Acidosis, osteoclast formation and cathepsin K expression in the loosening of total hip prostheses

Jami Mandelin

Institute of Biomedicine / Anatomy, University of Helsinki, Finland
ORTON Orthopaedic Hospital of the Invalid Foundation, Helsinki, Finland
Department of Medicine/Invärtes medicin and Orthopaedics and Traumatology, Helsinki University Central Hospital, Helsinki, Finland
and
The National Graduate School for Biomaterials and Tissue Engineering

ACADEMIC DISSERTATION

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki, in the large lecture hall (Iso luentosali) at Haartman Institute, University of Helsinki, Haartmaninkatu 3, Helsinki on March 18th, at 12 o'clock noon.

HELSINKI 2005

Supervised by:

Professor Yrjö T. Konttinen
Department of Internal Medicine
University of Helsinki
Helsinki, Finland

Professor Seppo Santavirta
Department of Orthopaedics and Traumatology
Helsinki University Central Hospital
Helsinki, Finland

Reviewed by:

Professor Esko Alhava
Department of Surgery
Kuopio University Hospital
Kuopio, Finland

Docent Teuvo Hentunen
Department of Anatomy, Institute of Biomedicine
University of Turku
Turku, Finland.

Official opponent:

Docent Juha Tuukkanen
Department of Anatomy and Cell Biology
University of Oulu
Oulu, Finland

Tieteellinen tutkimus ORTONin julkaisusarja, A:18
Publications of the ORTON Research Institute, A:18

ISBN 952-9657-25-0 (Paperback)
ISBN 952-10-2370-8 (PDF)
ISSN 1455-1330
http://ethesis.helsinki.fi
Yliopistopaino
Helsinki 2005

Work hard and try your best! If you then fail I am not angry.

-my father-

To Marja, Joel and Eetu

CONTENTS

1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications. They are referred to in the text by their Roman numerals I-V.

I Konttinen YT, Takagi M, **Mandelin J**, Lassus J, Salo J, Ainola M, Li TF, Virtanen I, Liljeström M, Sakai H, Kobayashi Y, Sorsa T, Lappalainen R, Demulder A, Santavirta S: Acid attack and cathepsin K in bone resorption around total hip replacement prosthesis. J Bone Miner Res 2001; 16: 1780-1786.

II **Mandelin J**, Li T-F, Liljeström M, Kroon ME, Hanemaaijer R, Santavirta S, Konttinen YT: Imbalance of RANKL/RANK/OPG system in interface tissue in loosening of total hip replacement. J Bone Joint Surg (Br) 2003;85-B:1196-1201.

III **Mandelin J**, Li T-F, Hukkanen M, Liljeström M, Salo J, Santavirta S, Konttinen YT: Interface tissue fibroblasts from loose total hip replacement prosthesis produce receptor activator of NF-kappaB ligand, osteoprotegerin and cathepsin K. J Rheumatol in press.

IV **Mandelin J**, Liljeström M, Li T-F, Ainola M, Hukkanen M, Salo J, Santavirta S, Konttinen YT: Pseudosynovial fluid from loosened total hip prosthesis induces osteoclast formation. J Biomed Mater Res in press.

V **Mandelin J**, Li TF, Hukkanen MV, Liljestrom M, Chen ZK, Santavirta S, Kitti U, Konttinen YT: Increased expression of a novel osteoclast-stimulating factor, ADAM8, in interface tissue around loosened hip prostheses. J Rheumatol 2003; 30: 2033-2038.

2. ABBREVIATIONS

ADAM	a disintegrin and a metalloproteinase domain
ATP	adenosine triphosphate
BSA	bovine serum albumin
cDNA	complementary DNA
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
ERK	extracellular signal-regulated kinase
FBS	fetal bovine serum
FC	fibrous capsule
Ig	immunoglobulin
IL	interleukin
JNK	c-Jun N-terminal kinase
kDa	kilo Dalton
LPS	lipopolysaccharide
MFR	macrophage fusion reseptor
M-CSF	macrophage colony stimulating factor
MMP	matrix metalloproteinase
mRNA	messenger RNA
NF	nuclear factor
PBGD	porphobilinogen deaminase
PBS	10 mM phosphate buffered 150 mM saline, pH 7.4
PCR	polymerase chain reaction
PMMA	polymethylmetacrylate
RANK	receptor activator of nuclear factor-κB
RANKL	receptor activator of nuclear factor-κB ligand
RNA	ribonucleic acid
RT-PCR	reverse-transcriptase PCR
SEM	standard error of mean
TBS	50 mM tris buffered 150 mM saline, pH 7.4
THR	total hip replacement
TNF	tumour necrosis factor
TRAF	TNF receptor-associated factor
TRAP	tartrate resistant acid phosphatase

3. ABSTRACT

Modern total hip replacement (THR) surgery relieves pain and improves functional ability of patients suffering from diseases of the hip, such as osteoarthritis, rheumatoid arthritis, congenital dysplasia or dislocation, and aseptic necrosis of the femoral head. At present, approximately half a million patients receive an artificial hip annually. THR surgery is a cost-effective treatment but the main problem is aseptic loosening of the initially properly fixed prosthesis in the long term (Takagi 1996, Wright and Goodman 2001b). A loosened prosthesis must be replaced in a revision operation that is more expensive and technically more demanding than the primary one. Despite extensive studies the mechanisms contributing to the loosening of the THR, which culminate in revision operation, are not fully understood (Wright and Goodman 2001b).

Aseptic loosening of THR prosthesis is always characterized by the formation of fibroblast-rich synovial membrane-like interface (Goldring et al. 1983, Goldring et al. 1986) tissue between the implant and host bone. Foreign bodies produced in gliding surfaces of the artificial joint migrate to this interface membrane and lead to a chronic, macrophage-dominated foreign body reaction. This is associated with periprosthetic bone lysis (Jasty et al. 1986, Murray and Rushton 1990).

Osteoclasts are responsible for bone resorption. Bone is mineralized and the mineral of the bone, hydroxyapatite, can not be degraded enzymatically. Bone resorption comprises two major steps: an acid dissolution of the bone hydroxyapatite followed by degradation of the demineralized collagen rich osteoid bone matrix. These two events are mediated by osteoclasts. The pH is lowered under osteoclast in Howship lacuna leading to demineralization of bone. After this, an acidic endoproteinase cathepsin K, which is secreted to the subosteoclastic space, degrades collagenous bone matrix (Kamiya et al. 1998, Yamaza et al. 1998). Receptor activator of nuclear factor-κB ligand (RANKL) system unquestionably controls osteoclast formation in bone. RANKL is an osteoblast-produced cytokine, which binds to receptor activator of nuclear factor-κB (RANK) on the surface of preosteoclasts / monocytes. The ligand-receptor interaction induces preosteoclasts to fuse and form multinuclear cells. This is inhibited by a soluble decoy receptor osteoprotegerin (Simonet et al. 1997, Quinn et al. 1998, Lacey et al. 1998, Yasuda et al. 1998a, Yasuda et al. 1998b).

The aim of this study was to advance a new theory for periprosthetic tissue destructive events. This is also based on acidification and bone mineral dissolution. This acidification, however, occurs in the extracellular, not in the subosteoclastic, space. This local acidification is associated with high production and extracellular release of cathepsin K. RANKL is present in interface tissue and may induce osteoclast formation without interference of osteoprotegerin, which localizes to different tissue compartment. Cultured fibroblasts from the interface tissue produce RANKL, osteoprotegerin and cathepsin K and may directly and indirectly contribute to periprosthetic osteolysis.

Pseudosynovial fluid generated in the synovial membrane of the artificial hip contains RANKL and induces osteoclast formation. Osteoclast fusion inducer ADAM8 is present in the interface tissue and it may facilitate macrophage or preosteoclast fusion. This knowledge may be applied in planning future clinical, pharmacological and mechanical studies that aim to prevent osteoclast formation, acidosis and cathepsin K production in the aseptic loosening of THR prostheses.

4. REVIEW OF THE LITERATURE

4.1 THR and loosening

The clinically successful artificial low friction THR constructions are well established (Figure 1). The original concept was based on polymethylmethacrylate (PMMA) fixed ultra-high molecular weight polyethylene acetabular cup and a femoral stem made of steel (Charnley 1961). Since the introduction of THR in the early 1960s, some significant changes have been introduced in design and materials. Steel proved to be too corrosive *in vivo* and it has relatively low fatigue strength and modulus of elasticity. Commercially pure titanium, titanium alloys and CoCr have replaced it. The moduli of elasticity of these metals are closer to that of cortical bone and they have greater fatigue strength than stainless steel. The decreased rigidity, abrasion, fretting, and corrosion makes CoCr superior to titanium alloy and stainless steel (Carter and Spengler 1978, Dunn and Maxian 1994, Black et al. 1998, Schmalzried et al. 1998). The favourable biocompatibility of titanium made it a good choice for femoral stems. However, titanium is not suitable for PMMA fixation due to its low resistance of abrasion and fretting. For the same reason excessive wear of the titanium femoral heads have occurred in the early trials when they have been used with polyethylene cups (Schmalzried et al. 1998). The properties of titanium have been improved with novel alloying techniques but it is still mainly used in noncemented techniques.

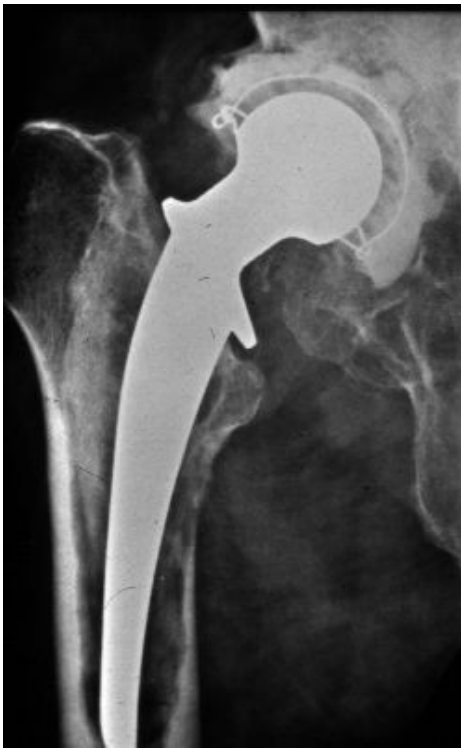

Figure 1. X-ray picture from a loose THR prosthesis. Metal wire surrounds the ultra-high molecular weight polyethylene acetabular cup, which is not otherwise visible in the picture. Radio-opaque agents make PMMA cement around the cup visible. Dark areas in the femur indicate osteolysis.

PMMA cement is used for THR fixation and to gain even stress distribution to the host bone. PMMA was chosen for being relatively inert, rapidly setting and biocompatible. It was soon discovered that in the long term cemented THR implants started to loose. First it was hypothesized

that this lysis was due to a chronic low-grade infection (Charnley et al. 1968) but recent research has shown massive osteolytic reactions around well-fixed THR prostheses in the absence of infection (Tallroth et al. 1989, Antti-Poika et al. 1990). Later the loosening of cemented totally replaced hips was thought to be due to the PMMA particles. The whole process was named "cement disease". This led the design of noncemented prostheses. However, it did not take long until shortcomings of also this approach became evident. In some instances failure was associated with considerable bone loss and release of particles from the bulk material even relatively early. The elimination of PMMA was not a solution and "cement disease" turned to be "particle disease" (Hungerford and Jones 1993, Evans et al. 1993). There are multiple surfaces and interfaces that can wear in THR implant and generate particles. The wear mechanisms include adhesion, abrasion, corrosion and fatigue. The most common or expected wear results from the two bearing gliding surfaces, the ball and the cup.

Abundant polyethylene wear is common in THR failure. It is estimated that the linear wear of average ultra-high molecular weight polyethylene is 0.1 to 0.2 mm/year (50 to 100 mm^3/year), corresponding to hundreds of millions of particles released to surrounding tissues (Wright and Goodman 2001a). There are studies that show how continuous polyethylene administration leads to massive osteolysis (Howie et al. 1988, Kim et al. 1998b). However, low frictional torque makes the use of ultra-high molecular weight polyethylene in the acetabular cup almost unavoidable in all THR designs (Santavirta et al. 1992). Wear and wear debris are increasingly becoming recognized as significant etiologic factors associated with progressive bone loss, loosening and failure of THR.

The host response to wear particles leading to osteolysis is a late appearing complication. Successful THR prosthesis remains well-fixed over a decade and signs of host response may remain modest (Santavirta et al. 1992, Wright and Goodman 2001b). It is difficult to study the causes of this complex adverse biological tissue response. Thus, the mechanism of osteolysis around technically well-fixed THR prostheses has remained puzzling. The recognition of synovial membrane-like interface tissue, which without exception grows between implant and bone, has led to research of biological aspects related to osteolysis (Goldring et al. 1983, Goldring et al. 1986, Jiranek et al. 1993, Santavirta et al. 1993, Goodman et al. 1998a). Synovial membrane-like interface tissue prevents an intimate contact between the host bone and prosthesis. It may also produce factors that activate osteoclastic bone resorption and affect bone formation. This results in a unique high-turnover periprosthetic bone remodelling with poor bone quality (Takagi et al. 2001).

Although the THR procedure has a very favourable impact socioeconomically, one of the challenges is to further prolong the functional life of the endoprosthesis. The major remaining issues of concern include the biologic response to particles, osteolysis due to wear debris, and problems related to revision surgery. Research into the biology of the osteolytic process and potential effects of

systemic distribution of wear debris is important (Wright and Goodman 2001b). Thus, the biocompatibility of the THR prosthesis is still a major issue.

4.2. Histopathology of interface tissue around loose THR prostheses

THR prosthesis is an artificial joint and after an initial wound healing and tissue remodelling process it is surrounded by similar structures that surround a natural joint. Acetabular cup and ball are surrounded by a structure called "pseudocapsular tissue". This tissue contains cells similar to those found in normal joint capsule and synovial membrane with a periprosthetic cavity lined by synovial lining-like structure (Santavirta et al. 1993, Santavirta et al. 1996). It also produces joint fluid called "pseudosynovial fluid". Pseudosynovial fluid can be studied for the content of different substances that have been released from the tissue to it (Saari et al. 1993, Takagi 1996, Kim et al. 2001).

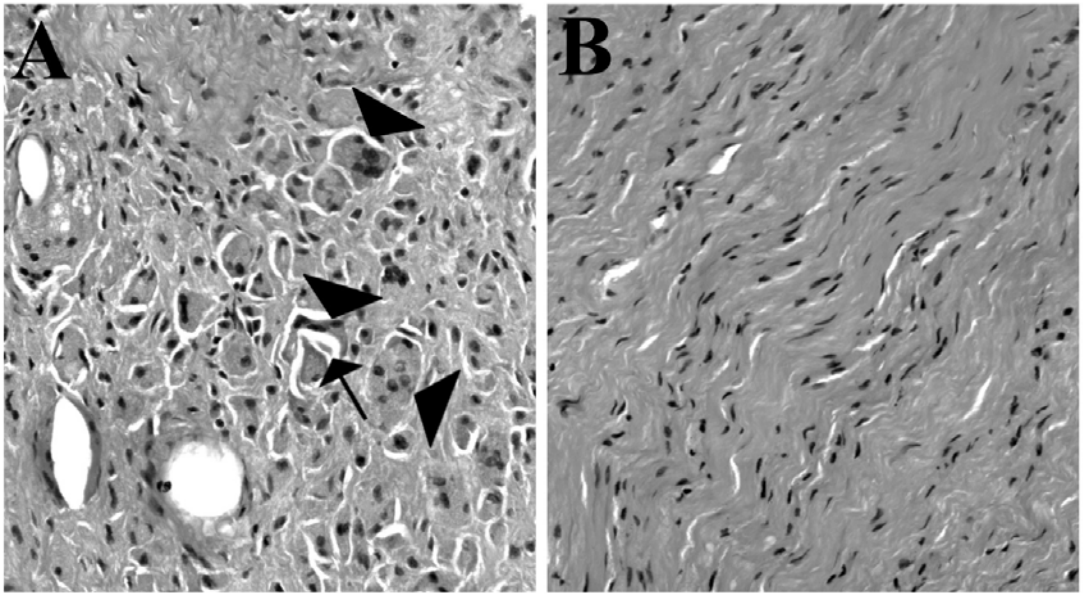

Figure 2. Representative pictures from synovial membrane-like interface tissue. (A) Polyethylene particle (arrow) surrounded by multinuclear giant cells (arrowheads). (B) Fibrotic area from the same sample as in panel A.

When THR prosthesis is inserted, the aim is to achieve such a contact between the prosthesis and the surrounding bone that the prosthesis is adequately fixed. Good osseointegration is essential for the successful outcome of the THR operation. However, synovial membrane-like fibrous tissue, which is referred to as "interface tissue", is always found between bone and loose THR implant during revision operation (Figure 2). Fibrous tissue ingrowth to a bone defect is not exceptional since a drilled hole in a bone is filled with fibrous tissue even without bone loading (Ashammakhi et al. 1995). If the drilled hole is loaded with different sized polyethylene particles, it is filled with tissue resembling the interface membrane that surrounds loose joint arthroplasty implant in humans

(Goodman et al. 1990). The biological role of both "pseudocapsular tissue" and "interface tissue" in loosening has been studied extensively and it is evident that both play essential roles in this process.

4.2.1 Development of interface tissue

There is evidence that the growth of interface tissue is most probably initiated by micromovement of the prosthesis. At least three reasons may contribute to micromovement. First, the initial movement of the THR prosthesis might be due to loss of fixation caused by necrosis of the surrounding bone (Aspenberg and Van der Vis 1998). Reaming of the femoral cavity causes damage to bone and its vascularity. In addition, the reamed cavity is flushed with high pressure that may damage the bone. Exothermal PMMA cement polymerization causes bone tissue damage. Because the dead bone must be removed, the normal bone resorption and formation cycle is disturbed leading to bone loss and micromovement. Second, there might be movement due to early loading of prosthesis. It has been shown that even movement without weight bearing can cause substantial loads to the prosthesis (Hodge et al. 1989). Micromovement of more than 140 μm allows fibrous tissue ingrowth (Engh et al. 1992, Karrholm et al. 1994). Early loading of cementless THR prosthesis prevents good osseointegration (Jasty et al. 1997). Movement leads to cyclic micromovement at the interface that leads to the formation of interface tissue (Aspenberg et al. 1992, Aspenberg and Herbertsson 1996). Third, different moduli of elasticity leading to stress shielding may cause bone loss and development of interface tissue (Engh et al. 1987, Rubash et al. 1998).

