OPG), all of which are involved in these disease processes, has stimulated not only the...
field of osteoimmunology, but has also triggered osteoimmunological therapy approaches.

More recently, the orphan receptor OSCAR has been implicated as a modulator of osteoimmune cell interactions. OSCAR was initially described by Kim et al. (9), although no naturally occurring ligand has been identified as of today. Compared to murine Oscar, whose expression is restricted to osteoclasts (9), human OSCAR is expressed more abundantly by osteoclasts, monocytes, granulocytes, macrophages, and monocyte-derived DCs (10). OSCAR has since been characterized as a costimulatory regulator of osteoclast differentiation (9) and modulator of DC maturation and survival (11). In addition, the expression and regulation of OSCAR in the immune system and the skeleton suggests an important function in the osteoimmune system. In this paper, we review the physiological role of OSCAR and discuss its potential implications for the bone:immune interface and the pathogenesis of human diseases.

Osteoclast biology and the role of the immune system

The generation of mature osteoclasts capable of resorbing bone is a multistep process that includes commitment of osteoclastic precursor cells, their multinucleation and maturation, and finally activation, which triggers specific cytoskeletal rearrangements, cell motility, attachment to bone matrix, and resorption. In addition, distinct collagenolytic enzymes such as cathepsin K are required to efficiently degrade bone (12).

The combination of M-CSF and RANKL is necessary and sufficient for in vitro osteoclastogenesis. M-CSF is crucial for the proliferation and survival of osteoclastic progenitor cells and binds the receptor c-fms (13, 14). RANKL represents the principal regulator for osteoclast differentiation and activation and promotes survival and motility of osteoclasts (15). When RANKL binds to RANK expressed on osteoclast precursors, a set of transcription factors (NF-κB, AP-1, and NFAT) are activated that modulate the expression of osteoclast-specific effector proteins (12).

Cytokines, chemokines, transcription factors, membrane receptors, and costimulatory molecules are induced during immune responses and directly or indirectly regulate bone biology and skeletal homeostasis. For instance, in a T cell-dependent murine arthritis model, inhibition of RANKL by OPG prevents both bone and cartilage destruction, but does not affect the inflammatory process (16). In contrast, mice with a disrupted Tnfsf11 gene encoding RANKL develop osteopetrosis due to the lack of osteoclasts and display defects in lymphocyte development and lymph node organogenesis (17).

In fact, targeted deletion of various immunomodulatory molecules is also associated with a distinct skeletal phenotype. Mice lacking PU.1, a transcription factor that regulates B cell functions, show a failure in macrophage differentiation (18) and inhibition of osteoclastogenesis (19). Deficiency of the ITAM-containing transmembrane signaling adapters, DNAX-activating protein of molecular mass 12 kDa (DAP12), or the FcRγ results in subtle osteoclastic defects, whereas deletion of both molecules causes severe osteopetrosis (20). In summary, the bone and immune system employ similar or identical signaling pathways.

Structure-function relationship of OSCAR

Initially, Oscar was identified and characterized in mice (9). Murine Oscar is localized to chromosome 7, adjacent to a region where the paired Ig-like receptors family resides. The human OSCAR (Mendelian Inheritance in Man 606862) gene maps to the leukocyte receptor complex on chromosome 19q13.4 (9, 21). This gene locus is close to the gene encoding CD85/Ig-like transcript/leukocyte Ig-like receptor and killer Ig-like receptor (KIR). Common features of these receptors are: 1) the presence of an Ig-like structure; and 2) use of FeRγ or DAP12 for signal transduction. Based on its transmembrane amino acid sequence, which shares 70% homology with KIR, and the fact that some of the CD85 and KIR molecules recognize certain MHC class I molecules, OSCAR may have evolved to interact with MHC class I molecules (22), such as classical Ags HLA-A3 and HLA-B27 as well as nonclassical HLA-G1 (23, 24).

The full-length OSCAR cDNA encodes a type I transmembrane protein of 282 aas and consists of four distinct domains (Fig. 1). Computerized structure analysis predicts one Ig motif (aa 38–121) and one Ig-like motif (aa 135–215). The transmembrane domain contains an arginine residue that is associated with ITAM-bearing signal transducing adaptor molecules such as FeRγ and DAP12 (9). In addition, a transmembrane region is predicted that involves the N-terminal aa 1–17 and 33–51. The extracellular domain features two potential cysteine residues that may stabilize the protein by facilitating disulfide bonds. The intracellular domain contains a short cytoplasmic tail that is presumably responsible for signal transduction. Analysis of the sequence homology suggests a putative receptor guanylate cyclase activity, although the functional relevance has not been demonstrated. Human OSCAR is present as a monomer with one N-glycosylation site (10).

