

COMMENTARIES

Costimulatory Blockade to Prevent Osteoclastogenesis, Inflammation, or Both?

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Commentary on: Joyce-Shaikh B, Bigler ME, Chao CC, Murphy EE, Blumenschein WM, Adamopoulos IE, Heyworth PG, Antonenko S, Bowman EP, McClanahan TK, Phillips JH, Cua DJ. Myeloid DAP12-associated lectin (MDL)-1 regulates synovial inflammation and bone erosion associated with autoimmune arthritis. *J Exp Med.* 2010 Mar 15;207(3):579-89.

Juxta-articular bone erosion at the interface of the inflammatory synovial pannus and the bone is characteristic of rheumatoid arthritis in humans. Osteoclasts are thought to be the primary cellular mediators of bone erosion at the site of invasion of the inflammatory pannus into cortical bone, which can extend through to the bone marrow (1-3). While synovial inflammation is a critical determinant of both the symptoms and promotion of bone erosion in inflammatory arthritis, it is also clear that these processes can be uncoupled (4). Deletion or blockade of the RANK/RANKL receptor pathway that is required for osteoclastogenesis can prevent bone erosion, with virtually no effect on inflammation and synovial pannus formation (1;5;6). Costimulatory signals for osteoclastogenesis in inflammatory arthritis are incompletely understood, yet are a topic of considerable interest because identification of costimulatory signals critical for pathological bone erosion in inflammation could enable more specific targeted therapy that would not affect basal osteoclastogenesis. A recent report by Joyce-Shaikh *et al.* in the *Journal of Experimental Medicine* adds significantly to our understanding of costimulatory receptors in inflammatory bone erosions (7). The authors demonstrate that MDL-1 (myeloid DAP12

associated lectin or CLEC5A), a DAP12-associated receptor, is a key regulator of both synovial inflammation and bony destruction in an inflammatory arthritis model.

Activation of immune cells is closely regulated by a requirement for engagement of multiple receptors simultaneously, which is better known as costimulation (8). Costimulation of osteoclasts was first described with the finding that mice deficient in the ITAM (immunoreceptor tyrosine-based activation motif) signaling chains were severely osteopetrotic with defective osteoclastogenesis despite the presence of intact RANK/RANKL and M-CSF signaling pathways (9;10). The ITAM signaling chains, DAP12 (DNAX associated protein 12 kD) and the FcR γ chain are transmembrane adaptor molecules that transduce activating signals via an ITAM for a group of cell surface receptors expressed on NK cells, granulocytes, macrophages and other cells in the myeloid lineage including dendritic cells, mast cells, osteoclasts and osteoclast precursors (8;11-13). Activation of an ITAM-associated receptor leads to phosphorylation of the associated ITAM, recruitment and activation of the cytoplasmic tyrosine kinase syk and initiation of a signaling cascade culminating in Ca²⁺ flux and NFATc1 activation. Absence of the ITAM signals abrogates the Ca²⁺ oscillations

and NFATc1 activation that are critical for osteoclastogenesis (reviewed in (14)).

MDL-1 is one of a number of innate receptors that can pair with the signaling adapter proteins DAP12 and DAP10 (DNAX-associated protein 12 kD or 10kD) (11;13). MDL-1 is expressed on inflammatory macrophages, particularly those that are TNF α -activated, neutrophils, and bone marrow-derived osteoclast precursors (11). MDL-1 was demonstrated early on to regulate myeloid cell-associated inflammatory responses (11;15). More recently, activation of MDL-1 was shown to stimulate *in vitro* osteoclastogenesis, and MDL knockdown in osteoclast precursor cells inhibited osteoclastogenesis (13). While RANKL is known to be upregulated on synovial fibroblasts, the coreceptors for osteoclastogenesis in these juxtaarticular regions is not known.

Joyce-Shaikh *et al.* examined the role of MDL-1 in inflammatory arthritis (7). They initially demonstrate that MDL-1 is highly expressed on myeloid cells in the inflammatory synovial pannus, colocalizing with CD68⁺ macrophages. MDL-1 is also upregulated on neutrophils in the bone marrow and peripheral blood in a murine collagen antibody-induced arthritis model (CAIA). Treatment with an agonist anti-MDL-1 antibody increased both disease incidence and severity in the CAIA model and in a CIA (collagen-induced arthritis) model. CAIA was diminished in severity and incidence in both *DAP12(-/-)* and *MDL-1(-/-)* animals. Their study demonstrates that activation of the MDL-1 receptor with agonist antibody during joint inflammation in CAIA enhances myeloid cell infiltration and promotes IL-1, IL-6, IL-17A, and TNF α expression. Corresponding with the increase in inflammation, severe cartilage damage and bone erosion was enhanced. In contrast, blockade of MDL-1 function with a soluble receptor fusion protein (MDL-1-Ig) downregulates TRAP, cathepsin K, and MMP9 expression, reducing the clinical signs of autoimmune joint inflammation, and preserving bone from damage. MDL-1-Ig-treated mice were highly resistant to either CAIA- or CIA-induced arthritis. Depletion of circulating granulocytes and/or monocytes significantly

reduced MDL-1-dependent joint inflammation during CAIA, suggesting that inflammatory macrophages and neutrophils expressing MDL-1 are important in inflammatory arthritis.

Ligands important for activating MDL-1 in inflammatory arthritis are not known, nor is it clear on what cell type those ligands might be expressed. Interestingly, dengue virus capsid protein has been shown to be an activating ligand for MDL-1. MDL-1 is important for the intense inflammatory response to dengue fever as blockade of MDL-1 inhibits cytokine storm and vascular leakage in a mouse model of dengue fever (16).

Given the remarkable effect of MDL-1 blockade on joint inflammation, it is not surprising that MDL-1-Ig treatment had a significant effect on decreasing bone erosion and osteoclastogenesis. The degree to which inflammation is suppressed by MDL-1 blockade is impressive. While it has been described previously that MDL-1 can contribute to the differentiation and function of myeloid cells, it is a pleasant surprise that blockade of a single DAP12-associated receptor has such a marked effect in an inflammatory arthritis model. Thus MDL-1 has a dual role, in that it can costimulate monocyte-macrophage differentiation or osteoclast differentiation depending upon which additional signals are present. Substantial evidence clearly suggests that MDL-1 can promote osteoclastogenesis separately from its effect on inflammation, however, it is difficult to say in the arthritis models that blocking MDL-1 has additional direct effects on osteoclasts and osteoclast precursors, because the effect on eliminating inflammation is so marked. Nonetheless, these findings suggest that the MDL-1 receptor may be a useful therapeutic target in inflammatory arthritis to both decrease joint inflammation and bone erosion, and further examination of MDL-1 in patients with rheumatoid arthritis will be of significant interest.

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