4.2.2 The cellular content of interface tissue and its impact on bone

Interface tissue consists mainly of fibroblasts, monocytes, macrophages, foreign body giant cells and endothelial cells. A small number of mast cells and lymphocytes are also detected in interface tissue. It is believed that macrophages and fibroblasts of the interface membrane play a fundamental role in aseptic THR prosthesis loosening (Moreschini et al. 1997). Fibroblasts proliferate *in situ* but monocytes and macrophages are recruited from the blood stream by the induction of chemokines, which are produced in the interface tissue (Ishiguro et al. 1997, Nakashima et al. 1999, Lassus et al. 2000, Konttinen et al. 2001). Proline 4-hydroxylase positive fibroblasts dominate the stroma of interface tissue (Santavirta et al. 1993). Fibroblasts proliferate and produce collagen actively in the interface tissue indicating that the tissue is activated mesenchymal tissue (Jiranek et al. 1993, Santavirta et al. 1998). The predominant cell in the lining cell layer of the periprosthetic cavity is CD11b, CD68, and nonspecific esterase positive but endogenous peroxidase-negative macrophage. There are also perivascular mononuclear cell infiltrations consisting mainly of CD11b/CD68 expressing macrophages with occasional CD4 expressing cells. Only few mononuclear cells were activated CD25 containing T cells or CD19 containing B lymphocytes (Santavirta et al. 1993). Approximately 8-30% of the total cells in the tissue express tartrate resistant acid phosphatase

(TRAP) activity and contain vitronectin receptor, and comprise a subset of the CD68 positive macrophages and macrophage polykaryons (Chun et al. 1999).

Interface tissue does not contain C-sensory peptidergic or sympathetic neural structures (Niissalo et al. 2002). This means that pain related to aseptic loosening cannot arise in the aneural interface membrane. Inflammation and cell activation in interface tissue and in aseptic loosening seems to be driven by non-neurogenic factors, such as loading, micro- and macromovement and foreign bodies. Cyclic loading, movement and particles, i.e. continuous stress, activate cells to produce several cytokines and proteolytic enzymes (Aspenberg and Herbertsson 1996). The production and activity of neutral proteinases are increased in interface tissue of loose THR prostheses (Takagi 1996). Similarly, production of pro-inflammatory and other cytokines is accelerated in interface tissue. Studies of tissues obtained from periprosthetic region and of cells incubated with debris particles suggest that osteolysis usually results indirectly from an inflammatory reaction amplified by macrophages in response to phagocytosis of debris particles, which they cannot degrade (Jiranek et al. 1993, Horowitz et al. 1993, Chiba et al. 1994, Haynes et al. 1997, Xu et al. 1997, Horowitz et al. 1998, Goodman et al. 1998b, Jones et al. 1999, Merkel et al. 1999).

Although macrophages most probably are the cells to keep up and even accelerate the local inflammatory process, connective tissue cells are also important in the reactive process. The production of the extracellular matrix and thus the control of its quality are dependent on connective tissue cells. The extracellular matrix and the quality of host bone finally determine the clinical success of THR (Takagi 1996, Takagi et al. 2001). Much emphasis is put on research of factors released by the cells in the interface tissue as these factors are thought to affect osteoblasts and osteoclasts of the surrounding bone. It has particularly been suggested that cytokines from interface tissue activate osteoclasts on bone (Jiranek et al. 1993, Horowitz et al. 1993, Chiba et al. 1994, Haynes et al. 1997, Xu et al. 1997, Horowitz et al. 1998, Goodman et al. 1998b, Jones et al. 1999, Merkel et al. 1999) and inhibit osteoblast function (Haynes et al. 1997). Recent studies have suggested that fibroblasts from interface tissue can support osteoclast differentiation like osteoblasts do (Haynes et al. 2001, Sakai et al. 2002). There is also evidence that pseudosynovial fluid contains cytokines, which may activate osteoclasts around the prosthesis (Kim et al. 1998a). This data combined with the fact that macrophages derived from interface tissue differentiate into osteoclastic bone resorbing cells *in vitro* (Sabokbar et al. 1997) makes the whole cascade leading to bone resorption even more complicated.

4.3. Osteoclasts and giant cells

The localized bone resorption adjacent to THR prostheses has been connected to macrophages and foreign-body giant cells, which invade the femoral cortices with abundant particulate debris, without the presence of sepsis or malignant disease (Jasty et al. 1986, Murray and Rushton 1990).

However, osteoclasts are specialized multinuclear cells that differentiate in bone from mononuclear phagocytes by fusion and they are the only cells capable to bone resorption (Sapp 1976, Horton et al. 1984, Baron et al. 1986). The nature of multinucleated giant cells has remained obscure. There is evidence that osteoclasts differ from giant cells but it has also been observed that both cells originate from the fusion of mononucleated precursors that belong to the mononuclear phagocyte lineage (Udagawa et al. 1990, Vignery 2000). There are several reports to show that multinuclear foreign-body type cells express many functional markers that characterize osteoclasts. Such markers include a high and polarized expression of H^+-ATPases, receptors for calcitonin (Vignery et al. 1989, Vignery et al. 1991a, Vignery et al. 1991b, Vignery et al. 1991c), TRAP, vitronectin receptors (Kadoya et al. 1994), cathepsin K (Kataoka et al. 2000, Nakase et al. 2000) and most importantly the capability of bone resorption (Flanagan et al. 1988, Athanasou et al. 1991, Athanasou and Quinn 1992, Neale et al. 1997). However, numerous reports show opposite results (Horton et al. 1986, Marks, Jr. and Chambers 1991, Boissy et al. 2001). While it is concluded that osteoclasts differ from giant cells, the similarities between them are striking. Osteoclasts are capable of particle phagocytosis and still retain their bone resorption ability (Wang et al. 1997a, Wang et al. 1997b). Macrophages as well as giant cells are able to adhere tightly to the substrate and are able to secrete H^+-ions, lysosomal enzymes and proteins under them (Silver et al. 1988, Baron 1995). However, formation of ruffled border that is segregated by a sealing zone is apparent only in osteoclasts (Glowacki et al. 1986). An actin ring delineates the sealing zone and it is visible only in bone resorbing osteoclasts (Lakkakorpi and Vaananen 1991). Recent discovery of so far the only true osteoclastogenic cytokine and its counterparts, namely RANKL and RANK and the soluble decoy receptor osteoprotegerin, has stimulated research on the nature of multinucleation and osteoclast development both *in vivo* and *in vitro*.

4.3.1 RANKL, RANK and osteoprotegerin in osteoclastogenesis

The adult skeleton is rigid yet living tissue, which is continually broken down by osteoclasts and reformed by osteoblasts in discrete temporary anatomic structures called basic multicellular units. Numerous factors affect formation of the basic multicellular units and bone homeostasis. Systemic hormones, like parathyroid hormone, vitamin D ($1\alpha,25(OH)_2D_3$) and calcitonin all have an impact on osteoclastic bone resorption. Parathyroid hormone also stimulates osteoblastic bone formation. Sex steroids have been recognized to play an important role in the regulation of osteoblast and osteoclast activities. Systemic hormones are mostly produced by some specific endocrine glands, secreted to circulation from where they diffuse to the tissues and mediate their effect by binding to their high affinity cellular receptors. Cytokines are secreted, small proteins that bind to cell-surface receptors on the target cells and affect their behaviour. Unlike systemic hormones cytokines can be produced virtually by any cell and they usually affect the target cells in the close proximity to their production site. Due to the discrete and distinct nature of basic multicellular unit these local factors may be even more important than systemic hormones for the initiation of bone resorption and the

control of osteoclast formation and their activity (Bilezikian et al. 2002).

It has been long speculated that some kind of osteoclast differentiation factor on osteoblasts should exist (Horton et al. 1972, Rodan and Martin 1981, Chambers 1988, Suda et al. 1992). Bone marrow derived osteoblastic stromal cells were essential for the modulation of the differentiation of osteoclast progenitors (Takahashi et al. 1988, Udagawa et al. 1989, Udagawa et al. 1990, Kukita et al. 1993). This could happen in many different ways: either by production of soluble factors (Wong 1984, Shiina-Ishimi et al. 1986) or then by cell-to-cell contact between osteoblastic stromal cells and osteoclast progenitors (Flanagan et al. 1988, Quinn et al. 1994, Jimi et al. 1996) or both of these ways could occur. Macrophage colony stimulating factor (M-CSF) was shown to be probably the most important soluble factor, which appeared to be necessary for not only proliferation of osteoclast progenitors, but also their differentiation into mature osteoclasts and osteoclast survival (Yoshida et al. 1990, Felix et al. 1990, Kodama et al. 1991, Takahashi et al. 1991a, Takahashi et al. 1991b, Tanaka et al. 1993). A number of local factors as well as systemic hormones induce osteoclast differentiation in the co-culture of osteoclast precursors and osteoblastic stromal cells. These factors are classified into three categories in terms of signal transduction: vitamin D receptor-mediated signals ($1\alpha,25(OH)_2D_3$); protein kinase A-mediated signals (parathyroid hormone, parathyroid hormone related peptide, prostaglandin E2 and interleukin (IL)-1); and gp130-mediated signals (IL-6, IL-11, oncostatin M and leukemia inhibitory factor). All of these osteoclast-inducing factors appeared to act on osteoblastic cells by inducing osteoclast differentiation factor (ODF), which recognized osteoclast progenitors and induced them to differentiate into mature osteoclasts (Suda et al. 1992, Mundy 1993).

The key factor required for normal osteoclast development has recently been characterised. It was first found on the surface of activated T-cells and named tumour necrosis factor (TNF) related activation-induced cytokine (TRANCE) (Wong et al. 1997) or RANKL (Anderson et al. 1997). Only later it was also found to be a membrane bound osteoblast cytokine that regulates osteoclast differentiation. It was isolated by utilizing binding to osteoprotegerin and has therefore also been named osteoprotegerin ligand (OPGL) (Lacey et al. 1998) Characterization of this factor confirmed its essential role in osteoclast differentiation and activation (Lacey et al. 1998, Fuller et al. 1998, Yasuda et al. 1998b, Jimi et al. 1999, Kong et al. 1999, Burgess et al. 1999). In bone, osteoclastogenesis starts when RANKL binds to RANK (Figure 3), which is expressed on the surface of osteoclast precursors (Nakagawa et al. 1998, Dougall et al. 1999, Hsu et al. 1999, Li et al. 2000).

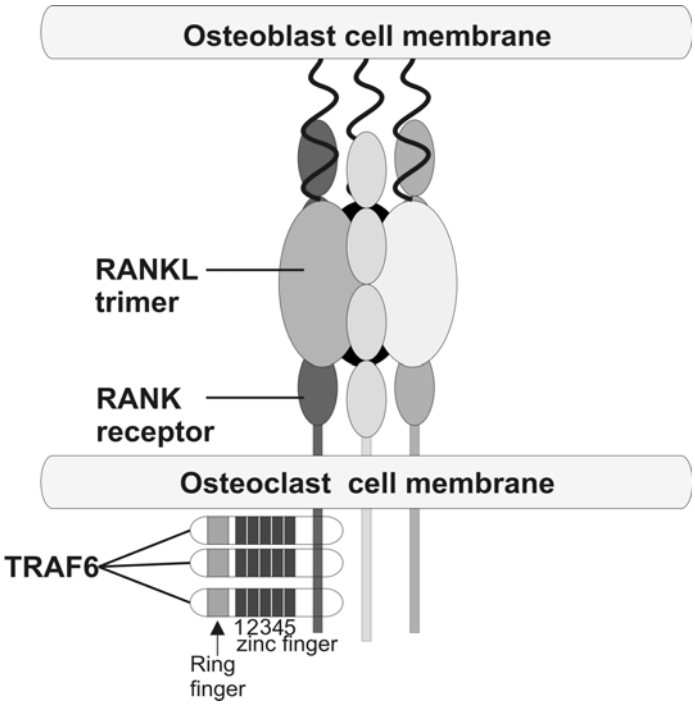

Figure 3. Trimerized RANKL docked with its receptor RANK. RANKL self-associates as a homotrimer. Either membrane bound or secreted trimeric RANKL binds to RANK. Like other TNF family cytokines, RANKL binds one elongated RANK molecule along each of the three clefts formed by the neighbouring monomers of the homotrimer. The binding leads to a triangle of RANK molecules in which three spatially distinct, but equivalent, RANK molecule bind to RANKL. The cytoplasmic domains of this complex can bind TRAF6 at three distinct cytoplasmic site of RANK molecule. TRAF6 uses different domains to initiate different signalling cascades upon stimulation. For complete NF-κB activation TRAF6 uses zinc finger domains 2-5 and for complete JNK and p38 activation it needs both RING and all zinc fingers (Kobayashi et al. 2001, Lam et al. 2001, Ye et al. 2002).

In the presence of M-CSF, the receptor ligand interaction induces osteoclast precursors to fuse and form multinuclear, active osteoclasts (Felix et al. 1990, Lacey et al. 1998, Van Wesenbeeck et al. 2002). This is inhibited by a soluble factor called osteoprotegerin, which binds to RANKL and blocks the RANKL-RANK interaction (Simonet et al. 1997, Lacey et al. 1998, Yasuda et al. 1998a) (Figure 4). Those local factors and systemic hormones that were shown to induce osteoclast differentiation in the co-culture systems actually modulate RANKL and osteoprotegerin production in osteoblastic stromal cells (O'Brien et al. 1999, Nagai and Sato 1999, Hofbauer et al. 1999a, Hofbauer et al. 1999b, Hofbauer et al. 1999c, Nakashima et al. 2000). Some of them may induce RANK expression on osteoclast precursors but may also directly enhance osteoclast formation and activity (Uy et al. 1995, Fuller et al. 2002).

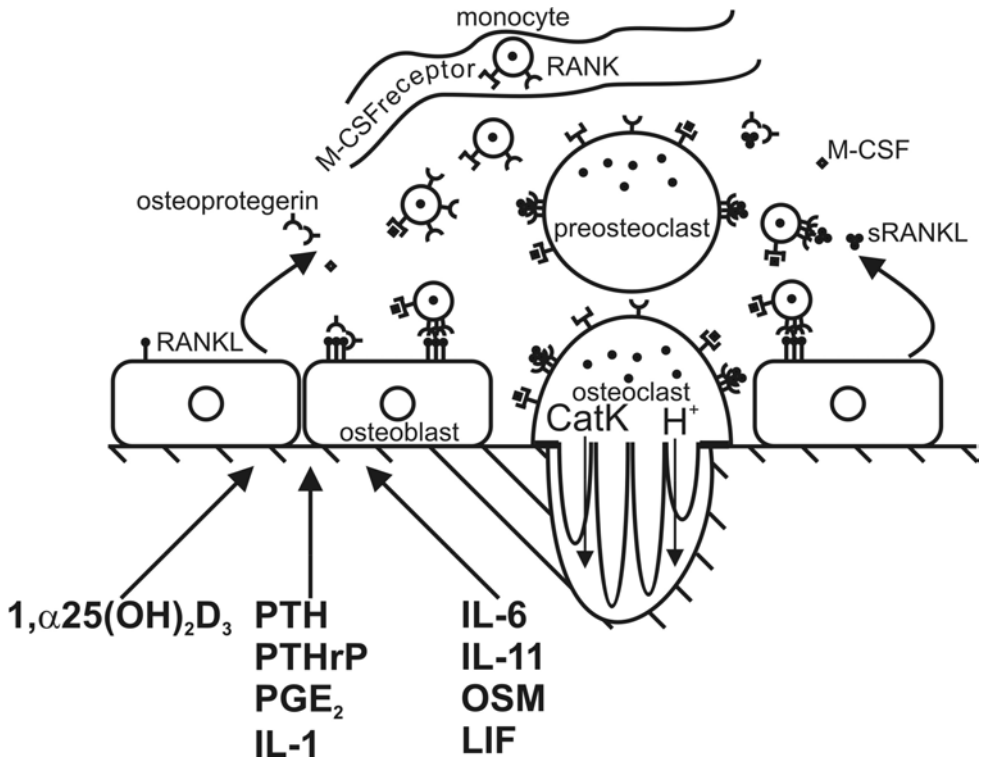

Figure 4. Osteoclastogenesis. Osteoblasts secrete M-CSF, which induces monocyte migration and proliferation. Monocytes arrive from bloodstream and increase in number. Cytokines and hormones induce RANKL production in osteoblasts. Trimeric RANKL homes three RANK receptors on the surface of monocyte. This activates the signalling cascades that lead to monocyte fusion and further activation. Osteoclasts attach to bone and form resorption lacunae called Howship lacunae. Activated osteoclasts secrete H^+-ions to acidify the lacunae and cathepsin K to degrade the non-mineralized organic matrix (Felix et al. 1990, Lacey et al. 1998, Jimi et al. 1999, Vaananen et al. 2000, Lam et al. 2001).