Overall, the structure-function relationship of OSCAR remains enigmatic, as its domains and sequence motifs do not reveal any activity with certainty and as there are no data on the natural ligand or the downstream signaling pathways. It is possible that OSCAR may, in fact, represent a cellular decoy receptor. Detection of a soluble OSCAR in serum of patients (25) could be due to an undefined shedding mechanism of the ectodomain. In addition, protein structure modeling predicts the presence of a signal peptide for human OSCAR, indicating a secretory function.

FIGURE 1. Structure-function relationship of human OSCAR. Human OSCAR contains two Ig-like motifs and one transmembrane domain, which have one arginine residue (R) associated with ITAM molecules. The extracellular domain contains one N-glycosylation site and two cysteine residues that contribute to protein stability. C, C terminus; EC, extracellular domain; IC, intracellular domain; N, amino terminus; SP, signal peptide; TM, transmembrane domain.

Amino Acid Domain

<table>
<thead>
<tr>
<th>Domain</th>
<th>1</th>
<th>19</th>
<th>131</th>
<th>146</th>
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</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>Ig domain</td>
<td>R</td>
<td>Ig-like domain</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>N = N-Glycosylation Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C = Disulfide Bond</td>
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Molecular analysis of the association of murine Oscar with ITAM-bearing signal transducing adaptor molecules indicates that FcRγ is involved in Oscar-mediated signaling. The expression of murine Oscar is upregulated in the presence of FcRγ, but Oscar is weakly expressed on the cell surface in the absence of FcRγ or DAP12. Therefore, it is likely that Oscar is expressed on the cell surface in the absence of adaptor proteins (22). Although FcRγ-deficient mice do not exhibit a defect in osteoclast differentiation (3), the administration of an Oscar-Ig fusion protein, an Oscar antagonist, inhibits osteoclastogenesis in vitro (9, 26). These data suggest that FcRγ-mediated signal transduction that follows OSCAR activation may not be essential for osteoclast development, but may be involved in an alternative signaling pathway for osteoclast differentiation (22).

The promoter of the murine and human OSCAR genes contains binding sites for transcription factors known to modulate osteoclastogenesis, including microphthalmia transcription factor (MITF), PU.1, and NFATc1 (27) (Fig. 2). Oscar is involved in the positive feedback circuit of the immunoreceptor–NFATc1 pathway by providing costimulatory signals required for RANKL-mediated activation of calcium signaling. The activation of NFATc1 is thought to be mediated by the ITAM of Oscar-associated FcRγ (3). How does Oscar interact with the NFATc1 promoter? In fact, chromatin immunoprecipitation demonstrated that NFATc1 is recruited to the promoter of Oscar in RANKL-stimulated osteoclast precursor cells (26, 27). Exposure to the calcineurin inhibitor FK-506 markedly suppressed murine Oscar expression, indicating that Oscar is transcriptionally regulated by NFATc1. In line with these data, there was no Oscar expression in RANKL-stimulated osteoclast precursor cells from NFATc1-deficient osteoclasts (26).

Activated MITF, PU.1 (21), and upstream stimulatory factors (Usfs) (28) bind to the E-box region (consensus sequence CACGTG) of the Oscar promoter and cooperate with NFATc1 to modulate Oscar in mice. Notably, activation of the RANKL/Mkk6/p38 signaling cascade further enhances transactivation of murine Oscar expression by MITF, PU.1, and Usf. Interestingly, IL-1 stimulates osteoclast differentiation via activation of MITF, a known inducer of Oscar (29).

In bone, murine Oscar is also modulated by inhibitory loops, and negative regulators of osteoclastogenesis are able to downregulate Oscar (Table I). For instance, the basic leucin zipper transcription factor MafB, the inhibitors of differentiation/DNA binding (IDs), and the protein inhibitor of activated Stat3 (Pias3) inhibit RANKL-induced osteoclastogenesis via downregulation of NFATc1 and Oscar (30–32). ID proteins interact with MITF and attenuate its DNA binding ability to the E-box of the Oscar promoter (32). The synergistic induction of Oscar promoter activity by MITF and NFATc1 can be abolished by Pias3, which mediates the recruitment of histone deacetylases to the promoter regions of NFATc1 and Oscar (31). The peroxisome proliferator-activated receptor-γ agonist KR62776 (33) as well as the flavonoid silibinin (34) concurrently suppress osteoclast differentiation and Oscar expression. In addition, the MHC class II transactivator CITA, a downstream target of NFATc1, attenuates RANKL-induced osteoclast formation through downregulation of NFATc1 and Oscar (35).

