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Review

# Does complement play a role in bone development and regeneration?

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ABSTRACT

The skeletal and the immune system are not two independent systems, rather, there are multifaceted and complex interactions between the different cell types of both systems and there are several shared cytokines. As a part of the innate immunity, the complement system was found to be an important link between bone and immunity. Complement proteins appear to be involved in bone development and homeostasis, and specifically influence osteoblast and osteoclast activity. This review describes the complex mutual regulation of the two systems, and indicates some of the negative side effects as a result of inappropriate or excessive complement activation.



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## Introduction

What began in the seventies with the discovery of a bone resorbing activity in cell culture supernatants of PBMNC (Horton et al. 1972) is now a steadily growing scientific field of intense interest, termed osteoimmunology. The subject of osteoimmunology is the multifaceted mutual regulation of the skeletal and the immune system that reaches far beyond the function of bone to provide the location of haematopoiesis and the formation of immune cells in the bone marrow. Bone cells, such as the bone-forming osteoblasts, their precursors the mesenchymal stem cells (MSC), and the bone resorbing osteoclasts are all influenced by cytokines released from

*Abbreviations:* BMP, bone morphogenetic protein; C3aR, C3a receptor; C5aR, C5a receptor; DAF, decay accelerating factor, CD55; DAMP, danger associated molecular pattern; ERK, extracellular-signal related kinase; GPCR, G protein-coupled receptor; IGF, insulin-like growth factor; IL, interleukin; MAC, membrane attack complex; MACIF, MAC inhibitory factor, CD59; MCP, membrane co-factor of proteolysis, CD46; M-CSF, macrophage-colony stimulating factor; MMP, matrix metallo proteinase; MSC, mesenchymal stem cell; OPG, osteoprotegerin; PAMP, pathogen associated molecular pattern; PBMNC, peripheral blood mononuclear cells; PDGF, platelet derived growth factor; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; TGF, transforming growth factor; TNF, tumour necrosis factor.

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immune cells while conversely immune cells, such as T cells, are a target for RANKL, a typical bone cytokine.

In this review we will first provide a short overview of the interactions of bone with immune cells or inflammatory cytokines and will then concentrate on an important pro-inflammatory protein cascade, the complement system. Finally, we will discuss some diseases, which are associated with various impaired complement-bone interactions.

## Bone and bone homeostasis

Bone is an organ with crucial functions in providing stability, serving as the main calcium depot, and containing the bone marrow with haematopoietic as well as mesenchymal precursor cells. It is continuously rebuilt in a process termed bone remodelling, a dynamic balance of bone formation by osteoblasts and resorption by osteoclasts. Remodelling is essential for constant renewal and repair of bone, and for adaptation to changing mechanical requirements. Diseases associated with pathologically low or high bone density such as osteoporosis or osteopetrosis, respectively, are linked to an imbalance of osteoblast and osteoclast activity.

Osteoblasts are derived from mesenchymal precursor cells and secrete the bone matrix, which mainly consists of collagen and non-collagenous proteins, *e.g.* alkaline phosphatase, osteopontin, and osteocalcin that are important for example for the mineralization of the matrix (Kassem et al. 2008). Mature osteoblasts that are completely embedded in bone substance become osteocytes. Although metabolically barely active, they have signalling functions in bone remodelling, and in phosphate and calcium metabolism as well as in mechanosensing (Bonewald 2010). Osteoblast differentiation and function is regulated by numerous factors, among them hormones, nerve signals, and vascular agents. Moreover, paracrine factors are involved in osteoblast regulation, *e.g.* transforming growth factor-ß (TGF-ß), bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs), and platelet-derived growth factors (PDGF), as well as inflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumour necrosis factor (TNF-α) (Heng et al. 2004; Kreja et al. 2010).