It has been shown that binding of RANKL to RANK activates at least five distinct cytoplasmic signalling cascades. During osteoclastogenesis and osteoclast activation at least the following pathways are activated: NF-κB (Anderson et al. 1997, Jimi et al. 1999, Kobayashi et al. 2001, Yamamoto et al. 2002, Xing et al. 2002), c-Jun N-terminal kinase (JNK) (Grigoriadis et al. 1994, Jimi et al. 1999, Lee et al. 2000, Matsuo et al. 2000, Kobayashi et al. 2001, Yamamoto et al. 2002), p38 (Matsumoto et al. 2000, Kobayashi et al. 2001, Hotokezaka et al. 2002, Mansky et al. 2002, Li et al. 2002), extracellular signal-regulated kinase (ERK) (Lee et al. 2000, Hotokezaka et al. 2002) and Src (Soriano et al. 1991, Lowe et al. 1993, Schwartzberg et al. 1997, Wong et al. 1999, Armstrong et al. 2002). The key preliminary step in RANK signalling is the binding of TNF receptor-associated cytoplasmic factor 6 (TRAF6) to specific cytoplasmic domains of RANK. Also TRAF2 and -5 can bind to RANK but only TRAF6 activates osteoclastogenesis (Wong et al. 1998, Darnay et al. 1998, Naito et al. 1999, Lomaga et al. 1999). TRAF6 plays essential roles in both

differentiation and activation of osteoclasts and activates so many distinct cytoplasmic signalling cascades because it is able to activate various kinases via its multiple domains (Kobayashi et al. 2001) and its capability to bind to RANK at several different cytoplasmic sites (Galibert et al. 1998, Darnay et al. 1999, Armstrong et al. 2002, Ye et al. 2002). Similarities to the signalling mechanisms utilized by IL-1 and TNF-α explain why they can be used to substitute RANKL under certain conditions (Jimi et al. 1999, Kobayashi et al. 2000, Roggia et al. 2001, Kaji et al. 2001, Zhang et al. 2001, Fuller et al. 2002). However, the very complex signalling mechanisms mediated through RANK explain why RANKL is the key factor capable of inducing survival, differentiation, fusion, maturation and activity of osteoclast precursors and osteoclasts.

4.3.2 ADAMs and other proteins in cell fusion

One critical step in osteoclast and giant cell formation is cell fusion. Cell fusion requires some important steps: cell accumulation, cell adhesion and membrane fusion. Cell accumulation may occur in two ways: A) some precursor cells migrate to or are present the site of the fusion and multiply locally in number before differentiation and fusion or B) all cells accumulate to the site from bloodstream and start to fuse. It is not well known which one is the predominant way. Cytokine and chemokine production by the host tissue is critical for cell accumulation (Mackay 2001, Muller 2001). Infectious agents evoke host tissue to release mediators, which attract monocytes and in most cases it is likely that both proliferation and differentiation occur at the inflammatory site. Monocyte chemoattractant protein-1 (MCP-1) has been shown to be essential for monocyte recruitment in several inflammatory models *in vivo* (Lu et al. 1998). Monocyte and even tissue macrophage proliferation can be induced by M-CSF (Chen et al. 1987, Naito et al. 1996). Interface tissue produces chemoattractant molecules e.g. MCP-1 and IL-8 (Ishiguro et al. 1997, Nakashima et al. 1999, Lassus et al. 2000, Konttinen et al. 2001) and M-CSF (Xu et al. 1997). Thus, it might be capable of both recruiting monocytes as well as inducing their proliferation. In normal osteoclastogenesis this is done by osteoblasts (Elford et al. 1987, Hanazawa et al. 1991, Takahashi et al. 1991a, Williams et al. 1992). Osteoblasts control cell fusion by producing RANKL as earlier described. It is possible that under certain circumstances particles (Goodman et al. 1990) or pathogen-induced cytokines, like TNF-α, induce fusion (Merkel et al. 1999, Zou and Bar-Shavit 2002, Sabokbar et al. 2003).

Membrane fusion in general is a normal event in a wide range of biological processes. In all cases the fusion requires accessory molecules, so called docking proteins, which overcome the repulsive forces between lipid membranes. They mediate and control membrane fusion by bringing the membranes close enough for the fusion to occur (Jahn et al. 2003, Spear and Longnecker 2003). The most important cell fusion for the maintenance of life is the sperm-egg fusion in fertilization. The proteins involved in sperm-egg fusion are metalloprotease-disintegrins (ADAMs) 1 and 2, better known as fertilin-α and -β or PH-30 α and -30 β and integrins (Blobel et al. 1992, Wolfsberg et al.

1993). It has been shown that also other proteins belonging to ADAM family are involved in cell-cell fusion processes. Meltrin-α (ADAM12) participates in myoblast fusion (Yagami-Hiromasa et al. 1995) and most probably in osteoclast fusion (Abe et al. 1999). ADAM8 (Figure 5) may mediate monocyte fusion since it increases the number of multinucleated cells when present in the fusion stage of osteoclast formation (Choi et al. 2001).

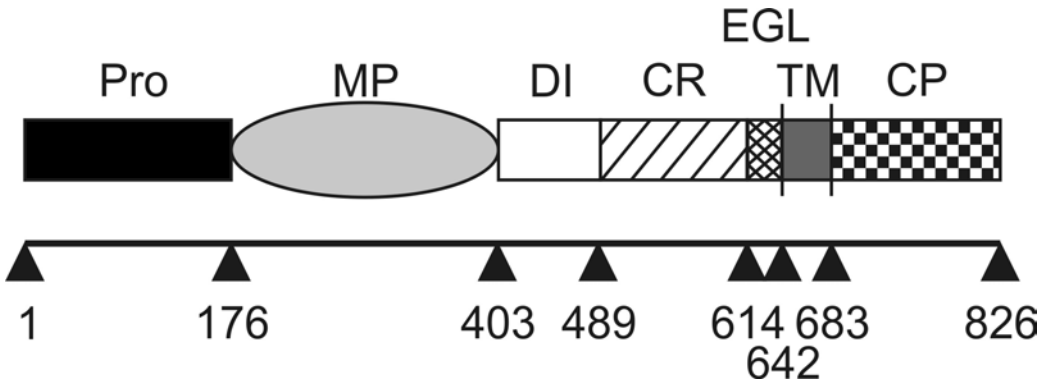

Figure 5. Domain organization of ADAM8. Protein domains are in scale and the amino acid positions are given below. Abbreviations for domains: Pro, prodomain; MP, metalloprotease; DI, disintegrin; CR, cysteine-rich; EGL, epidermal growth factor-like; TM, transmembrane; CP, cytoplasmic. Modified from Sclomann et al. 2002.

How these proteins mediate cell-cell adhesion and fusion is not well known. It has been speculated that they can bind to integrins via their disintegrin domains (Almeida et al. 1995, Zhang et al. 1998). Another possibility is that they form part of multimeric complexes and need co-factors to promote fusion. Signalling and cytoskeletal changes are essential for both osteoclast formation and their function. It is now evident members of ADAM family are able to interact with cytoskeletal proteins and induce intracellular signalling (Iba et al. 2000, Cao et al. 2001, Kawaguchi et al. 2003). A recent study indicates that at least ADAMs 8, 9, 10, 12, 15, 17, and 28 are expressed in osteoclast precursors and in mature osteoclasts (Verrier et al. 2004). This suggests that at least some ADAM proteins might be essential for osteoclastogenesis and osteoclast function.

Other proteins shown to be involved and essential for macrophage fusion, and most probably for osteoclastogenesis, are macrophage fusion receptor (MFR) known as P84/SHPS-1/SIRPα/BIT and CD44 and CD47. There is evidence that CD47 and MFR interact in cell adhesion (Seiffert et al. 1999, Jiang et al. 1999). Blockage of their immunoglobulin (Ig) variable domain inhibits fusion (Han et al. 2000). In a similar way expression of a soluble CD44 extracellular domain or antibodies against CD44 prevent multinucleation (Kania et al. 1997, Saginario et al. 1998) and CD44

deficiency in mice prevents osteoclastic fusion. Interestingly, this does not have any clear effect on bone phenotype (Suzuki et al. 2002). It is not clear how CD44 induces cell fusion since the known ligands for CD44 are extracellular matrix components e.g. hyaluronan, osteopontin and fibronectin (Miyake et al. 1990, Jalkanen and Jalkanen 1992, Weber et al. 1996) and it has been suggested that migration rather than fusion is affected when CD44 is blocked (Suzuki et al. 2002, Chellaiah and Hruska 2003). It is not known whether ADAMs can co-operate with MFR/CD47 complex or CD44 and if they do, how this happens. Further studies are required to identify and characterize the molecules involved in cell accumulation, cell adhesion and membrane fusion in the formation of osteoclasts and giant cells.

4.3.3 Osteoclast function

After fusion, the sequence of cellular events needed for bone resorption is called the resorption cycle. The resorption cycle comprises the following events: migration to the resorption site, attachment to bone, polarization, formation of new membrane domains, dissolution of hydroxyapatite, degradation of organic matrix, transcellular removal of degradation products from the resorption lacuna, and finally return to the non-resorption stage (Vaananen et al. 2000, Vaananen and Zhao 2002).

Osteoclasts (Figure 6) need to migrate to the site, which is about to be resorbed. Migration is tightly controlled and requires formation of specialized focal adhesions called podosomes (Lehto et al. 1982, Lakkakorpi and Vaananen 1991). M-CSF, RANKL and $\alpha_v\beta_3$-integrin are essential to podosome rearrangement, and the secondary target of these molecules is c-Src (Grey et al. 2000, Pfaff and Jurdic 2001, Armstrong et al. 2002, Faccio et al. 2003a). Deficiency of c-Src leads to formation of dysfunctional osteoclasts due to failure to organize podosomes and, thus, failure to form an actin ring and ruffled border (Schwartzberg et al. 1997). Also matrix metalloproteinases (MMPs) have been shown to be essential for osteoclast migration (Blavier and Delaisse 1995, Sato et al. 1998). Especially MMP-9 is highly expressed in osteoclasts (Reponen et al. 1994) and it is essential for osteoclast recruitment in developing long bones (Engsig et al. 2000). MMP-9 is upregulated in chemotaxis (Yu et al. 2003), and downregulated by hyaluronan-CD44 interaction hampering the migration of osteoclasts (Spessotto et al. 2002). It has been suggested that MMPs affect the migration by increasing the disassembly of podosomes (Goto et al. 2002).

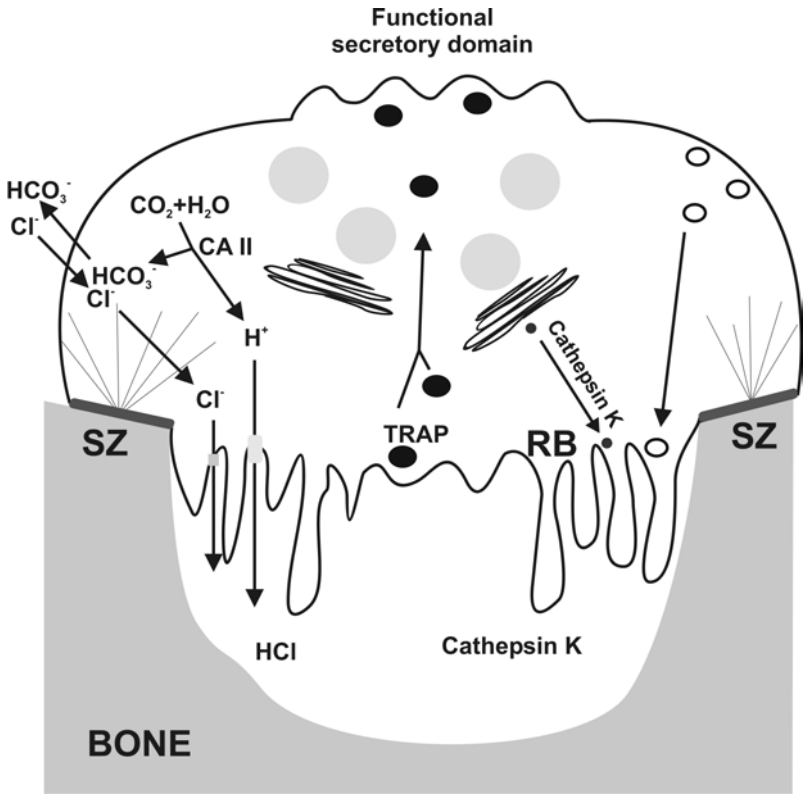

Figure 6. Resorbing osteoclast. Cathepsin K and protons are secreted to the resorption lacunae. CAII, carbonic anhydrase II; RB, ruffled border; SZ, sealing zone. Modified from Vaananen and Zhao 2002.

Having reached the resorption site osteoclasts attach to bone. Bone must be cleared from protective organic surface layer to reveal the underlying mineralized substrate. Osteoblasts are responsible for this clearance and, thus, regulate the initiation of resorption (Chambers 1981, Chambers et al. 1984). Attachment to bone leads to the formation of the actin ring and the tight sealing zone, which encloses the resorption lacuna (Lakkakorpi et al. 1991, Lakkakorpi and Vaananen 1991). The $\alpha_v\beta_3$-integrin is essential for actin ring formation and osteoclast polarization (McHugh et al. 2000, Faccio et al. 2003a, Faccio et al. 2003b). It is located in the margin of the sealing zone and its "activated" form is located in the ruffled border (Lakkakorpi et al. 1991, Faccio et al. 2002). Ruffled border is the resorbing membrane domain, an organelle under the osteoclast, which is formed by fusion of intracellular acidic vesicles with the plasma membrane facing the bone. It has finger like projections that penetrate to the bone matrix. The plasma membrane domain of ruffled border has several features that are typical for late endosomal membranes, and several late endosomal markers, such as small GTPase Rab7 (Zhao et al. 2001), vacuolar proton pump (V-type H^+-ATPase) (Baron et al. 1985, Blair et al. 1989) and lysosomal glycoprotein lgp110 (Palokangas et al. 1997).

Osteoclasts secrete H^+-ions, which are produced by carbonic anhydrase II (Sly et al. 1983, Silverton et al. 1987, Silver et al. 1988, Lehenkari et al. 1998), to Howship lacunae with V-type H^+-ATPase

(Rousselle and Heymann 2002). At the same time passive transport of Cl^--ions through chloride channels occur to maintain electroneutrality in the lacunae. Chloride channel encoded by *ClCN7* gene is shown to be essential for osteoclast function. Mutations in this gene lead to dysfunctional osteoclasts and severe osteopetrosis in human and mouse (Kornak et al. 2001). Cathepsin K is also secreted to lacunae (Kamiya et al. 1998, Yamaza et al. 1998). Acidosis causes mineral dissolution (Lemann, Jr. et al. 1966) and cathepsin K degrades the remaining organic matrix (Garnero et al. 1998). Both of them are essential for osteoclast function (Gelb et al. 1996, Li et al. 1999). Osteoclast removes resorbed material from Howship lacuna by transcytosis via a functional secretory domain on top of the osteoclast (Salo et al. 1997, Nesbitt and Horton 1997). TRAP is located in the transcytotic vesicles (Clark et al. 1989, Halleen et al. 1999) and lack of it leads to decreased resorption activity (Hayman et al. 1996).

4.4 Cathepsin K

Cathepsins, except cathepsin G, have an acidic pH optimum and they are located mainly in lysosomes where they degrade proteins, which are about to be destroyed (Barrett et al. 1998). Cathepsin K is expressed abundantly in the osteoclasts (Drake et al. 1996). The deletion cathepsin K causes pycnodysostosis, an autosomal recessive osteochondrodysplasia characterized by osteosclerosis and short stature, due to osteoclast dysfunction (Gelb et al. 1996, Saftig et al. 1998, Gowen et al. 1999). It has a unique role in osteoclastic bone collagen degradation.

Cathepsin K belongs to a papain-like cysteine proteinase family. Cathepsin K has two potent N-glycosylation sites Asn^{103} and Asn^{161} (Bromme and Okamoto 1995, Inaoka et al. 1995, Li et al. 1995, Shi et al. 1995) but it is glycosylated only at Asn^{103} (McQueney et al. 1997). The non-active, glycosylated procathepsin K is transported to endosomes via the mannose-6-phosphate recognition system (Alberts et al. 1994, McQueney et al. 1997). Based on the amino acid structure cathepsin K has a predicted molecular mass of 35 kDa and in mass spectrometry the proenzyme has a 36366 Da molecular mass. In electrophoresis, procathepsin K runs approximately as 40 kDa sized protein. Autocatalytic activation of cathepsin K in acidic conditions gives a mixture of Gly^{113}, Arg^{114}, and Ala^{115} N-terminal containing non-glycosylated active cathepsin K molecules, which have a mass of 23696 Da in mass spectrometry. Electrophoresis discloses active cathepsin K of approximately 27 kDa size (Drake et al. 1996, Bromme et al. 1996, McQueney et al. 1997). The active site cysteine, histidine and asparagine catalytic triad, which is involved in the proteolytic catalysis of the papain-like cysteine proteinases, is located at sites 139, 276, and 296, respectively, as assessed in multiple sequence alignment (Inaoka et al. 1995, Bossard et al. 1996, Barrett et al. 1998).

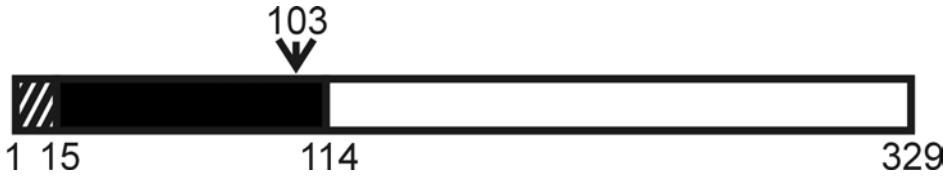

Figure 7. Schematic structure of cathepsin K. Signal peptide 1-14, propeptide 15-113 and active cathepsin K 114-329. N-glycosylation site at Asn^{103} is marked with arrow.

Cathepsin K gene is located in chromosome 1q21. The structure of the promoter region suggests that cathepsin K expression is tightly controlled. The promoter region includes a PU.1 consensus sequence and two activator protein (AP)1 consensus sequences (Rood et al. 1997). Lack of PU.1 transcription factor leads to a complete lack of both macrophages and osteoclasts showing that it is an essential transcription factor regulating the initial stages of myeloid differentiation (Tondravi et al. 1997). Similarly, lack of c-Fos, a component of AP1, affects osteoclast differentiation leading to a lineage shift between osteoclasts and macrophages that results in increased numbers of bone marrow macrophages (Grigoriadis et al. 1994). The promoter region contains also four E-boxes binding sites for Mitf family transcription factors (Motyckova et al. 2001). This transcription factor family is linked to osteoclasts (Walker 1975a, Walker 1975b, Thesingh and Scherft 1985, Weilbaecher et al. 1998), and a recent study indicates that they regulate cathepsin K promoter activity (Motyckova et al. 2001). Production of cathepsin K in mature osteoclasts is increased by bone morphogenetic protein (BMB)-2 (Kaneko et al. 2000), fibroblast growth factor (FGF)-2 (Chikazu et al. 2000), IL-1 (Kamolmatyakul et al. 2001), and RANKL (Corisdeo et al. 2001, Wittrant et al. 2003) whereas estrogen (Mano et al. 1996), interferon-γ (Kamolmatyakul et al. 2001), osteoprotegerin (Wittrant et al. 2002), and transforming growth factor (TGF)-β (Karsdal et al. 2003) abrogate it.

