

# INFLAMMATORY REACTIONS TO IMPLANT MATERIALS & BONE RESORPTION: OBSERVATIONS AND MECHANISMS

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**INTRODUCTION:** Bone resorption is a complex process with a multitude of regulatory steps at cellular and systemic levels. Under physiological conditions bone is continuously remodeled to allow for adjustments to changing environmental requirements and for the repair of microfractures. During remodeling, resorption and formation are in equilibrium, ensuring the maintenance of bone mass. If local or systemic resorption increases over formation, bone mass decreases, as is the case in postmenopausal osteoporosis, in conditions of inflammatory arthritis, or in the formation of osteolytic lesions in periimplant bone.

The recruitment and activation of the bone resorbing cells, the osteoclasts (OC), are modulated by a multifactorial haematopoietic microenvironment, which includes the extra-cellular matrix (ECM), cells of various origins and functional types (osteoblasts, marrow and endothelial cells), and local and systemic growth factors and cytokines (1). Two growth factors were found to be necessary and sufficient for the development of functional OC, colony-stimulating factor-1 (CSF-1) and receptor activator of NF- $\kappa$ B ligand (RANKL). CSF-1, binding to the receptor tyrosine kinase *c-fms*, belongs to the family of lineage-specific haematopoietic growth factors, inducing haematopoietic precursor cells to develop to the monocyte/macrophage (MO/M $\Phi$ ) lineage (2). RANKL is a member of the membrane-bound TNF $\alpha$  family of growth factors. It binds to the specific receptor RANK and its activity is, at least in part, controlled by an inactivating soluble decoy receptor, osteoprotegerin (OPG) (3).

Besides CSF-1 and RANKL, there are numerous growth factors, which exert profound effects on the recruitment, activation and survival of OC. One of these factors is TNF $\alpha$ , a major product of MO and M $\Phi$ . TNF $\alpha$  was suggested to be the causative agent in osteoporosis, to be contributing to the bone loss in inflammatory forms of arthritis, and to be critically involved in aseptic loosening of orthopaedic implants. Furthermore, the spectrum of TNF $\alpha$  activity in bone resorption is gaining versatility through its capacity to synergise with other growth factors and cytokines, such as RANKL.

Within the present study, we describe 1) that metal ions induce the release of TNF $\alpha$  in MO and M $\Phi$ , and 2) some aspects of the effects of the cytokine on the

formation of OC, which may contribute to the loosening of orthopaedic implants.

## METHODS:

*Release of TNF $\alpha$  by monocytes.* Mononuclear cells from peripheral blood were purified by Ficoll/Hypaque gradient centrifugation. The cells were seeded into culture dishes and incubated with either metal particles or salts. After 40 h, the culture supernatants were collected and the levels of TNF $\alpha$  were determined by ELISA.

*TNF $\alpha$  in osteoclastogenesis.* The recruitment of OC was studied *in vitro* in two culture systems. (1) Murine bone marrow cells were grown in the presence of RANKL and CSF-1. In the presence of the two growth factors, haematopoietic precursor cells develop into multinucleated, TRAP<sup>+</sup> OC-like cells (OCL). (2) Bone marrow cells were grown in a co-culture together with primary osteoblasts. Upon addition of 1,25(OH)<sub>2</sub>D<sub>3</sub> to the culture, OCL form within 6 days (4). Using these culture systems, it is possible to investigate the effects of growth factors and cytokines on the formation of OC. It is possible to differentiate between direct effects on haematopoietic cells or indirect effects through accessory cells. Furthermore, these culture systems serve as tools to investigate the expression of growth factors, receptors, and other components of the haematopoietic microenvironment during OC recruitment *in vitro*.

*TNF $\alpha$  in ovariectomy.* Given the importance of TNF $\alpha$  in the regulation of bone resorption, it was investigated, whether the cytokine occupies an essential role in bone loss induced under estrogen deficiency. For this purpose, mice deficient in TNF $\alpha$  or in the p55 receptor for TNF $\alpha$  were ovariectomized (OVX). Bone mass was followed in weekly intervals over 3 weeks by pQCT.