Currently, natural or pharmacological ligands of OSCAR have not been identified. A putative OSCAR ligand is induced on osteoblasts exposed to vitamin D, which also induces RANKL, suggesting a sequential RANKL–OSCAR activation (9). Whether putative OSCAR ligands are membrane-bound and require cell–cell contact or whether the ligand is a soluble protein remains unclear. Identification of an OSCAR ligand would expedite the understanding of the osteoimmunological implications of OSCAR. Because most of the transcription factors regulating OSCAR gene expression are downstream of RANKL, a critical osteoblast-derived differentiation factor for osteoclast functions, OSCAR may be involved in the coupling process between bone resorption and formation.

**Immune system**

Recent studies revealed diverse functions of human OSCAR in the immune system, including the promotion of cellular activation and maturation of immune cells, prevention of apoptosis, uptake and presentation of Ags, and enhancement of proinflammatory circuits (10, 11, 36). Thus, OSCAR modulates the innate and adaptive immune response.

Most immunological studies of human OSCAR have focused on its effects on monocytes or macrophage-derived DCs, which share their precursor cells with osteoclasts (i.e., hematopoietic cells of the myelomonocytic lineage) (37). DCs act as potent professional APCs involved in triggering and orchestrating adaptive immunity (38–41). Immature DCs constantly screen the surrounding environment for pathogens. DCs endocytose Ag, produce a variety of cytokines, differentiate to mature DCs, and present pathogen-derived peptides to T cells, thereby inducing T cell activation. These DC responses are...
triggered by recognition of microbial molecules through TLRs, which decipher specific chemical signatures of pathogens. Whereas TLRs and FcR\(\gamma\) are differentially regulated during DC maturation (42), OSCAR is apparently expressed independently of the maturation state on the surface of DCs. As described above, OSCAR specifically associates with the FcR\(\gamma\), which upon phosphorylation of its ITAM motif recruits protein tyrosine kinases to initiate cellular activation (10, 36).

Ligation of human OSCAR triggers intracellular calcium release (10) and is responsible for the sustained secretion of high levels of IL-8, high levels of chemokines that attract Th2 effectors and regulatory T cells, and lower levels of IL-12p40 (11). IL-8 belongs to the CXC family of chemokines with various biological functions that include chemotaxis, generation of reactive oxygen species, cell adhesion, and promotion of angiogenesis (43). IL-8 modulates the function of a variety of inflammatory cells, including monocytes (44).

Upon human OSCAR ligation on DCs, the expression of numerous phenotypic markers for cellular activation and maturation, like CD40, CD86, and HLA-DR, is upregulated (11). Moreover, human OSCAR ensures DC survival (11, 36). Activation of human OSCAR could rescue DCs that were harvested in the absence of survival factors or the cytokine GM-CSF from apoptosis. Activation of the inhibiting

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**Table 1. Regulators of OSCAR gene expression in osteoclasts**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Function</th>
<th>References</th>
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<tbody>
<tr>
<td>Stimulator</td>
<td>RANKL</td>
<td>Cytokine</td>
</tr>
<tr>
<td></td>
<td>IL-1</td>
<td>Cytokine</td>
</tr>
<tr>
<td></td>
<td>FcR(\gamma)</td>
<td>Signaling adaptor</td>
</tr>
<tr>
<td></td>
<td>NFATc1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td></td>
<td>MITF</td>
<td>Transcription factor</td>
</tr>
<tr>
<td></td>
<td>PU.1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td></td>
<td>USF</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>MaB</td>
<td>Transcription factor</td>
</tr>
<tr>
<td></td>
<td>PIAS3</td>
<td>Repressor of transcription</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>Transcription factor</td>
</tr>
<tr>
<td></td>
<td>KR62776</td>
<td>PPAR-(\gamma) agonist</td>
</tr>
<tr>
<td></td>
<td>Silibinin</td>
<td>Polyphenolic flavonoid</td>
</tr>
<tr>
<td></td>
<td>MHC CIITA</td>
<td>Non–DNA-binding coactivator</td>
</tr>
</tbody>
</table>

PPAR-\(\gamma\), peroxisome proliferator-activated receptor-\(\gamma\).
receptor CD85j, which is expressed at a similar density on the surface of DCs as OSCAR, abolishes the induction by OSCAR of antiapoptotic proteins Bcl-2 and Bcl-xL (45).