Cathepsin K is activated in intracellular lysosomal compartment of osteoclasts prior their attachment to bone and initiation of resorption (Dodds et al. 2001, Rieman et al. 2001). The secretion to Howship lacuna requires c-Src and phosphatidylinositol (PI) 3-kinase (Furuyama and Fujisawa 2000, Rieman et al. 2001) indicating that intact cytoskeletal structures are required for proper targeting and processing of the enzyme. Howship lacuna has a pH, which is optimal for both autocatalytic activation and collagenolytic activity of cathepsin K (Bromme et al. 1996, McQueney et al. 1997, Garnero et al. 1998).

Cathepsin K shares the enzyme type-specific inhibitors with other cysteine proteinases. These prototypic protease inhibitors are L-trans-Epoxysuccinyl-leucylamido(4-guanidino)butane (E-64), peptidyl diazomethanes, iodoacetic acid, peptide aldehydes and cystatins (Bromme et al. 1996, Bossard et al. 1996, Barrett et al. 1998). Specific synthetic inhibitors such as SB 240314 (Thompson

et al. 1997, Dodds et al. 2001) and CLIK-166 (Katunuma et al. 2000) have been developed. Cathepsin K activity is efficiently controlled by pH. Activation occurs in acidic conditions, the optimal catalytic activity is between pH 6.0 and 6.5, and the half-life of the activity at pH 6.5 is approximately 60 minutes (Bromme et al. 1996). Osteoclastic cathepsin K is also controlled by cystatin C, which locates to the same compartments as cathepsin K (Yamaza et al. 2001).

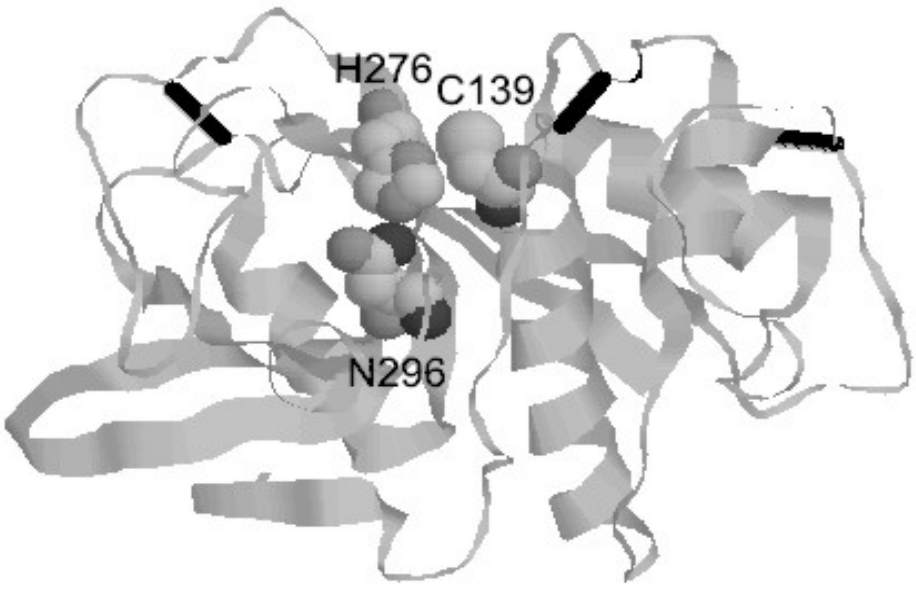

Figure 8. Ribbon structure of active cathepsin K. Active site cysteine, asparagine and histidine are marked with space fill structure displaying Van der Waals radii. Figure is generated with Protein Explorer.

There are no reports of human bone diseases due to overexpression of cathepsin K in osteoclasts indicating that its activity is highly controlled or that compensatory bone formation is accelerated in this situation as is seen in a mouse model (Kiviranta et al. 2001). Some human diseases, like rheumatoid arthritis (Hummel et al. 1998) or osteoclastoma (Inaoka et al. 1995, Li et al. 1995, Drake et al. 1996), seem to be associated with increased cathepsin K production and bone destruction. In these conditions, the production is more likely to be secondary and related to enhanced macrophage activation and osteoclast formation (Atkins et al. 2000, Gravallese et al. 2000) since also cystatin C is increased (Hansen et al. 2000, Hansen et al. 2001).

5. AIMS OF THE STUDY

Despite extensive studies, the mechanisms leading to osteolysis around initially well fixed prosthesis have remained unclear. New molecules have been found and proven to be involved in

osteoclast formation and bone resorption. Normal bone remodelling requires bone resorption, which results from the action of osteoclasts. We adopted the elements of this biological process directly to our study on aseptic loosening and investigated in the interface tissue the same events and molecules, which are involved in normal bone remodelling. The aim was to gain new information on the mechanism of osteolysis in bone around loosened hip prostheses by answering the following specific questions:

- Is interface tissue acidic to be able to cause demineralization of bone and is cathepsin K expressed in interface tissue so that it could degrade demineralised bone matrix?

- Are RANKL, RANK and osteoprotegerin expressed and involved in the osteolytic process *in vivo*?

- Is fibroblast a potential source of RANKL in interface tissue and how is its RANKL production controlled *in vitro*?

- How are the multinucleated cells in the interface tissue formed and are they capable of bone resorption?

- Is there any evidence of multinucleation in interface tissue *in situ*?

6. MATERIALS AND METHODS

6.1 Patients and samples

6.1.1 Patients with loose totally replaced hip implants

All patients with aseptic loosening recruited to the present study (n=28) had hip pain, and their radiographs disclosed periprosthetic osteolysis consistent with loosening. The time between the primary and revision operations, i.e. the life in service of the implants, was 14.5 ± 1 (mean ± standard error of mean (SEM)) years. Microbiological culture samples taken during operations were negative (I-III, V).

6.1.2 Controls

Patients with well fixed but mechanically broken acetabular ultra-high molecular weight polyethylene plastic cups (n=8) served as controls together with rheumatoid arthritis (n=8) and trauma (n=8) patients. One of the patients was operated due to septic loosening caused by *Pseudomonas aeruginosa* and *Flavimonas oryzihabitans* (I, II, V).

Hip joint capsule tissue samples (n=6, five women and one man) were obtained from patients

undergoing primary THR for osteorthritis of the hip. Their mean age was 60 ± 4 years. Dense collagenous capsular tissue was selected as a source of true connective tissue fibroblasts. Loose connective tissue was cut away from the samples before culture (III).

Femoral heads from patients (n=6) undergoing a hemiarthroplasty due to a fracture of the femoral neck were used for osteoblast isolation. Five of the patients were women and one was man. The mean age of these patients was 83 ± 11 years (III).

6.2 Cell cultures

6.2.1 Monocytes (II, IV)

Human monocytes from healthy subjects were isolated from buffy coat cells with Ficoll-Paque (Pharmacia Biotech) at 2500 g for 20 minutes at room temperature. The mixed mononuclear cell band was removed by aspiration and the cells were washed with Ca^{2+}/Mg^{2+} free 100 mM phosphate buffered 150 mM saline (PBS, pH 7.4) and centrifuged at 1250 g for 5 minutes. PBS was removed and washing step was repeated 3 times.

Mononuclear cells were resuspended in 50 ml serum- free macrophage medium (SFM, GIBCO) with 1 % penicillin/ streptomycin and counted. After cell counting, 4 x10^6 cells/well were allowed to adhere to 6-well plates for 1 h at +37 °C in a 5 % CO_2 in air in incubator. Non-adherent cells were washed away and adherent cells were stimulated to activate monocytes for 24 hours with or without 10 µg/ml lipopolysaccharide (LPS, Sigma) (II), for 1, 3, 7 and 14 days with 15 µl/ml of pseudosynovial fluid or 25 ng/ml M-CSF (R&D Systems) and 40 ng/ml RANKL (Alexis Biochemicals) (IV). The media with pseudosynovial fluid or with cytokines was replaced twice a week.

6.2.2 Fibroblasts and osteoblasts (III)

Soft tissue samples were minced with a sterile scalpel in a laminar flow hood. The explants were left overnight in RPMI 1640 medium containing 10 % fetal bovine serum (FBS, BioWhittaker) with 1000 U/ml penicillin and 1 mg/ml streptomycin (10x) solution. Next day, the media were changed to basal RPMI with 10 % FBS media and 100 U penicillin and 0.1 mg streptomycin (1x solution). The medium was changed twice a week for three weeks and when approximately 80 % confluence was reached the explants were removed and the cells were subcultured 1:3 until confluent. Passages 2-4 were used for subsequent experiments.

Trabecular bone of the femoral heads was cut to pieces. The pieces were washed several times with PBS and subjected to collagenase (Collagenase XI, Sigma) treatment for 4 times 15 minutes. After washes with PBS the explants were washed once with Dulbecco's Modified Eagle Medium (D-MEM) without D-glucose and sodium pyruvate (Invitrogen), supplemented with 10 % FBS and 10x

penicillin and streptomycin and cultured over night in this medium. On the following day, the medium was changed to D-MEM with 10 % FBS and 1x antibiotics. After this samples were processed as described above.

For experiments, fibroblasts and osteoblasts were grown to confluence in 6-well plates. The number of cells was $2x10^5$ cells/well. The cells were stimulated with TNF-α (0.05 ng/ml, R&D Systems), IL-1β (0.01 ng/ml, R&D Systems), IL-6 (0.5 ng/ml, R&D Systems), IL-11 (0.1 ng/ml, R&D Systems) or 1α,25-$(OH)_2D_3$ ($1.0x10^{-8}$ mol/l, Calbiochem) for 72 hours after which the culture media were collected. Each of the stimulations was made in triplicate. Cells were washed in PBS and lysed with 1 % Triton X-100 (Sigma) in PBS. Samples were centrifuged to remove cell debris and cell homogenates were stored at –20 °C until used. For RNA isolation, the cells were stimulated for 24 h, washed with PBS and lysed with TRIzol (Invitrogen).

6.3 Immunohistochemistry, immunofluorescence and histochemistry (I-V)

Six µm thick cryostat sections were mounted on DAKO Capillary Microscope slides (TechMate™, Dako) and fixed in cold acetone for 5 minutes at +4 °C (I - III), then washed in 10 mM PBS (pH 7.4) for 5 minutes. After that the slides were washed with washing buffer and installed in DAKO TechMate™ Horizon immunostainer and stained automatically at +22°C using the following protocol: 1) the primary antibody, diluted in DAKO ChemMate™ antibody diluent, for 25 minutes. The antibodies were rabbit anti-cathepsin K C-terminal peptide IgG (0.17 µg/ml, Yamaza et al. 1998) or mouse anti-human cathepsin K IgG_1 (1 µg/ml, Calbiochem), goat anti-human RANK IgG (0.25 µg/ml, R&D Systems), mouse anti-human RANKL IgG_{2b} (1 µg/ml, R&D Systems) and mouse anti-human osteoprotegerin IgG_1 (1.25 µg/ml, R&D Systems), 2) biotinylated secondary antibody for 25 minutes, 3) block peroxidase for 25 minutes, 4) peroxidase-conjugated streptavidine 3 times for 3 minutes, 5) horseradish peroxidase (HRP) Substrate Buffer and finally 6) substrate working solution containing 3,3'-diaminobenzidine tetrahydrochloride (ChemMate™ detection kit) for 5 minutes. Between steps, the sections were washed with DAKO ChemMate™ washing buffer three times and dried in absorbent pads. After staining the sections were removed from the machine, counterstained with hematoxylin, washed, dehydrated in ethanol series, cleared in xylene and mounted in permanent mounting media. Normal goat IgG or monoclonal mouse IgG_{2b} or IgG_1 of irrelevant specificity were used at the same concentration as and instead of the primary antibodies as negative staining controls.

In article V, six µm thick tissue sections were fixed in cold acetone at -20 °C for 15 minutes. Endogenous peroxidase activity was blocked with 0.3 % H_2O_2 in absolute methanol for 30 minutes. The sections were then incubated with the following reagents at room temperature: 1) normal goat serum (Vector Laboratories) diluted 1:50 in 50 mM tris buffered 150 mM saline (TBS, pH 7.4) containing 0.1 % bovine serum albumin (BSA, Sigma) for 20 minutes, 2) rabbit antiserum to

ADAM8 (1:1000, Choi et al. 2001) in TBS containing 0.1 % BSA overnight, 3) biotinylated goat anti-rabbit IgG (1:100, Vector Laboratories) in TBS containing 0.1 % BSA for 30 minutes, 4) avidin-biotin-peroxidase complex (Vector Laboratories) for 30 minutes, 5) a combination of 0.023 % 3,3'-diaminobenzidine tetrahydrochloride (Sigma) and 0.006 % H_2O_2 for 5 minutes. Between the steps, the sections were washed for 3 x 5 minutes in TBS. Finally, the slides were counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene and mounted in permanent mounting media. Nonimmune rabbit serum was used at the same concentration as and instead of the primary antiserum as a negative staining control.

The ADAM8-stained areas were measured using a computer-assisted image analysis system (SensiCam). Under a x200 magnification, the sections without counterstaining were inspected with Leitz Diaplan microscope (Wetzlar) coupled to a 12-bit PC digital image camera (SensiCam). Images were analyzed using a semiautomatic Analysis Pro 3.0 image analysis and processing system (Soft Analysis System). The whole section area and ADAM8-stained area were measured. Results are reported as percentages of the positively stained areas.

For double immunofluorescence staining (I, II) sections were fixed in cold acetone for 20 minutes at -20 °C. After fixation sections were incubated with normal donkey serum (Vector Laboratories), diluted 1:20 in PBS containing 1.25 % BSA, for 30 minutes at +22 °C followed by blotting of excess serum. Cathepsin K was detected with rabbit anti-cathepsin K C-terminal peptide IgG (2 µg/ml in TBS containing 0.1 % BSA) and CD68 (as a monocyte/macrophage marker) with mouse anti-human CD68 KP 1 IgG_1 (2 µg/ml in TBS containing 0.1 % BSA, Dako). After washing, the primary antibodies were labeled using tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG, respectively (I). RANK was labelled using goat anti-human RANK IgG (1 µg/ml in TBS containing 0.1 % BSA) and RANKL using mouse anti-human RANKL IgG_{2b} (4 µg/ml in TBS containing 0.1 % BSA). Secondary antibodies were TRITC-conjugated donkey anti-goat IgG and FITC-conjugated donkey anti-mouse IgG (II). All secondary antibodies were purchased from Jackson ImmunoResearch Laboratories and diluted 1:100 in PBS containing 12.5 % BSA and 0.8 g/L human IgG (Vector Laboratories) to reduce nonspecific staining. After washing sections were air-dried and mounted in Vectashield mounting medium (Vector Laboratories).

Phenotype assessment and the purity of the cultures (III) was done by immunofluorescence with the following antibodies: mouse anti-human prolyl 4-hydroxylase 5B5 IgG_1, (Dako) as fibroblast marker, mouse anti-human CD68 KP 1 IgG_1 (Dako) as monocyte marker, mouse anti-human CD31 IgG_1 (Dako) as endothelial cell marker and monoclonal anti human smooth muscle myosin heavy chains IgG_1 (BioGenex) as smooth muscle cell marker. Cells were cultured on glass coverslips, washed, fixed with 2 % paraformaldehyde (Sigma) for 20 minutes at +22 °C, treated with 0.2 %

Triton X-100 for 10 minutes at +22 °C and after washing blocked with 3 % goat normal serum followed by incubation with the antibodies for 30 minutes at +22 °C. After washing, anti-mouse Alexa Fluor 488 (Molecular Probes) secondary antibody was applied to the coverslips for 30 minutes at +22 °C. Finally, the coverslips were washed and stained with 4,6-Diamidino-2-phenylindole (DAPI, Sigma) to visualize the nuclei, mounted in Glycergel (Dako) on microscope slides and kept in the dark at +4 °C until analysis. Osteoblasts were identified by their capacity to form mineralized matrix, which was stained with the method of von Kossa.

In osteoclast induction studies (IV) monocytes and osteoclasts were stained for TRAP using TRAP staining kit (Leukocyte Acid Phosphatase kit, Sigma). The cytoskeletal actin was stained using Alexa Fluor 633 phalloidin reagent (Molecular Probes) and the nuclei were visualized using 4,6-Diamidino-2-phenylindole (DAPI) reagent (Sigma).

ADAM8 and TRAP double staining (V) was performed as follows: after ADAM8 immunostaining, the slides were washed 3 x 10 minutes with PBS and 3 x 10 minutes with distilled water. TRAP staining was done with TRAP staining kit. After a slight hematoxylin counterstaining, the slides were air-dried, mounted in Glycergel and kept in the dark at +4 °C.

6.4 RNA isolation and cDNA synthesis (I-V)

Total RNA was isolated by TRIzol reagent (Invitrogen) according to manufacturer's instructions. RNA was measured spectrophotometrically and its quality was confirmed with ethidium bromide stained 1% agarose gels under ultraviolet light. RNA was DNase treated (Promega) and 2 µg was used to prepare primary cDNA using $(dT)_{12\text{-}18}$ primers and SuperScript enzyme, followed by RNase H treatment (Invitrogen).