## RESULTS:

*Release of TNF $\alpha$  by monocytes in response to the exposure to metal particles and salts.* Cells of the MO/M $\Phi$  lineage responded to the exposure to metal particles (cpTi of various sizes, AlOx, stainless steel) by releasing TNF $\alpha$ . The release, however, was primarily dependent on the size of the particles, rather than on the material that was used. When MO were exposed to metal ions (Co<sup>2+</sup>, Ni<sup>2+</sup>, Ti<sup>3+</sup>, Cr<sup>3+</sup>), they reacted by releasing osteoclastogenic cytokines. The patterns of released cytokines varied among MO from different donors and were dependent on the metal ions the cells were treated with. Co<sup>2+</sup> induced a significant

release of TNF $\alpha$  in most cell preparations, while the expression of other cytokines (IL-6, IL-1 $\alpha$  and -1 $\beta$ ) was affected to a lesser extent. Ni<sup>2+</sup> induced a broader response than did Co<sup>2+</sup>, the response, however, was less uniform among the cell preparations. Ti<sup>3+</sup> and Cr<sup>3+</sup> were considerably less efficient in inducing the release of osteoclastogenic cytokines.

*The effects of TNF $\alpha$  on OC formation in vitro.* When TNF $\alpha$  was added to cultures of bone marrow cells grown in the presence of CSF-1 and RANKL, the growth factor had no effect on the formation of OCL. Addition of the same amount of TNF $\alpha$  to co-cultures of marrow cells and osteoblasts caused a significant decrease in the formation of OC. If the osteoblasts were derived from p55<sup>-/-</sup> animals, the inhibitory effect of TNF $\alpha$  was not only blocked, but the growth factor caused an increase in OC formation.

*The role of TNF $\alpha$  in bone loss induced by estrogen deficiency.* Deficiency in estrogen caused bone loss in mice deficient in TNF $\alpha$  or in the p55 TNF receptor. The extent of bone loss in the ko strains was not different than the OVX-induced bone loss in wt animals.

**DISCUSSION:** TNF $\alpha$  is a major inflammatory cytokine and has been shown to be a potent stimulator of bone resorption *in vivo* (5). The growth factor has been implicated in aseptic loosening of prosthetic implants, in bone loss after estrogen deficiency and in the increase in bone resorption accompanying inflammatory diseases.

In the present study it was demonstrated that MO and M $\Phi$  are induced to release elevated levels of TNF $\alpha$  upon exposure to metal particles or ions. Elevated levels of the cytokine, either in the periprosthetic tissue or systemically, may contribute to the formation of osteolytic lesions and subsequently lead to loosening of the implant.

While TNF $\alpha$  is paramount in the regulation of bone resorption, it acts in concert with other growth factors and cytokines, such as CSF-1, IL-1, and IL-6. In contrast to previous studies, and underlining this concept, we describe here that TNF $\alpha$  is not necessary of OVX-induced bone loss. Other factors can compensate for the loss of TNF $\alpha$ , or, the effect of the cytokine on bone cell biology is more complex than anticipated.

Most interesting was the finding that TNF $\alpha$  *in vitro* exerts dual effects on the formation of OCL. While TNF $\alpha$  stimulated the development of OCL through a direct action on marrow cells, it inhibits the formation of OCL indirectly via osteoblasts.

In inflammatory diseases such as psoriatic arthritis, serum TNF $\alpha$  levels are increased and concomitantly the pool of circulating OC precursors is expanded (6). Circulating TNF $\alpha$  will act directly on the OC precursors, with no accessory cells around mediating a potential inhibitory effect. In the bone

microenvironment, however, the effects of TNF $\alpha$  may be quite to the contrary.

Many aspects of the effects of TNF $\alpha$  on bone cell development and function have been elucidated in the past. However, in particular the inhibitory effects of TNF $\alpha$  on the formation of OC are little investigated and will be the topic of further studies.

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