Similar to OSCAR, RANKL also has an important role in DC biology, because RANKL inhibits apoptosis of DCs and enhances DC activity (46). Conversely, survival and cytokine production by DCs are inhibited by soluble OPG (47). It will be interesting to explore combined effects of OSCAR and RANKL on human DC survival.

Moreover, human OSCAR is involved in Ag uptake and presentation in DCs (10), which initiates adaptive immune response. The role of OSCAR in endocytosis was derived from its ability to internalize the human anti-OSCAR mAb in DCs. Endocytosis of OSCAR in DCs was observed with kinetics similar to that of the anti-mannose receptor mAb (10). The receptor complex is then transported to Lamp-1- and HLA-DR+–containing vesicles. These compartments contain receptors that are involved in MHC class II-mediated Ag presentation. Furthermore, proliferation of naïve T cells is synergistically enhanced by OSCAR after costimulation with TLR ligands (11).

Endothelial cells express costimulatory molecules on their surface with positive or negative regulatory effects on T cell responses (48). Cognate recognition of the endothelium by trafficking T cells may be critical in initiating the migration and extravasation of Ag-specific lymphocytes into sites of inflammation. Endothelial cells also express OSCAR (C. Goettsch, N. Al-Fakhri, M. Schoppet, and L.C. Hofbauer, unpublished observations); however, the functional role of OSCAR in the vascular system remains to be defined. Established and putative functions of OSCAR within the skeleton, immune system, and vascular system are summarized in Fig. 3.

OSCAR and human diseases

Knowledge about the role of OSCAR for human disease is limited to reports on skeletal and joint disorders. In 2005, Kim and colleagues (49) identified 10 polymorphisms in the human OSCAR gene in a Korean population. One single nucleotide polymorphism (OSCAR-2322A > G) located in the promoter region and the respective specific haplotype (OSCAR-h1) were associated with low bone mass and osteoprotective fractures in postmenopausal women (49). The authors speculated that the sequences flanking the OSCAR–2322A > G site contain a putative binding site for the transcription factor CREB, which binds to the cAMP response element. Based on this hypothesis, analysis of OSCAR in osteoclast-specific CREB mutant mice will be required to elucidate the expression control of OSCAR. Moreover, OSCAR was analyzed in patients with osteoarthrosis as compared with patients with periprosthetic osteolysis (50). Synovial fluid of patients with osteoarthritis induced significantly higher OSCAR and NFATc1 mRNA expression in mouse calvaria bone explants compared with periprosthetic osteolysis patients, suggesting that high levels of OSCAR and NFATc1 may facilitate the degenerative process of osteoarthritis.

A recent study evaluated the role of OSCAR in rheumatoid arthritis (25). OSCAR was localized on mononuclear cells surrounding synovial microvessels and was found to be enhanced in peripheral blood monocytes of patients with rheumatoid arthritis compared with healthy subjects. Moreover, monocytic OSCAR expression was positively associated with disease activity, C-reactive protein serum levels, and the erythrocyte sedimentation rate. In this study, TNF-α, a key cytokine and target in rheumatoid arthritis, was identified as the main inducer of OSCAR expression in monocytes. It needs to be clarified whether expression of OSCAR is a bystander effect or whether it is a consequence of TNF-α induction. Taken together, several studies implicate OSCAR as a regulator of disease and thus as a potential pharmacological target at the osteoimmunological interface. However, as our understanding of OSCAR biology is still very limited with even the natural ligand remaining elusive, intensive research efforts are required to understand the potential of targeting OSCAR for therapeutic purposes.

Conclusions

The field of osteoimmunology is rapidly evolving to encompass a wide range of molecular and cellular interactions, among them intracellular and cell-to-cell signaling pathways. Detailed knowledge of these pathways will provide a scientific basis for future therapeutic approaches to diseases of the immune system and the skeleton. Research in osteoimmunology may delineate new strategies to develop novel therapies for bone loss in inflammatory and autoimmune diseases as well as in osteoporosis. OSCAR, an IgG-like receptor, has been identified, to our knowledge, as a new and potentially relevant factor to be studied in the field of osteoimmunology. OSCAR represents an important costimulatory receptor for osteoclast differentiation by activating NFATc1 and a modulator of the activation and maturation of immune cells of the macrophagic/monocytic lineage. Human studies indicate that OSCAR may contribute to the pathogenesis and severity of diseases at the bone/immune interface, including osteoporosis, rheumatoid arthritis, and osteolysis adjacent to joint implants. Identification of the cognate ligand of OSCAR and subsequent signaling pathways will shed more light onto this molecule and will thus foster a more detailed and complete understanding of this branch of regulation of bone metabolism and the immune system.

Disclosures

The authors have no financial conflicts of interest.

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