6.5 Quantitative reverse transcriptase polymerase chain reaction qRT-PCR (I-V)

Quantitative PCR was run using 0.2 µg first strand cDNA, 0.5 mM primers and 0.2 mM TaqMan probes in LightCycler™ PCR mix by LightCycler™ PCR machine (Roche Molecular Biochemicals). The primer sequences are provided in the original publications. The identity of the product of one sample from each group and of the positive control was verified by sequencing 50 ng isolated (QIAquick, Qiagen Inc.) amplicon using automated Applied Biosystems 373 A sequencer. Probes had reporter dye FAM (6-carboxy-fluorescein) at the 5' ends and quencher dye TAMRA (6-carboxy-tetramethyl-rhodamine) at the 3' ends, which were also phosphorylated (Heid et al. 1996). Serial 1:10 dilutions of human genomic DNA were used to determine the copy number of the amplicon in relation to porphobilinogen deaminase (PBGD) mRNA copies. The housekeeping gene PBGD copy numbers were similar in all samples. PBGD was used as a standard gene and as a marker of successful cDNA synthesis. All primers were designed so that they were located inside one exon to make it possible to use genomic DNA as a standard. The copy numbers

of mRNA molecules were determined at least twice for all samples. The cDNA synthesis reaction was also performed without reverse transcriptase followed by amplification of PBGD to exclude the possibility of genomic DNA contamination.

6.6 pH measurements (I)

For peroperative measurement of pH, the Portamess 910/Type 911 pH system (Elektronische Meßgeräte GMBH&CO) was used once but the rest of the measurements were done using the Sentron 1001 pH System (Sentron Europe B.V.). While both systems are equipped with a sting electrode, Sentron 1001 pH System was chosen because it tolerates the harsh circumstances of sterilization. The equipment was calibrated, after which sterilization was performed in a vacuum chamber injected with hydrogen peroxide, which after evaporation yielded microbicidic plasma in an electrical field produced using a radiowave frequency in Sterrad 100 S Sterilizer. Sterilized electrodes were used for peroperative pH measurements and recalibrated using standard pH solutions and corrections for ambient temperature. Sentron has automatic temperature compensation between 0°C and 60°C. It has a pH measurement range between 0 and 14, and a high resolution (pH ± 0.01, mV ± 1 and temperature ± 0.5°C). pH measurements were performed under sterile conditions during revision THR operation. The five measure points were the bottom of the acetabulum, the inner surface of the femoral cavity 5 cm from the calcar area, the surfaces of the loosened revised hip implant components (measured immediately after removal), and the gluteus medius muscle as a control point. Special attention was paid to locate pathologic interface tissue. Surgeons, who performed the measurements, chose the measure points by visualizing the operation area. Measurements of the acetabular and femoral cavity interface membranes were performed immediately after the implants had been removed and the surfaces had been washed. All blood was evacuated with suction. Measurements were done directly after removal of the implants to avoid any changes in pH values due to air contact. Between individual measurements, the sting electrode was carefully washed with deionized water and dried. Equipment used for the measurement of pH was calibrated and used strictly according to manufacturer instruction during each step of the measurement. A two-point calibration was used to achieve optimal accuracy of data.

6.7 Villanueva bone stain (I)

Prior to revision surgery, each patient received oral tetracycline hydrochloride 250 mg four times daily for 2 days, followed by a 14-day drug-free interval, and then a second 2 day course of tetracycline. The bone samples for confocal laser scanning were fixed in 70 % ethanol and stained with Villanueva bone stain (18,19) for 42 hours. 50 μm sections were prepared of the methyl methacrylate resin-embedded bone-implant interface by grinding. For confocal laser scanning microscopy (TCS SP, Leica Microsystems), the excitation wavelength was set at 488 nm using an acoustic optical tunable filter system. The autofluorescence of the Villanueva bone stain was detected at 590 - 650 nm using a spectral confocal microscope. Ten random x250 fields of the

interface tissue-to-bone area were used to perform histomorphometric analysis with National Institute of Health Image public domain software using the point counting principle. Each image was analysed to determine the mature, mineralized bone volume (green) and low- or non-mineralized bone volumes (yellow or orange, respectively).

6.8 Immunoblot analysis (I, III-V)

For immunoblotting samples were boiled for 5 minutes in sodium dodecyl sulphate (SDS) gel loading buffer before electrophoresis. Electrophoresis was performed in 10 % polyacrylamide slab gel. After electrophoresis, the gels were blotted onto nitrocellulose membrane (Bio-Rad Laboratories). The membrane was blocked overnight using 3 % BSA in TBS and washed in a washing buffer (0.1 % Tween 20, 50 mM Tris-HCl, 0.5 M NaCl, pH 7.5). After washes, the membranes were incubated for one hour in biotinylated rabbit anti-human sRANKL IgG detection antibody (0.25 µg/ml, PeproTech), affinity purified goat anti-human cathepsin K IgG (0.4 µg/ml, Santa Cruz Biotechnology) or polyclonal rabbit antiserum to ADAM8 (1:10,000, Choi et al. 2001) in washing buffer containing 2 % BSA. This was followed with washes and incubation for one hour at +22°C with alkaline phosphatase conjugated ExtrAvidine (1:5000 in washing buffer containing 2 % BSA, Sigma) in combination with the corresponding primary antibody specific alkaline phosphatase conjugated goat anti-rabbit or rabbit anti-goat IgG (1:5000 in washing buffer containing 2 % BSA, Jackson Immunoresearch Laboratories). The membranes were washed with washing buffer for 30 minutes with at least 3 changes of the buffer followed by a final wash in TBS. The alkaline phosphatase-binding sites were revealed in color development solution (Alkaline Phosphatase Conjugate Substrate Kit, Bio-Rad Laboratories) containing a mixture of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT). The color reaction was stopped at 30 minutes by washing the membranes in distilled water for ten minutes.

6.9 RANKL and osteoprotegerin ELISA (III)

RANKL ELISA was performed from cell lysates with sRANKL kit (Biomedica). Samples were diluted 1:5 with PBS. 100 µl of the diluted sample was added to 96-well microtiter plate wells and 100 µl of detection antibody was added. A seven-point standard diluted from 2 ng/ml to 31.25 pg/ml was prepared from kit's stock standard in PBS. Duplicates were used for samples and standards. Plates were incubated for 16-24 hours at +4 °C and washed 3 times with 350 µl washing buffer. After washes, 200 µl streptavidin-HRP conjugate was added to the wells followed by incubation for one hour at +22 °C under continuous gentle shaking. Plates were washed three times, 200 µl of tetra methyl benzidine (TMB) colour substrate solution was added to the wells and incubated at dark for 20 minutes. The reaction was stopped with 50 µl stop solution and the absorbance was measured at 450 nm.

For osteoprotegerin ELISA Nunc MaxiSorp™ (Nunc) plates were coated with monoclonal mouse

anti-human osteoprotegerin IgG_{2a} (2 µg/ml in PBS) (R&D Systems, clone 69127.11) over night at room temperature. The plates were washed three times with PBS containing 0.05 % Tween 20, and blocked with 1 % BSA and 5 % sucrose in PBS for one hour. The blocking buffer was removed and 100 µl of media samples, diluted 1:100 in 1% BSA in PBS to adjust the sample to the range of the assay, were added to each well for 1.5 hours at +22 °C. Recombinant human osteoprotegerin/Fc chimera (R&D Systems) at 2 ng/ml-31.25 pg/ml was used as a standard. After washes the bound osteoprotegerin was incubated with biotinylated anti-human osteoprotegerin IgG (100 ng/ml, R&D Systems) diluted in 1% BSA in PBS. The plates were washed and 1:70,000 diluted alkaline phosphatase conjugated ExtrAvidin (Sigma) was added. Unbound ExtrAvidin was washed away and the colour was developed using Sigma Fast™ p-Nitrophenyl Phosphate tablet set (pNPP substrate). The absorbance reaction was measured at 405 nm.

6.10 *In vitro* analysis of bone resorption (IV)

In vitro bone resorption analysis was performed by first stimulating monocytes with pseudosynovial fluid or M-CSF and RANKL for 7 days. $4x10^5$ cells were transferred to 12-well plates containing a dentin slice 5 mm in diameter (Immunodiagnostic Systems). Cells were incubated on dentin in the presence of media with cytokines (replaced twice) for 7 days. The cells were then brushed away and the dentin slices were stained with toluidine blue to visualize the resorption pits. The surface areas of the resorption pits were determined using AnalySIS 3.2 software (Soft Imaging System) and a confocal microscope.

6.11 *In situ* hybridization (V)

Nine µm thick frozen sections from synovial membrane-like interface tissue (n = 6) and control OA synovial membrane (n = 6) samples were processed for *in situ* hybridization. RNA probes were produced by *in vitro* transcription from cDNA of ADAM8. Digoxigenin-conjugated UTP (Roche Molecular Biochemicals) was incorporated to the RNA probes during the synthesis phase. Hybridization (overnight at +58 °C) and post-hybridization washes were done under stringent conditions. Digoxigenin was labelled by alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche Molecular Biochemicals) and the colour was developed in a mixture of nitroblue tetrazolium (NBT), 5-bromo-4-chloro-3-indolylphosphate (BCIP) and levamisole. The slides were counterstained with methylene green for 15 minutes. After thorough washes, the slides were air-dried, mounted in Glycergel and stored in the dark at +4 °C.

6.12 Statistical analysis

Repeated measures ANOVA with Bonferroni's multiple comparison test was used to compare differences between patients or between different cell stimulations unless otherwise mentioned. Spearman correlations were used to analyse the correlations between mRNA and protein levels. All tests were performed with GraphPad Prism version 3.02 for Windows (GraphPad Software). All

results are expressed as mean ± SEM.

7. RESULTS AND DISCUSSION

7.1 Interface tissue expresses markers indicating active bone resorption capacity (I, III)

7.1.1 Interface tissue is acidic

Bone resorption requires low pH for mineral dissolution. Osteolysis has been described around loosened THR prostheses and is currently the major issue of concern in THR surgery. Direct pH measurements were done during revision operations to reveal if interface tissue itself provides environment, which can lead to bone mineral dissolution. It was noticed that interface tissue is clearly acidic. The lowest pH value recorded from pathological interface tissue or implant surfaces was 6.1 ± 0.2, and thus was significantly below the control measure point value. The pH at the gluteus medius control measure point was 7.39 ± 0.05 and the pH of the interface area in the patient with a well-fixed but broken cup was 7.75 in the acetabular base and 7.85 in the acetabular cup. In contrast, the pH value in the patient with septic loosening was 4.38 in the femoral cavity and 5.80 in the femoral stem.

Acidosis can arise locally at tissue level as a result of reduced vascular supply due to inflammation, infection or tumour. Interface tissue is hypovascular, i.e. suffers from reduced vascular supply and displays signs of ischemia-reperfusion injury (Santavirta et al. 1996). There are no other data available of bone pH but the pH in skin has been reported to be near 7.1. In pH 7 osteoclasts are very sensitive to small changes in extracellular pH and resorption is markedly enhanced in acidic conditions (Arnett and Dempster 1986, Meghji et al. 2001). Acidosis also inhibits extracellular matrix gene expression in osteoblasts (Frick and Bushinsky 1998) and stimulates RANKL RNA expression in bone (Frick and Bushinsky 2003). Thus, besides direct effect on bone, low pH has an impact on both osteoclast and osteoblast activity that may lead to periprosthetic bone weakening.

7.1.2 Periprosthetic bone is demineralized

To obtain direct evidence of the effects of low pH on periprosthetic bone Villanueva bone stain and confocal laser scanning were performed. The Villanueva stained periprosthetic bone in contact with the acidic interface disclosed wide zones of mineral poor organic bone matrix. The mineral poor organic bone surface was covered with macrophages or multinuclear giant cells, but part of it was uncovered, i.e. free of cells. A seam of bone surface in direct contact with the interfacial tissue was partially or totally devoid of hydroxyapatite. Demineralized bone was often also seen as patchy islands in the little deeper intermediate bone layer. Deep layers contained mature, mineralized bone. The volumetric proportions of non- and low-mineralized bone were significantly higher both in the acetabulum and femur around aseptically loosened than around well-fixed samples. Vice versa, more mature mineralized bone was present around well-fixed implants than around aseptically

loosened implants. In many places the demineralized areas were not subosteoclastic indicating that demineralization of the bone was due to general acidosis in the interface tissue.

7.1.3 Cathepsin K and TRAP are present in interface tissue

Cathepsin K and TRAP are markers for osteoclasts. The essential role of cathepsin K in osteoclast function led us to investigate its presence in periprosthetic tissues. All interface membrane extracts from patients suffering from loosened THR prostheses contained 42-kDa pro- and 27-kDa proteolytically processed, activated forms of cathepsin K. Synovial membrane extracts from trauma and rheumatoid arthritis contained only apparently latent 42-kDa cathepsin K bands. Pseudosynovial fluid from patients suffering from aseptic loosening contained 42-kDa cathepsin K, indicating extracellular *in vivo* release and secretion of cathepsin K. Acidosis lead to autocatalytic activation of cathepsin K (McQueney et al. 1997). This may explain the presence of active cathepsin K in interface tissue and lack of it in synovial membrane samples.

Immunohistochemisty indicated that cathepsin K was particularly prominent in foreign body type giant cells, some of which contained intracytoplasmic, phagocytosed material or were attached to large ultra-high molecular weight polyethylene particles. Less than 5 % of all local cathepsin K containing cells were multinuclear foreign body giant cells. More than 95 % of all local cathepsin K containing cells were mononuclear cells. Almost all of these cells displayed CD68 in double staining and were also TRAP-positive. Endothelial cells did not express cathepsin K, and double staining of proline-4-hydroxylase as a fibroblast marker and cathepsin K disclosed that fibroblasts did not contain cathepsin K. Some cathepsin K expressing cells were present in rheumatoid arthritis and even in trauma samples, but they were mostly confined to the synovial lining cell layer and were all lacking TRAP. In contrast, strong cathepsin K staining was found on the rim of cells and in the matrix along the edge of the acidic interface membrane bordering the host bone. Polyclonal rabbit and monoclonal mouse antibodies gave identical staining results. Control staining, using normal rabbit IgG or mouse IgG_1 instead of the primary antibodies, was totally negative.

Cathepsin K mRNA copy number per one thousand β-actin copies was significantly increased in interface tissue 1875 ± 302 mRNA copies (n=10) compared to 501 ± 48 in rheumatoid arthritis ($p<0.001$, n=8) or to 268 ± 61 in trauma ($p<0.001$, n=8) patients (I).

Cathepsin K has been thought to be exclusively expressed by cells of the monocytic lineage and, accordingly, we did not detect cathepsin K protein in proline-4-hydroxylase expressing cells in tissue sections. However, cultured interface tissue fibroblasts, which contained proline-4-hydroxylase and did not express markers for other cell types investigated, produced and released pro- and active cathepsin K protein as revealed by immunoblotting. Also the mRNA expression of cathepsin K in these cultures was regulated. Unstimulated fibroblasts produced 9 ± 1 cathepsin K

mRNA copies per one PBGD copy. 1α,25-$(OH)_2D_3$ stimulation increased its production to 31 ± 10 copies ($p<0.01$). TNF-α (9 ± 1 mRNA copies) and IL-1β (9 ± 1 mRNA copies) did not alter fibroblasts-mediated cathepsin K mRNA production whereas IL-6 (13 ± 4 mRNA copies) and IL-11 (17 ± 5 mRNA copies) slightly increased its production but the increase was not statistically significant (III).

Proteinases have been shown to be present in many diseases including loosening of total hip prostheses. Before this study only neutral endoproteinases like MMPs were thought to be important in extracellular matrix destruction and periprosthetic bone degradation in loosening of hip implants. Here we show that cathepsin K is expressed, released and activated in interface tissue. Even cultured fibroblasts from interface tissue were able to produce active cathepsin K, and cytokines and 1α,25-$(OH)_2D_3$ regulate the synthesis of cathepsin K mRNA. It is not clear why we did not detect cathepsin K protein in fibroblasts when we stained tissues. One reason might be that it is readily secreted by fibroblasts. Thus, the amount of cathepsin K inside the cells might be so low that it is impossible with our staining technique to show fibroblasts cathepsin K production *in vivo*. However, also rheumatoid fibroblasts have been shown by others to produce cathepsin K (Hou et al. 2002) indicating that fibroblasts really can be a source of cathepsin K, probably also in interface tissue. Interface tissue has optimal conditions for full cathepsin K activity. Based on this and the fact that it is the most effective collagenase against type I collagen, it can be assumed that cathepsin K may be the major collagenolytic enzyme in interface tissue.

7.2 Interface tissue is capable of osteoclast induction (II, III)

7.2.1 RANK and RANKL coexist in stroma in the absence of osteoprotegerin

Multinuclear cathepsin K positive giant cells were seen in the interface tissue. Although macrophages are able to fuse spontaneously, the presence of RANKL has been shown to be essential for bone resorption activity in these cells (Boissy et al. 2001). RANK protein was found in interface tissue. It seems that especially multinuclear foreign body giant cells contain RANK protein. RANKL protein was also found in interface membranes. In contrast, control synovial membrane from femoral neck fracture did not display any cells staining for RANK or RANKL proteins. Osteoprotegerin protein was found in both synovial membrane-like interface tissue and synovial membrane of femoral neck fracture but the staining was restricted to vascular endothelial cells whereas macrophage-like cells, foreign body giant cells and fibroblasts did not contain osteoprotegerin. Double immunofluorescence staining for RANK and RANKL disclosed both double and single positive cells. Negative staining controls were negative.

7.2.2 The levels of RANK and RANKL mRNAs are increased in interface tissue

Quantitative RT-PCR revealed that synovial membrane-like interface tissue contained more RANK and RANKL mRNA copies than synovial tissue from femoral neck fracture. Interface tissue

samples contained 6 ± 1 (mean ± SEM) copies whereas synovial membrane samples contained only 3 ± 1 copies of RANK mRNA ($p<0.05$). The difference in RANKL was even more significant 18 ± 3 vs. 5 ± 2 ($p<0.01$). In contrast, the average osteoprotegerin mRNA level was the same in both groups: 41 ± 11 vs. 41 ± 10 copies.

