**OPG/OPGL/M-CSF CYTOKINE EXPRESSION DURING FRACTURE HEALING**

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**Methods**

All animal protocols received prior approval by the institutional animal care and use committee, and conformed to the federal guidelines for the care and use of laboratory animals. Closed, transverse, mid-diaphyseal fractures of the tibiae were generated in eight to ten week old male Balb/c mice and fixed with an intramedullary rod using a modification of the technique developed for generating fractures in rats (6). Animals were euthanized by cervical dislocation on days 0 (unoperated control), 1, 3, 7, 14, 21 and 28 postoperatively. For the histologic examination, the fracture callus or the whole tibia was fixed, decalcified, embedded in paraffin, and stained with hematoxylin and eosin using standard techniques. For RNA analysis, the callus tissue was harvested including about 3 mm of both proximal and distal fracture fragments and total RNA was isolated. Messenger RNA expression of osteoprotegerin (OPG), osteoprotegerin ligand (OPGL/TRANCE), and macrophage colony stimulating factor (M-CSF), have been shown to be key regulators in the control of bone mass through their modulation of the bone resorptive cycle (1,2,3). OPG is a secreted soluble TNF receptor family member that binds to OPGL and prevents it from stimulating osteoclastogenesis (4,5). In order to investigate the potential roles for these cytokines in fracture healing, their temporal expression patterns were analyzed.

**Introduction**

Fracture healing is a unique postnatal process characterized by a definable temporal sequence of endochondral bone formation, bone resorption and bone remodeling, that is orchestrated for the purpose of restoring mechanical integrity. While the role of the pro-inflammatory cytokines in bone remodelling is well established, their participation in the response of skeletal tissue to injury is not well understood. Recently, a novel member of the TNF receptor family, osteoprotegerin (OPG), and its specific ligand (OPGL/TRANCE), in conjunction with macrophage colony stimulating factor (M-CSF), have been shown to be key regulators in the control of bone mass through their modulation of the bone resorptive cycle (1,2,3). OPG is a secreted soluble TNF receptor family member that binds to OPGL and prevents it from stimulating osteoclastogenesis (4,5). In order to investigate the potential roles for these cytokines in fracture healing, their temporal expression patterns were analyzed.

**Results**

Maximal immune-cell response was seen one day after fracture. Maximal formation of cartilage occurred between days 7 and 14, and the cartilage resorption peaked between days 14 and 21. Peak bone formation occurred between days 21 and 28 while bone remodeling and marrow formation were initiated at day 14 and continued through the end of the experimental period. The expression of OPG was detected in unfractured bones and showed elevated levels of expression throughout the repair process with two distinct peaks of expression observed: the first occurring immediately after the fracture at day one; and the second at the time of maximal cartilage formation at day 7. In contrast, the expression of OPGL was nearly undetectable in unfractured bones and was strongly induced throughout fracture repair period showing inverse times of peak expression to that of OPG with maximal levels of expression seen at days 3 and 14 when OPG levels were decreasing. M-CSF expression followed the temporal profile of OPGL but was expressed at relatively high basal levels in unfractured bones.

**Discussion**

These data demonstrate that the ratios of expression for OPG, OPGL, and M-CSF are tightly coupled, and suggest that these cytokines are key regulators of both cartilage resorption and bone remodeling. Previous data from our laboratory have shown that the expression of other cytokines such as IL-6, TNF-α and IL-1 appear to play less of a role in the resorption of cartilage and are associated primarily with initial inflammation and later remodelling stages of the healing process (7). These findings demonstrate the critical role of the TNF-related cytokine family in the repair and regeneration of bone and cartilage, and specifically implicate OPG, OPGL and M-CSF as important regulators of endochondral cartilage resorption and subsequent bone remodeling.

**References**


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