Osteoclasts are highly specialized multinucleated cells derived from haematopoietic cells of the monocyte/macrophage lineage. Osteoclasts are able to degrade the bone matrix by providing an acid environment and secreting proteolytic enzymes (Boyle et al. 2003). Interactions of osteoclast precursors with osteoblasts and stromal cells are essential for osteoclast differentiation. The key molecules regulating osteoclastogenesis are receptor activator of nuclear factor-κB ligand (RANKL), a member of the tumour necrosis factor (TNF) family, and osteoprotegerin (OPG). Both factors are expressed by osteoblasts. Osteoclast formation is induced by the binding of RANKL to its receptor RANK on osteoclast precursors. The presence of macrophage-colony stimulating factor (M-CSF) is also important. M-CSF binds to the c-fms receptor of early osteoclast precursors, regulating their proliferation, differentiation, and survival. Osteoclast formation and activity is inhibited by OPG, the soluble decoy receptor for RANKL, which is also released by osteoblasts. The RANK/RANKL/OPG and M-CSF/c-fms receptor regulatory axes tightly couple osteoblast and osteoclast activity, thus controlling skeletal mass homoeostasis (Asagiri and Takayanagi 2007; Boyce and Xing 2008; Boyle et al. 2003). This finely tuned regulatory mechanism can be disturbed for example by inflammatory cytokines during the systemic or local inflammatory processes of fracture healing, particularly in the case of severe multiple trauma and in diseases such as osteoarthritis.

## Interactions between the immune system and bone

Although the interactions of the skeletal and the immune system are manifold and complex, a key component of the mutual regulation is the RANK/RANKL/OPG system. RANKL is mainly produced not only by osteoblasts but also by immune cells such as T cells and neutrophils (Chakravarti et al. 2009; Maruotti et al. 2010). Binding of RANKL to its receptor RANK on monocytes is essential for the formation of osteoclasts by fusion of monocytes. However, the RANK/RANKL system also influences immune cell interactions, such as dendritic cell-T cell interactions and is necessary for the maturation of dendritic cells (Page and Miossec 2005; Schiano de Colella et al. 2008). The RANK/RANKL signalling is regulated by the expression of OPG, a decoy receptor for RANKL, which like RANKL is expressed by osteoblasts. The expression of these cytokines and the proliferation and differentiation of osteoblasts during the transition from bone resorption to bone formation is regulated by coupling factors expressed by osteoclasts. These coupling factors are secreted or membrane bound proteins expressed by osteoclasts or are liberated from the bone matrix during osteoclastic bone resorption (Matsuo and Irie 2008; Zhao et al. 2006). However, the RANK/RANKL/OPG system is also regulated by immune cells. B cells for example can up-regulate OPG expression and down-regulate RANKL expression (Djaafar et al. 2010).

In addition to the RANK/RANKL/OPG system, there are also interactions between bone and the immune system that are mediated by cytokines secreted by immune cells. Some of the most important pro-inflammatory cytokines produced by immune cells, such as IL-1β, IL-6, and TNF-α, induce osteoclastogenesis and bone resorption (Fig. 1). Other cytokines, such as the B cell mitogen IL-14, produced by Th1 and Th2 cells, have an osteoprotective effect (Maruotti et al. 2010).

In the following sections we will concentrate on the interaction between bone and the complement system, as complement activation is a central part of inflammatory processes and may play a central role in both bone and the immune system.

## The complement system

The complement system is an ancient system for sensing and fighting danger, and is an essential part of the innate immunity. It defends the organism against foreign materials and pathogens by direct lysis or by recruitment of leukocytes, which perform phagocytosis. Normally, complement activation appears locally at sites of danger such as injuries, cell damage, and invasion of pathogens, helping to fight infections or to degrade dead or damaged cells. In contrast, severe incidents, such as multiple trauma or sepsis as well as chronic inflammatory processes, such as in rheumatoid arthritis and autoimmune diseases, can cause systemic, excessive, or persisting complement activation (Ricklin et al. 2010).

Most of the complement serine proteases are present in plasma as zymogens, inactive precursors that require proteolytic cleavage for activation. Several danger- or pathogen-associated patterns (DAMPs, PAMPs) including DNA, ATP, and pathogen surfaces can trigger complement activation via three different established pathways: the classical, the alternative, and the lectin pathway. All three pathways lead to the formation of one of two different C3 convertases and to the cleavage of C3 into C3a and C3b (Fig. 2). The larger fragment, C3b, can become a part of a newly formed C3 convertase or bind to a C3 convertase and form a C5 convertase that cleaves C5 to C5a and C5b. C5b promotes the acquisition of the terminal complement components C6 to C9 to the pathogen surface and the formation of the membrane attack complex (MAC) (Fig. 2). C3b and C5b bound to the cell surface of pathogens also act as opsonins and promote phagocytosis by macrophages and neutrophils.

In addition to these three activation pathways, other, complement-independent mechanisms for the cleavage of the