The mRNA expression of RANK, RANKL and osteoprotegerin in interface tissue indicates that these proteins are produced *in situ* and that interface tissue really is more potent osteoclast inducer than healthy or "normal" synovial membrane. Since interface tissue consists mainly fibroblasts, monocytes, macrophages, foreign body giant cells and endothelial cells but not osteoblasts or lymphocytes it is likely that fibroblasts are an important source of RANKL but the presented data do not exclude the possibility of monocytes being in part responsible for RANKL production (Crotti et al. 2004). Although it has been shown that neuropeptides can regulate RANKL and osteoprotegerin (Mukohyama et al. 2000), it is likely that in aneural interface tissue (Niissalo et al. 2002) other factors may regulate the RANKL system and osteoclastogenesis.

7.2.3 Monocytes can enhance their RANK expression

The reason for increased RANK production in interface tissue can be either on increase in monocyte number or level of RANK expression per monocyte. Preosteoclasts are able to increase their RANK production upon TGF-β stimulation (Yan et al. 2001). At the time of the study, little was known about RANK expression in osteoclasts and monocytes. We stimulated cultured human peripheral blood monocytes with LPS to investigate if monocytes are able to alter RANK expression after stimulation. Monocytes produced 52 ± 9 copies of RANK mRNA after 24 h LPS stimulation compared to 11 ± 1 copies without LPS stimulation (mean ± SEM). There are still not many studies concerning the regulation of RANK expression but it has been shown that LPS decreases RANK expression in murine bone marrow macrophages (Zou and Bar-Shavit 2002). In contrast, we showed that RANK was induced by LPS indicating that the RANK gene can be regulated. The discrepancy between our studies and that of Zhou et al. may due to different source of stimulated cells, i.e. human versus mouse and different dosage of LPS (we used 500-fold stronger LPS solution). There might also be species-specific time-dependent RANK downregulation after LPS induction because of the activation of NF-κB, three mitogen-activated protein kinases (MAPKs), and ERK-1 and -2 by LPS (Guha and Mackman 2001).

7.2.4 Fibroblasts from interface tissue produce RANKL and osteoprotegerin

Normally osteoblasts are responsible for osteoclast development but the synovial membrane-like interface tissue lacks osteoblasts. Our results suggest that RANKL in interface tissue can stimulate RANK expressing responder cells to differentiate to osteoclasts (II). It has also been shown that synovial fibroblasts from rheumatoid arthritis form a potential source for RANKL (Takayanagi et al. 2000). To further characterise the source of osteoclastogenic molecules in interface tissue we

cultured interface tissue fibroblasts from patients with loosened THR prostheses and studied the effects of cytokines and 1α,25-$(OH)_2D_3$ on RANKL and osteoprotegerin production. The effects on interface tissue fibroblasts were compared to the corresponding effects on osteoblasts from bone and on fibroblasts from dense connective tissue.

Western blotting revealed a 35 kDa RANKL protein in osteoblast, interface tissue fibroblast and fibrous capsule fibroblast lysates. A protein of similar size was present in giant cell tumour lysate. The antibody also recognized recombinant human 28 kDa soluble RANKL protein, which was used as a positive sample control.

Basal and stimulated production of osteoprotegerin protein was highest in osteoblast culture media. TNF-α stimulation increased osteoprotegerin production two fold ($p<0.001$) in osteoblasts and 1.5 fold in interface tissue fibroblasts ($p<0.001$). Fibroblasts from fibrous capsules also increased their osteoprotegerin release upon TNF-α stimulation but the amount always remained two to three fold lower than that produced by interface tissue fibroblasts (Table 1).

The amount of RANKL protein in all cell lysates was always at least 10 fold lower than that of osteoprotegerin in the corresponding culture media. Surprisingly, the amount of RANKL protein was highest in unstimulated fibroblasts from interface tissue. There were no statistically significant differences between fibroblasts of different origin or different stimulations although the amount of RANKL was always higher in interface tissue fibroblasts than in fibrous capsule fibroblasts. Osteoblastic RANKL production was very similar to that of fibroblasts. The highest RANKL-osteoprotegerin ratios were always seen in non-stimulated cells for all cell types studied (Table 1).

Table 1. The amount of RANKL protein in cell lysates and osteoprotegerin protein in culture media, and their ratio.

cell	*stimulant*	*RANKL (ng/ml)**	*osteoprotegerin (ng/ml)*	*RANKL-osteoprotegerin ratio¶*
osteoblast	TNF-α	484 ± 113	24220 ± 5083†	0.048 ± 0.015
rTHR fibroblast		**407 ± 55**	**18201 ± 2159‡**	**0.022 ± 0.002**
FC fibroblast		223 ± 155	10318 ± 2438 §	0.022 ± 0.021
osteoblast	IL-1 β	226 ± 64	13403 ± 1651	0.037 ± 0.024
rTHR fibroblast		**417 ± 57**	**14425 ± 1839**	**0.029 ± 0.005**
FC fibroblast		139 ±163	11032 ± 2392	0.013 ± 0.023
osteoblast	IL-6	329 ± 129	17407 ± 2405	0.032 ± 0.023
rTHR fibroblast		**459 ± 120**	**15830 ± 2170**	**0.027 ± 0.011**
FC fibroblast		318 ± 87	8957 ± 2417	0.035 ± 0.013
osteoblast	IL-11	319 ± 85	14439 ± 2528	0.050 ± 0.028
rTHR fibroblast		**292 ± 70**	**11760 ± 1743**	**0.026 ± 0.004**
FC fibroblast		54 ± 37	7988 ± 2111	0.009 ± 0.007
osteoblast	1α,25-$(OH)_2D_3$	282 ± 159	13511 ± 1791	0.017 ± 0.021
rTHR fibroblast		**531 ±147**	**12522 ± 1678**	**0.038 ± 0.016**
FC fibroblast		0	6180 ± 1589	0
osteoblast	none	413 ± 102	11458 ± 1846	0.054 ± 0.021
rTHR fibroblast		**586 ± 116**	**12250 ± 1772**	**0.046 ± 0.013**
FC fibroblast		316 ±252	7719 ± 1876	0.041 ± 0.043

*No statistical differences were found in RANKL concentrations inside or between the groups when analysed by one way ANOVA.

†TNF-α stimulation increased osteoblast-mediated osteoprotegerin production compared to IL-1 β, IL-11, 1α,25-$(OH)_2D_3$, non-stimulated ($p<0.001$) or IL-6 stimulated ($p<0.05$) osteoblasts. Osteoprotegerin production by TNF-α stimulated osteoblasts was higher than that of interface tissue or fibrous capsule fibroblasts in all stimulation ($p<0.05$) except for interface tissue fibroblasts stimulated with TNF-α, IL-1β or IL-6 and fibrous capsule fibroblasts stimulated with IL-1β.

‡Osteoprotegerin production by TNF-α stimulated interface tissue fibroblasts was higher than the production by IL-11 ($p<0.001$), 1α,25-$(OH)_2D_3$ ($p<0.01$) or non-stimulated ($p<0.001$) interface tissue fibroblasts but not different from IL-1β and IL-6 stimulated cells. IL-6 increased the osteoprotegerin production when compared to unstimulated cells ($p<0.01$)

§TNF-α stimulated fibrous capsule fibroblasts produce more osteoprotegerin than IL-11 ($p<0.01$), 1α,25-$(OH)_2D_3$ stimulated ($p<0.001$) or non-stimulated ($p<0.01$) cells. The amount of osteoprotegerin is highest in IL-1 β stimulated cells and the difference is significant when compared to IL-6 ($p<0.01$) and IL-11, 1α,25-$(OH)_2D_3$ stimulated or non-stimulated cells ($p<0.001$). No statistical difference was seen between TNF-α and IL-1 β stimulated cells.

¶The ratio was calculated so that the RANKL value of cell lysate from an individual well was divided with the osteoprotegerin value from a medium sample from exactly the same well.

Quantitative RT-PCR confirmed that both RANKL and osteoprotegerin mRNAs are expressed in interface tissue fibroblasts. All cells produced considerable amounts of osteoprotegerin mRNA. The expression of RANKL mRNA was very low in all cell types and stimulations. The expression was highest in fibrous capsule fibroblasts. The highest RANKL mRNA levels were measured after 1α,25-$(OH)_2D_3$ stimulation but even in such cultures RANKL protein in the cell lysates remained relatively low. The RANKL-osteoprotegerin mRNA ratio was extremely low. The number of RANKL mRNA copies was 25 000 to 60 000 times lower than that of osteoprotegerin mRNA in osteoblasts and 10 000 to 20 000 times lower in interface tissue and fibrous capsule fibroblasts. Fibrous capsule fibroblasts produced highest amounts of RANKL mRNA when compared to osteoprotegerin mRNA production. The RANKL-osteoprotegerin mRNA ratio was lowest in osteoblasts. Like for the corresponding protein levels, the RANKL-osteoprotegerin mRNA ratios were usually highest in non-stimulated cells (Table 2).

Table 2. The number of RANKL and osteoprotegerin mRNA and their ratio.

cell	*stimulant*	*RANKL / PBGD*	*osteoprotegerin/ PBGD*	*RANKL-osteoprotegerin ratio**
osteoblast	TNF-α	0.0024 + 0.0004	110 + 51	6.7 + 5.2
rTHR fibroblast		**0.0032 + 0.0012**	**65 +16**	**5.0 + 1.8**
FC fibroblast		0.0082 + 0.0060	70 + 24	11.8 + 8.6
osteoblast	IL-1 β	0.0020 + 0.0001	126 + 71	7.7 + 6.4
rTHR fibroblast		**0.0038 + 0.0010**	**154 + 70**	**2.5 + 0.7**
FC fibroblast		0.0126 + 0.0088	80 + 37	15.6 + 10.9
osteoblast	IL-6	0.0024 + 0.0006	93 + 53	6.3 + 4.7
rTHR fibroblast		**0.0045+ 0.0030**	**93 + 36**	**4.8 + 3.2**
FC fibroblast		0.0093 + 0.0066	77 + 31	12.1 + 8.7
osteoblast	IL-11	0.0033 + 0.0005	58 + 18	8.4 + 4.5
rTHR fibroblast		**0.0053 + 0.0029**	**94 + 39**	**5.6 + 3.1**
FC fibroblast		0.0107 + 0.0074	75 + 30	14.3 + 9.8
osteoblast	1α,25-$(OH)_2D_3$	0.0036 + 0.0008	83 + 36	6.1 + 2.0
rTHR fibroblast		**0.0098 + 0.0052**	**125 + 47**	**7.9 + 4.1**
FC fibroblast		0.0157 + 0.0106	70 + 23	22.6 + 15.2
osteoblast	none	0.0022 + 0.0009	66 + 37	8.3 + 5.6
rTHR fibroblast		**0.0060 + 0.0027**	**76 + 22**	**7.9 + 3.5**
FC fibroblast		0.0104 + 0.0071	68 + 20	15.2 + 10.4

*Note that the RANKL-osteoprotegerin mRNA ratio is shown as one RANKL mRNA copy per 100 000 osteoprotegerin mRNA copies.

Cultured cells exhibited low basal mRNA expression and protein levels of RANKL. Interestingly, RANKL mRNA levels were not significantly changed and cellular RANKL was even lowered after stimulation with cytokines and 1α,25-$(OH)_2D_3$. This is in high contrast to the experiments with

mouse osteoblasts/stromal cells or cell lines. Indeed, little is known about the RANKL production in primary human cells. Primary human osteoblasts have been used only to study estrogen effects on osteoprotegerin production (Viereck et al. 2002). However, studies with cell lines or murine osteoblasts show that osteoblasts decrease their RANKL, and increase their osteoprotegerin production during osteoblast differentiation (Gori et al. 2000, Thomas et al. 2001) and loose their ability to induce RANKL expression in response to stimulation (Thomas et al. 2001, Kitazawa and Kitazawa 2002). This might be due to the CpG methylation around the transcription start site at RANKL gene that is the case at least in mouse (Kitazawa and Kitazawa 2002). We did not determine the CpG methylation status of RANKL promoter in the cells that we studied but the methylation may be one reason for weak cytokine and 1α,25-$(OH)_2D_3$ response in our *in vitro* experiments.

Although RANKL production was quantitatively low in cultured interface tissue fibroblasts, RANKL was still clearly produced. A recent study also shows that fibroblasts derived from the inner granulation tissue of the pseudocapsule in loosened total hip prostheses are capable to support osteoclastogenesis (Sakai et al. 2002). RANKL production and subsequent osteoclastogenesis has also been shown to be induced in mouse whole bone marrow cultures after PMMA particle stimulation (Clohisy et al. 2003). The study, which shows that osteoprotegerin gene transfer protects against PMMA induced bone resorption, confirms the essential role of RANKL in particle-induced osteolysis (Yang et al. 2002). If the effects of the particles that activate stromal cells are direct or occur via cytokine production after phagocytosis is still unknown. At least the soluble factors studied here were not able to induce RANKL production. We used relatively low cytokine concentrations and higher concentrations might have induced stronger response on RANKL. However, the used concentrations were higher than those measured in the pseudosynovial fluid (Inomoto et al. 2000) but it is possible that local concentration of the cytokines in the interface tissue may exceed the *in vitro* used concentrations. It should be also noted that osteoprotegerin production was affected after stimulations. Further studies are needed to characterize the factors that are responsible for RANKL induction in interface tissue. However, apart from soluble factors there are other possible explanations for increased RANKL production in interface tissue. Cyclic loading and micromotion cause mechanical stress, which may induce RANKL production in interface tissue (Kanzaki et al. 2002). It is also possible that the fluid pressure waves and shear stress, which are generated during walking, induce fibroblasts to produce RANKL.

The balance between RANKL and osteoprotegerin is important in the regulation of osteoclast formation (Takai et al. 1998, Yasuda et al. 1998b). Our findings indicate that soluble osteoprotegerin is not able to prevent interactions between RANKL and its cell bound receptor RANK in interface tissue. Clear reason for the lack of osteoprotegerin in interface tissue fibroblasts but the strong production in cultured interface tissue fibroblasts can not be given. Although the

passage number was low, culture conditions may change the phenotype of the cells. It is also extremely difficult to simulate the complex effects of cytokines, micromovement, particles, extracellular matrix and nutrients, which are present in the interface tissue. However, similar restricted osteoprotegerin localization to vascular endothelium has been described in the synovial membranes of psoriatic and rheumatic arthritis and also in interface tissue (Ritchlin et al. 2003, Haynes et al. 2003, Crotti et al. 2004). One explanation for this may be that the antibody, which we used, only recognizes free but not RANKL bound osteoprotegerin. However, it does not explain the differences in the mRNA expression. In interface tissue RANKL mRNA expression was increased compared to control synovium whereas osteoprotegerin mRNA expression was similar. More likely the reason for the striking *in vivo* and *in vitro* discrepancy is that we were not able to simulate the situation that occurs in the interface tissue. It can be concluded that the localization of RANKL and osteoprotegerin in different tissue compartment and the accumulation of RANK expressing mononuclear cells in the interface tissue may be the reason for the formation of multinuclear cathepsin K and TRAP expressing cells surrounding the loosened of THR prostheses.

7.3 RANKL in pseudosynovial fluid induces osteoclast formation from peripheral blood monocytes (IV)

The origin and properties of the multinucleated cells in the interface tissue requires further analysis. It is likely, that many if not all the monocytes or preosteoclasts arrive from circulation and are not produced by proliferation *in situ* (Santavirta et al. 1998). Pseudosynovial membrane surrounds the artificial hip joint and generates the pseudosynovial fluid and most of the substances in it. The fluid is likely to contribute to the development of the interface tissue. As a result of fluid pressure waves during cyclic loading it gradually penetrates between the implant and host bone (Schmalzried et al. 1992). Pseudosynovial fluid has earlier been recognized as a liquid transport medium for particulate ultra high molecular weight polyethylene debris produced between the gliding pair from the acetabular cup (Robertsson et al. 1997). We hypothesized, like Takei and his coworkers have suggested (Takei et al. 2000), that pseudosynovial fluid may also form a transport vehicle for potent biologically active osteoclastogenic factors synthesized in pseudosynovial membrane. This led us to investigate if the peripheral blood derived monocytes are able to differentiate to bone resorbing osteoclast and if the pseudosynovial fluid is capable to induce osteoclastogenesis.

7.3.1 Pseudosynovial fluid contains RANKL and stimulates osteoclast phenotypic mRNA expression

At the final stage of differentiation osteoclasts start to express cathepsin K and TRAP, which are needed for bone resorption. The production of these late stage osteoclastic markers are also seen in interface membrane. All analyzed pseudosynovial fluid samples contained approximately 30 kDa soluble RANKL. During pseudosynovial fluid stimulated differentiation cathepsin K production increased from 3 ± 1 mRNA copies (day 3) to 20 ± 15 mRNA copies (day 14, $p<0.01$) in monocytes. TRAP expression was also significantly induced from day 3 to day 14 (224 ± 22 vs. 434

± 76 $p<0.05$, respectively). Combination of M-CSF and RANKL was more potent cathepsin K inducer (27 ± 17 and 71 ± 15 mRNA copies, respectively) but interestingly, weaker TRAP inducer (77 ± 19 and 314 ± 157 mRNA copies, respectively).

7.3.2 Pseudosynovial fluid stimulates cell fusion

RANKL induced cell fusion is a key step in osteoclast formation. Pseudosynovial fluid was able to induce cell fusion as observed by counting of nuclei in TRAP positive cells. At day three, cells were small, round and no fusion had occurred. After 14 days of psudosynovial fluid stimulation, the number of cells containing three or more nuclei was 120 ± 35 per cm^2. M-CSF and RANKL stimulation was used for positive control and it generated 246 ± 24 multinuclear cells per cm^2 after 14 days stimulation. Osteoprotegerin (40 ng/ml) was able to diminish the fusion (70 ± 25, $p<0.05$) but not completely even with higher concentrations. Synovial fluid from osteoarthritis was significantly weaker fusion inducer (36 ± 23 multinuclear cells per cm^2, $p<0.01$).

Cell fusion is critical for osteoclast formation. The results here show that pseudosynovial fluid contains all necessary elements to induce monocyte fusion. The most important factor is probably RANKL. Osteoprotegerin, at least in part, inhibited fusion indicating that functionally active RANKL indeed is present in pseudosynovial fluid.

7.3.3 Multinuclear cells that contain cathepsin K and TRAP resorb bone

Cathepsin K and TRAP are markers for osteoclasts. They were both shown to be present in multinuclear giant cells in interface tissue. Peripheral blood monocytes started to display markers of osteoclast phenotype after stimulation with pseudosynovial fluid. The multinuclear cells formed showed intense TRAP staining in the central part of the cell indicating that TRAP is located in the perinuclear transcytotic vesicles. Cells also express cathepsin K mRNA and contain cathepsin K protein. However, by definition osteoclasts are multinucleated cells responsible for bone resorption. We were able to show that pseudosynovial fluid induced multinucleated cells did resorb bone. The resorption was not as extensive as was that of the cells generated with M-CSF and RANKL. The number of pits per osteoclast was 0.06 ± 0.01 per mm^2 after pseudosynovial fluid stimulation and 56 ± 11 per mm^2 after M-CSF and RANKL stimulation ($p < 0.01$, t-test). The average resorbed area per osteoclast was 3.4 ± 2.3 μm^2 when the osteoclasts were generated with pseudosynovial fluid whereas the resorbed area per osteoclast generated with M-CSF and RANKL was 16.4 ± 3.9 μm^2 ($p < 0.05$, t-test). M-CSF and RANKL induced osteoclasts generated significantly larger pits than pseudosynovial fluid stimulated cells (64.4 ± 13.2 μm^2 vs. 2.2. ± 1.1 μm^2, $p < 0.01$, t-test). One reason for the weaker resorption by pseudosynovial fluid stimulated cells might be that the cells are not fully maturated osteoclasts. It is also quite natural that optimized system with pure cytokines is superior to other induction systems. Thus, based on the definition of bone resorption capacity it can be concluded that pseudosynovial fluid induces osteoclast formation.

7.4 Cell fusion is evident *in situ* in interface tissue (V)

It is apparent that the multinucleation in interface tissue occurs *in situ*. RANKL is produced in interface tissue and cells of the monocyte lineage are present and probably driven to fuse to become bone resorbing osteoclasts. However, direct evidence for fusion could help to understand and perhaps provide means to prevent the adverse host response against host bone leading to periprosthetic osteolysis and loosening. ADAM8 has been shown to induce osteoclast fusion in culture conditions (Choi et al. 2001). We analyzed interface tissue samples for the presence of ADAM8 to obtain information about the fusion capacity of the cells in the interface tissue.

7.4.1 ADAM8 mRNA is expressed in interface tissue

We first examined the amount of ADAM8 mRNA in interface tissue and compared it to the expression of ADAM8 in osteoarthritic synovial membrane. The number of ADAM8 mRNA copies (10.3 ± 2.7 per 10,000 β-actin mRNA copies) was significantly ($p < 0.05$, t-test) higher in interface tissue samples than in osteoarthritic synovial membrane samples (1.7 ± 0.3 per 10,000 β-actin mRNA copies).

To localise the cells and areas, that produce ADAM8 mRNA in interface tissue, we performed *in situ* hybridization. The signal was especially strong in the lining-like layers and sublining areas in interface tissue. Occasionally mRNA transcripts appeared in the deep stroma of the interface tissue. ADAM8 mRNA was also found in the lining and sublining areas in control OA synovial membrane samples but the intensity of the signal was substantially lower than that observed in the interface tissue samples. The control sense RNA probe used for ADAM8 did not show any labelling.

7.4.2 ADAM8 protein is expressed in mononuclear cells

Intense ADAM8 immunoreactivity was detected in the synovial lining-like layers and sublining areas in interface tissue. The ADAM8 staining intensity varied in different regions in the stroma of the interface tissue samples. The immunoreactivity was always strong in the cell-rich areas with macrophage-like cell infiltration. In contrast, it was hardly detectable in the fibrotic areas. Small capillaries showed strong immunoreactivity, while large blood vessels often exhibited weak staining. ADAM8/TRAP double staining disclosed the close and sometimes overlapping ADAM8 immunoreactivity with TRAP positive cells. A close spatial relationship was also found between ADAM8 positive mononuclear cells, and between ADAM8 positive mono- and TRAP positive multinuclear cells. Synovial lining cell layer of the control osteoarthritic synovial membrane samples contained ADAM8 although the staining was usually very weak. The percentage area of ADAM8 staining was significantly higher ($p < 0.001$, t-test) in the interface tissue (56.4 ± 2.1) than in osteortritic synovial membrane (12.7 ± 1.3).

ADAM8 clearly has a role in cell adhesion. It has been shown to induce osteoclast fusion but the

mechanism of this effect is unclear. Based on the data of other ADAM family members it is likely that the effect of binding may be mediated via activation of the precursor cells to facilitate fusion, rather than directly mediating the cell-to-cell contact (Schlondorff and Blobel 1999). However, it has been shown that a homophilic interaction of ADAM8 molecules, which are lacking the metalloprotease domains, is sufficient for cell adhesion (Schlomann et al. 2002). We detected the proteolytically cleaved 65 kDa ADAM8 molecule in tissue extracts. This is the size that corresponds to ADAM8 that lacks its metalloprotease domain. Our antibody recognizes the cysteine/disintegrin domain of ADAM8. This indicates that ADAM8 in interface tissue is in such form that facilitates homophilic interaction. Further, the released catalytically active metalloprotease domain can perhaps contribute to extracellular matrix destruction. It is concluded that ADAM8 is induced in interface tissue and its expression may contribute to active cell fusion and extracellular matrix degradation.

8. CONCLUDING REMARKS

Host reaction against wear debris induces periprosthetic bone loss around THR prosthesis and contributes to loosening of the implant. It has been speculated that interface tissue represents aggressive tissue, which would be compatible with its histology and osteolytic capacity. Development of an acidic and cathepsin K-rich interface tissue and the formation of osteoclast-like multinuclear foreign body giant cells, which are often associated with bone loss, may represent the key steps in this adverse host reaction. The events that are normally well controlled in osteoclast formation and function in basic multicellular units seem to occur uncontrolled in interface tissue. This was shown in the present study:

- Interface tissue is acidic and produces cathepsin K, which probably cause demineralization of bone followed by degradation of the organic matrix.

- The osteoclastogenic molecules RANK and RANKL are present in interface tissue and may interact without osteoprotegerin interference.

- Cultured interface tissue fibroblasts produce RANKL and osteoprotegerin almost as well as osteoblasts and are probably in this respect capable to induce and promote osteoclastogenesis.

- Pseudosynovial fluid is capable of inducing bone resorbing osteoclast from monocytes.

- ADAM8 is produced in interface tissue indicating that the local cells may fuse and acquire osteoclastic phenotype in interface tissue.

A schematic representation of the elements that contribute to the loosening of THR prosthesis is illustrated in figure 9.

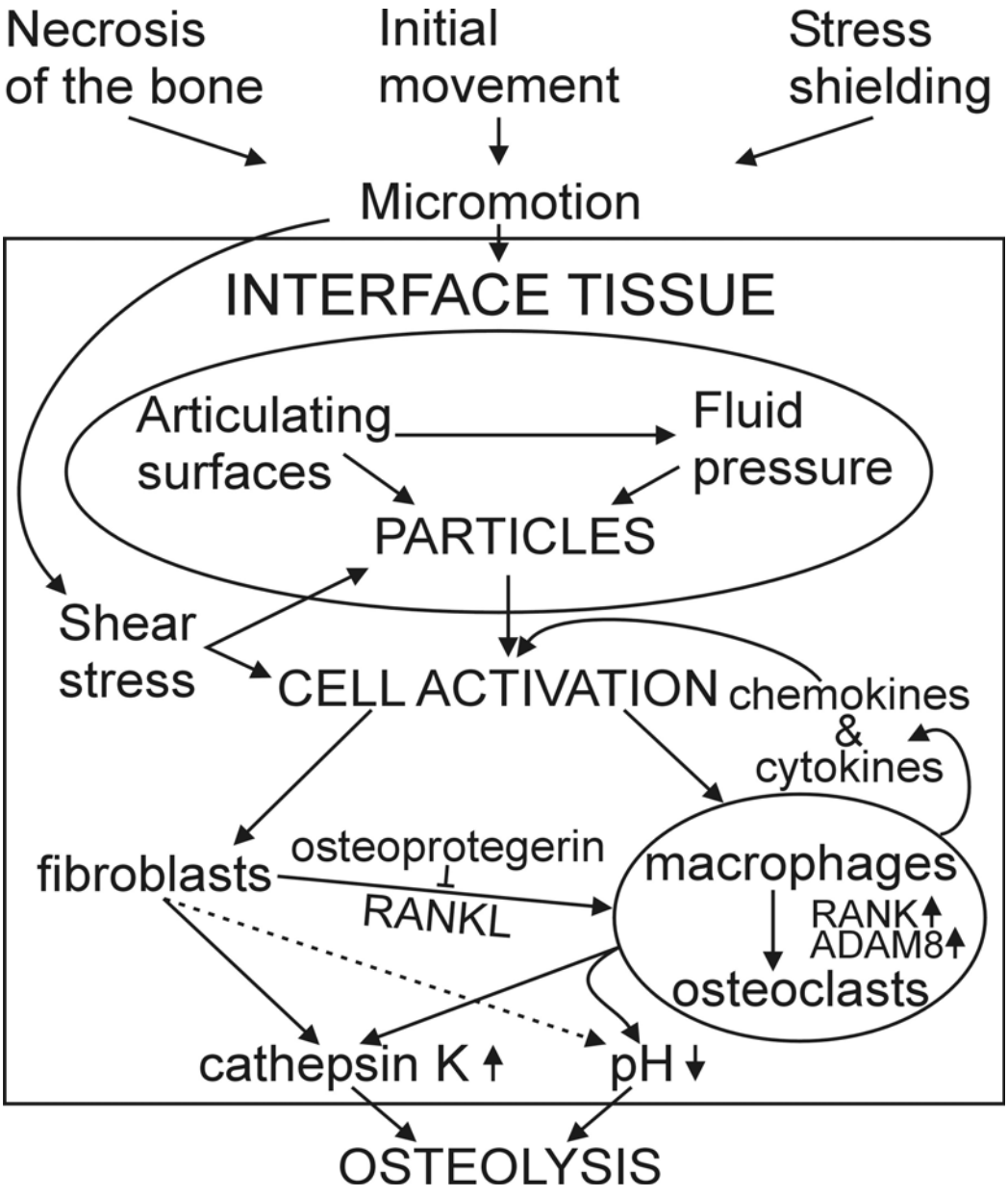

Figure 9. A schematic representation of loosening of THR prosthesis.

The prevention of interface tissue ingrowth between the prosthesis and host bone could be best solution to prevent bone loss seen around loosening THR prosthesis. If this is impossible, attempts should be made to reduce the number of particles should in interface tissue. Amorphous diamond coating of the articulating surfaces may be one solution to this. The best solution to increase the lifetime of the prostheses, which are already installed, might be neutralization of pH and inhibition of cathepsin K and RANKL either alone or simultaneously.

9. ACKNOWLEDGEMENTS

This study was carried out at the Institute of Biomedicine / Anatomy in University of Helsinki, at ORTON Orthopaedic Hospital of the Invalid Foundation, Helsinki, Department of Medicine/Invärtes medicin and Orthopaedics and Traumatology, Helsinki University Central Hospital, Helsinki, Finland during years 1999-2004. I thank professor Ismo Virtanen, head of Anatomy, for the excellent research facilities and for helping me during the thesis preparation. I also thank Seppo Seitsalo and research director Dietrich Schlenzka at ORTON Hospital, Eero Hirvensalo, head of Department of Orthopaedics and Traumatology and Carola Grönhagen-Riska, head of Department of Medicine for the positive attitude to research and for the opportunity to partly carry out this work at their institutes.

My deepest gratitude goes to my supervisor, professor Yrjö T. Konttinen for encouraging, guiding and trusting me. Your enthusiasm to work, endlessly caring of your personnel and enormous energy have made work in the lab extremely enjoyable and rewarding. It has always been extremely easy to share problems with you. Your tremendous knowledge and efficiency have been of great value during this work. I am also deeply indebted to my other supervisor professor Seppo Santavirta. Your kindness, intelligence and knowledge on the orthopaedics and implants have been important for this work. The way how you can explain difficult and complex events in the loosening process so that even I can understand them has always amazed me. I also want to thank both of you for caring about my future and supporting me in my future plans.

I also thank professor Eero Vuorio, member of my supervisory board. I appreciate you for giving me the possibility to learn lab skills in your laboratory and all the support you have given to me. I also admire the way you run your lab. The atmosphere in the lab is open and supportive. I believe that it reflects your kindness to other people.

I owe my warmest thanks to docent Jarl-Erik “Iblo” Michelsson who started to guide a young man and taught me the mysteries of research. Science was a hobby for you but your love to research, extreme care of the projects, excellent bookkeeping, well organized and systematic research still makes me very impressed.

Pre examiners of my thesis, professor Esko Alhava and docent Teuvo Hentunen are also greatly valued. I appreciate you for giving your time for reading my work and the valuable comments to improve it.

I also thank my official opponent docent Juha Tuukkanen. I appreciate that a busy person like you gave me the opportunity for a good conversation.

I want to thank people in the Surgical hospital lab, especially Saara and Kaija, for teaching me RNA isolation and for letting a young student to learn and work in the lab.

TULES-group members and Johanna, I do not know how to thank you. The atmosphere you created, the support when I had difficult times in my life and the knowledge you have have enormously helped my life and working. I want to thank Dr. Li who left the group couple years ago for friendship, most wonderful food, enjoyable conversations and valuable help in science. I have always admired your hard and precise working. I am also deeply grateful for Mika, Mari, Mikko and Johanna for your valuable help and impressed your enormous knowledge and skills. I also want to thank Erkki for extremely skillful technical help and Eija for being such a supportive person. I also appreciate Tong and Tarvo for their valuable comments and criticism. I want to thank Ahmed, Arzu, Arnoldas, Uula, Hannele, Mikael, Lauri, Nalle, Jian Ma, Guofeng Ma, Oliver, Sirkku, Laura, and Eve-Kai for enjoyable conversations. Eero and Ville, it has always been a pleasure to work with you. Although you are nowadays seldom present at work, it is always nice to see you.

I have had a great pleasure to work with several collaborators. I thank Juha Paloneva for enjoyable moments in the lab and for trusting me your valuable samples. I thank Jari Salo and Jan Lassus for helping us to collect samples from Töölö hospital. I also thank Jan (twice) for functional knee. I thank Heikki Valleala for good cooperation and for trusting me your samples.

I want to thank the stuff in the Anatomy, especially Outi and Aili. Your help and kindness to me have made my life easier. Pipsa and Marja-Leena for taking care of the cell culture room and for preparing all the reagents for everybody to use. Paula and Hanna for cutting my samples. I also thank all others who are working at Anatomy for enjoyable conversations and help.

I am grateful to all my friends for friendship, for moments worth memorizing and for keeping me in sense. Especially to Janne who provided home for me when I most needed it. Petteri who actually started my scientific career by taking care of the paper work to enter the university and by introducing me to Iblo. Mikko who has been always helping and supporting me when needed. My referee friends Toni, Marko and Emppu have always given me the right criticism.

I cannot thank enough my parents Pentti and Saija. You have made me what I am. The attitude to life, to work, and to family comes from you. I also want to thank my brothers Riku and Samu for enjoyable moments and for all the help and support what you have given to me.

Finally, I want to express my warmest gratitude to my wife Marja sharing life with me. You have given me the most valuable gifts, our children. I cannot express how grateful I am for everything that you are. Your support and understanding have been endless.

This study was financially supported by the Helsinki University Research fund, Eemil Aaltonen foundation, Biomedicum Helsinki foundation, Magnus Ehrnrooth foundation, the Finnish Society for Rheumatolgy, Academy of Finland and TEKES. Two year salary from TULES-graduate school is greatly appreciated.

Helsinki 14.1.2005

Jami

10. REFERENCES

Abe E, Mocharla H, Yamate T, Taguchi Y, Manolagas SC: Meltrin-alpha, a fusion protein involved in multinucleated giant cell and osteoclast formation. Calcif Tissue Int 1999; 64: 508-515.

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (eds): Molecular Biology of the Cell. 3rd Ed.,1994.

Almeida EA, Huovila AP, Sutherland AE, Stephens LE, Calarco PG, Shaw LM, Mercurio AM, Sonnenberg A, Primakoff P, Myles DG: Mouse egg integrin alpha 6 beta 1 functions as a sperm receptor. Cell 1995; 81: 1095-1104.

Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L: A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997; 390: 175-179.

Antti-Poika I, Josefsson G, Konttinen Y, Lidgren L, Santavirta S, Sanzen L: Hip arthroplasty infection. Current concepts. Acta Orthop Scand 1990; 61: 163-169.

Armstrong AP, Tometsko ME, Glaccum M, Sutherland CL, Cosman D, Dougall WC: A RANK/TRAF6-dependent signal transduction pathway is essential for osteoclast cytoskeletal organization and resorptive function. J Biol Chem 2002; 277: 44347-44356.

Arnett TR, Dempster DW: Effect of pH on bone resorption by rat osteoclasts in vitro. Endocrinology 1986; 119: 119-124.

Ashammakhi N, Makela EA, Vihtonen K, Rokkanen P, Tormala P: Repair of bone defects with absorbable membranes. A study on rabbits. Ann Chir Gynaecol 1995; 84: 309-315.

Aspenberg P, Goodman S, Toksvig-Larsen S, Ryd L, Albrektsson T: Intermittent micromotion inhibits bone ingrowth. Titanium implants in rabbits. Acta Orthop Scand 1992; 63: 141-145.

Aspenberg P, Herbertsson P: Periprosthetic bone resorption. Particles versus movement. J Bone Joint Surg [Br] 1996; 78: 641-646.

Aspenberg P, Van der Vis HM: Migration, particles, and fluid pressure. A discussion of causes of prosthetic loosening. Clin Orthop 1998; 75-80.

Athanasou NA, Quinn J, Ferguson DJ, McGee JO: Bone resorption by macrophage polykaryons of giant cell tumour of tendon sheath. Br J Cancer 1991; 63: 527-533.

Athanasou NA, Quinn JM: Bone resorption by macrophage polykaryons of a pilar tumor of scalp. Cancer 1992; 70: 469-475.

Atkins GJ, Haynes DR, Graves SE, Evdokiou A, Hay S, Bouralexis S, Findlay DM: Expression of osteoclast differentiation signals by stromal elements of giant cell tumors. J Bone Miner Res 2000; 15: 640-649.

Baron R: Molecular mechanisms of bone resorption. An update. Acta Orthop Scand Suppl 1995; 266:66-70.: 66-70.

Baron R, Neff L, Louvard D, Courtoy PJ: Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. J Cell Biol 1985; 101: 2210-2222.

Baron R, Neff L, Tran VP, Nefussi JR, Vignery A: Kinetic and cytochemical identification of osteoclast precursors and their differentiation into multinucleated osteoclasts. Am J Pathol 1986; 122: 363-378.

Barrett AJ, Rawlings ND, Woessner JF (eds): Handbook of Proteolytic Enzymes. Academic Press, London, UK, 1998.

Bilezikian JP, Raisz LG, Rodan GA (eds): Principles of Bone Biology. 2nd Ed., Academic Press, San Diego, CA, 2002.

Black J and Hastings G (eds): Handbook of Biomaterial Properties. 1st Ed., Chapman & Hall,1998.

Blair HC, Teitelbaum SL, Ghiselli R, Gluck S: Osteoclastic bone resorption by a polarized vacuolar proton pump. Science 1989; 245: 855-857.

Blavier L, Delaisse JM: Matrix metalloproteinases are obligatory for the migration of preosteoclasts to the developing marrow cavity of primitive long bones. J Cell Sci 1995; 108: 3649-3659.

Blobel CP, Wolfsberg TG, Turck CW, Myles DG, Primakoff P, White JM: A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion. Nature 1992; 356: 248-252.

Boissy P, Destaing O, Jurdic P: RANKL induces formation of avian osteoclasts from macrophages but not from macrophage polykaryons. Biochem Biophys Res Commun 2001; 288: 340-346.

Bossard MJ, Tomaszek TA, Thompson SK, Amegadzie BY, Hanning CR, Jones C, Kurdyla JT, McNulty DE, Drake FH, Gowen M, Levy MA: Proteolytic activity of human osteoclast cathepsin K. Expression, purification, activation, and substrate identification. J Biol Chem 1996; 271: 12517-12524.

Bromme D, Okamoto K: Human cathepsin O2, a novel cysteine protease highly expressed in osteoclastomas and ovary molecular cloning, sequencing and tissue distribution. Biol Chem Hoppe Seyler 1995; 376: 379-384.

Bromme D, Okamoto K, Wang BB, Biroc S: Human cathepsin O2, a matrix protein-degrading cysteine protease expressed in osteoclasts. Functional expression of human cathepsin O2 in Spodoptera frugiperda and characterization of the enzyme. J Biol Chem 1996; 271: 2126-2132.

Burgess TL, Qian Y, Kaufman S, Ring BD, Van G, Capparelli C, Kelley M, Hsu H, Boyle WJ, Dunstan CR, Hu S, Lacey DL: The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. J Cell Biol 1999; 145: 527-538.

Cao Y, Kang Q, Zolkiewska A: Metalloprotease-disintegrin ADAM 12 interacts with alpha-actinin-1. Biochem J 2001; 357: 353-361.

Carter DR, Spengler DM: Mechanical properties and composition of cortical bone. Clin Orthop 1978; 192-217.

Chambers TJ: Phagocytic recognition of bone by macrophages. J Pathol 1981; 135: 1-7.

Chambers TJ: The regulation of osteoclastic development and function. Ciba Found Symp 1988; 136:92-107.: 92-107.

Chambers TJ, Thomson BM, Fuller K: Effect of substrate composition on bone resorption by rabbit osteoclasts. J Cell Sci 1984; 70:61-71.: 61-71.

Charnley J: Arthroplasty of the hip: A new operation. Lancet 1961; 1: 1129-1132.

Charnley J, Follacci FM, Hammond BT: The long-term reaction of bone to self-curing acrylic cement. J Bone Joint Surg Br 1968; 50: 822-829.

Chellaiah MA, Hruska KA: The integrin alpha(v)beta(3) and CD44 regulate the actions of osteopontin on osteoclast motility. Calcif Tissue Int 2003; 72: 197-205.

Chen BD, Chou TH, Clark CR: Delineation of receptor-mediated colony-stimulating factor (CSF-1) utilization and cell production by precursors of mononuclear phagocytic series at various stages of differentiation. Br J Haematol 1987; 67: 381-386.

Chiba J, Rubash HE, Kim KJ, Iwaki Y: The characterization of cytokines in the interface tissue obtained from failed cementless total hip arthroplasty with and without femoral osteolysis. Clin Orthop 1994; 304-312.

Chikazu D, Hakeda Y, Ogata N, Nemoto K, Itabashi A, Takato T, Kumegawa M, Nakamura K, Kawaguchi H: Fibroblast growth factor (FGF)-2 directly stimulates mature osteoclast function through activation of FGF receptor 1 and p42/p44 MAP kinase. J Biol Chem 2000; 275: 31444-31450.

Choi SJ, Han JH, Roodman GD: ADAM8: a novel osteoclast stimulating factor. J Bone Miner Res 2001; 16: 814-822.

Chun L, Yoon J, Song Y, Huie P, Regula D, Goodman S: The characterization of macrophages and osteoclasts in tissues harvested from revised total hip prostheses. J Biomed Mater Res 1999; 48: 899-903.

Clark SA, Ambrose WW, Anderson TR, Terrell RS, Toverud SU: Ultrastructural localization of tartrate-resistant, purple acid phosphatase in rat osteoclasts by histochemistry and immunocytochemistry. J Bone Miner Res 1989; 4: 399-405.

Clohisy JC, Frazier E, Hirayama T, Abu-Amer Y: RANKL is an essential cytokine mediator of polymethylmethacrylate particle-induced osteoclastogenesis. J Orthop Res 2003; 21: 202-212.

Corisdeo S, Gyda M, Zaidi M, Moonga BS, Troen BR: New insights into the regulation of cathepsin K gene expression by osteoprotegerin ligand. Biochem Biophys Res Commun 2001; 285: 335-339.

Crotti TN, Smith MD, Findlay DM, Zreiqat H, Ahern MJ, Weedon H, Hatzinikolous G, Capone M, Holding C, Haynes DR: Factors regulating osteoclast formation in human tissues adjacent to peri-implant bone loss: expression of receptor activator NFkappaB, RANK ligand and osteoprotegerin. Biomaterials 2004; 25: 565-573.

Darnay BG, Haridas V, Ni J, Moore PA, Aggarwal BB: Characterization of the intracellular domain of receptor activator of NF-kappaB (RANK). Interaction with tumor necrosis factor receptor- associated factors and activation of NF-kappab and c-Jun N-terminal kinase. J Biol Chem 1998; 273: 20551-20555.

Darnay BG, Ni J, Moore PA, Aggarwal BB: Activation of NF-kappaB by RANK requires tumor necrosis factor receptor-associated factor (TRAF) 6 and NF-kappaB-inducing kinase. Identification of a novel TRAF6 interaction motif. J Biol Chem 1999; 274: 7724-7731.

Dodds RA, James IE, Rieman D, Ahern R, Hwang SM, Connor JR, Thompson SD, Veber DF, Drake FH, Holmes S, Lark MW, Gowen M: Human osteoclast cathepsin K is processed intracellularly prior to attachment and bone resorption. J Bone Miner Res 2001; 16: 478-486.

Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J: RANK is essential for osteoclast and lymph node development. Genes Dev 1999; 13: 2412-2424.

Drake FH, Dodds RA, James IE, Connor JR, Debouck C, Richardson S, Lee-Rykaczewski E, Coleman L, Rieman D, Barthlow R, Hastings G, Gowen: Cathepsin K, but not cathepsins B, L, or S, is abundantly expressed in human osteoclasts. J Biol Chem 1996; 271: 12511-12516.

Dunn MG, Maxian SH: Biomaterials used in orthopaedic surgery. In: Greco RS (ed) Implantation biology: the host response and biomedical devices, CRC Press, Boca Raton, 1994, pp 229-252.

Elford PR, Felix R, Cecchini M, Trechsel U, Fleisch H: Murine osteoblastlike cells and the osteogenic cell MC3T3-E1 release a macrophage colony-stimulating activity in culture. Calcif Tissue Int 1987; 41: 151-156.

Engh CA, Bobyn JD, Glassman AH: Porous-coated hip replacement. The factors governing bone ingrowth, stress shielding, and clinical results. J Bone Joint Surg Br 1987; 69: 45-55.

Engh CA, O'Connor D, Jasty M, McGovern TF, Bobyn JD, Harris WH: Quantification of implant micromotion, strain shielding, and bone resorption with porous-coated anatomic medullary locking femoral prostheses. Clin Orthop 1992; 13-29.

Engsig MT, Chen QJ, Vu TH, Pedersen AC, Therkidsen B, Lund LR, Henriksen K, Lenhard T, Foged NT, Werb Z, Delaisse JM: Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. J Cell Biol 2000; 151: 879-889.

Evans BG, Salvati EA, Huo MH, Huk OL: The rationale for cemented total hip arthroplasty. Orthop Clin North Am 1993; 24: 599-610.

Faccio R, Grano M, Colucci S, Villa A, Giannelli G, Quaranta V, Zallone A: Localization and possible role of two different alpha v beta 3 integrin conformations in resting and resorbing osteoclasts. J Cell Sci 2002; 115: 2919-2929.

Faccio R, Novack DV, Zallone A, Ross FP, Teitelbaum SL: Dynamic changes in the osteoclast cytoskeleton in response to growth factors and cell attachment are controlled by beta3 integrin. J Cell Biol 2003a; 162: 499-509.

Faccio R, Takeshita S, Zallone A, Ross FP, Teitelbaum SL: c-Fms and the alphavbeta3 integrin collaborate during osteoclast differentiation. J Clin Invest 2003b; 111: 749-758.

Felix R, Cecchini MG, Fleisch H: Macrophage colony stimulating factor restores in vivo bone resorption in the op/op osteopetrotic mouse. Endocrinology 1990; 127: 2592-2594.

Flanagan AM, Nui B, Tinkler SM, Horton MA, Williams DM, Chambers TJ: The multinucleate cells in giant cell granulomas of the jaw are osteoclasts. Cancer 1988; 62: 1139-1145.

Frick KK, Bushinsky DA: Chronic metabolic acidosis reversibly inhibits extracellular matrix gene expression in mouse osteoblasts. Am J Physiol 1998; 275: F840-F847.

Frick KK, Bushinsky DA: Metabolic acidosis stimulates RANKL RNA expression in bone through a cyclo-oxygenase-dependent mechanism. J Bone Miner Res 2003; 18: 1317-1325.

Fuller K, Murphy C, Kirstein B, Fox SW, Chambers TJ: TNFalpha potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. Endocrinology 2002; 143: 1108-1118.

Fuller K, Wong B, Fox S, Choi Y, Chambers TJ: TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med 1998; 188: 997-1001.

Furuyama N, Fujisawa Y: Regulation of collagenolytic protease secretion through c-Src in osteoclasts. Biochem Biophys Res Commun 2000; 272: 116-124.

Galibert L, Tometsko ME, Anderson DM, Cosman D, Dougall WC: The involvement of multiple tumor necrosis factor receptor (TNFR)-associated factors in the signaling mechanisms of receptor activator of NF-kappaB, a member of the TNFR superfamily. J Biol Chem 1998; 273: 34120-34127.

Garnero P, Borel O, Byrjalsen I, Ferreras M, Drake FH, McQueney MS, Foged NT, Delmas PD, Delaiss: The collagenolytic activity of cathepsin K is unique among mammalian proteinases. J Biol Chem 1998; 273: 32347-32352.

Gelb BD, Shi GP, Chapman HA, Desnick RJ: Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. Science 1996; 273: 1236-1238.

Glowacki J, Jasty M, Goldring S: Comparison of multinucleated cells elicited in rats by particulate bone, polyethylene, or polymethylmethacrylate. J Bone Miner Res 1986; 1: 327-331.

Goldring SR, Jasty M, Roelke MS, Rourke CM, Bringhurst FR, Harris WH: Formation of a synovial-like membrane at the bone-cement interface. Its role in bone resorption and implant loosening after total hip replacement. Arthritis Rheum 1986; 29: 836-842.

Goldring SR, Schiller AL, Roelke M, Rourke CM, O'Neil DA, Harris WH: The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg Am 1983; 65: 575-584.

Goodman SB, Fornasier VL, Lee J, Kei J: The histological effects of the implantation of different sizes of polyethylene particles in the rabbit tibia. J Biomed Mater Res 1990; 24: 517-524.

Goodman SB, Huie P, Song Y, Schurman D, Maloney W, Woolson S, Sibley R: Cellular profile and cytokine production at prosthetic interfaces. Study of tissues retrieved from revised hip and knee replacements. J Bone Joint Surg Br 1998a; 80: 531-539.

Goodman SB, Lind M, Song Y, Smith RL: In vitro, in vivo, and tissue retrieval studies on particulate debris. Clin Orthop 1998b; 25-34.

Gori F, Hofbauer LC, Dunstan CR, Spelsberg TC, Khosla S, Riggs BL: The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. Endocrinology 2000; 141: 4768-4776.

Goto T, Maeda H, Tanaka T: A selective inhibitor of matrix metalloproteinases inhibits the migration of isolated osteoclasts by increasing the life span of podosomes. J Bone Miner Metab 2002; 20: 98-105.

Gowen M, Lazner F, Dodds R, Kapadia R, Feild J, Tavaria M, Bertoncello I, Drake F, Zavarselk S, Tellis I, Hertzog P, Debouck C, Kola I: Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. J Bone Miner Res 1999; 14: 1654-1663.

Gravallese EM, Manning C, Tsay A, Naito A, Pan C, Amento E, Goldring SR: Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum 2000 2000; 43: 250-258.

Grey A, Chen Y, Paliwal I, Carlberg K, Insogna K: Evidence for a functional association between phosphatidylinositol 3-kinase and c-src in the spreading response of osteoclasts to colony-stimulating factor-1. Endocrinology 2000; 141: 2129-2138.

Grigoriadis AE, Wang ZQ, Cecchini MG, Hofstetter W, Felix R, Fleisch HA, Wagner EF: c-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. Science 1994; 266: 443-448.

Guha M, Mackman N: LPS induction of gene expression in human monocytes. Cell Signal 2001; 13: 85-94.

Halleen JM, Raisanen S, Salo JJ, Reddy SV, Roodman GD, Hentunen TA, Lehenkari PP, Kaija H, Vihko P, Vaananen HK: Intracellular fragmentation of bone resorption products by reactive oxygen species generated by osteoclastic tartrate-resistant acid phosphatase. J Biol Chem 1999; 274: 22907-22910.

Han X, Sterling H, Chen Y, Saginario C, Brown EJ, Frazier WA, Lindberg FP, Vignery A: CD47, a ligand for the macrophage fusion receptor, participates in macrophage multinucleation. J Biol Chem 2000; 275: 37984-37992.

Hanazawa S, Takeshita A, Tsukamoto Y, Kawata Y, Takara KO, Kitano S: Transforming growth factor-beta-induced gene expression of monocyte chemoattractant JE in mouse osteoblastic cells, MC3T3-E1. Biochem Biophys Res Commun 1991; 180: 1130-1136.

Hansen T, Petrow PK, Gaumann A, Keyszer GM, Eysel P, Eckardt A, Brauer R, Kriegsmann J: Cathepsin B and its endogenous inhibitor cystatin C in rheumatoid arthritis synovium. J Rheumatol 2000; 27: 859-865.

Hansen T, Petrow PK, Gaumann A, Keyszer GM, Otto M, Kirkpatrick CJ, Kriegsmann J: Expression of cysteine proteinases cathepsins B and K and of cysteine proteinase inhibitor cystatin C in giant cell tumor of tendon sheath. Mod Pathol 2001; 14: 318-324.

Hayman AR, Jones SJ, Boyde A, Foster D, Colledge WH, Carlton MB, Evans MJ, Cox TM: Mice lacking tartrate-resistant acid phosphatase (Acp 5) have disrupted endochondral ossification and mild osteopetrosis. Development 1996; 122: 3151-3162.

Haynes DR, Barg E, Crotti TN, Holding C, Weedon H, Atkins GJ, Zannetino A, Ahern MJ, Coleman M, Roberts-Thomson PJ, Kraan M, Tak PP, Smith MD: Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology (Oxford) 2003; 42: 123-134.

Haynes DR, Crotti TN, Potter AE, Loric M, Atkins GJ, Howie DW, Findlay DM: The osteoclastogenic molecules RANKL and RANK are associated with periprosthetic osteolysis. J Bone Joint Surg [Br] 2001; 83: 902-911.

Haynes DR, Hay SJ, Rogers SD, Ohta S, Howie DW, Graves SE: Regulation of bone cells by particle-activated mononuclear phagocytes. J Bone Joint Surg [Br] 1997; 79: 988-994.

Heid CA, Stevens J, Livak KJ, Williams PM: Real time quantitative PCR. Genome Res 1996; 6: 986-994.

Hodge WA, Carlson KL, Fijan RS, Burgess RG, Riley PO, Harris WH, Mann RW: Contact pressures from an instrumented hip endoprosthesis. J Bone Joint Surg [Am] 1989; 71: 1378-1386.

Hofbauer LC, Gori F, Riggs BL, Lacey DL, Dunstan CR, Spelsberg TC, Khosla S: Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis. Endocrinology 1999a; 140: 4382-4389.

Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL: Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology 1999b; 140: 4367-4370.

Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S: Interleukin-1beta and tumor necrosis factor-alpha, but not interleukin- 6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Bone 1999c; 25: 255-259.

Horowitz SM, Doty SB, Lane JM, Burstein AH: Studies of the mechanism by which the mechanical failure of polymethylmethacrylate leads to bone resorption. J Bone Joint Surg [Am] 1993; 75: 802-813.

Horowitz SM, Luchetti WT, Gonzales JB, Ritchie CK: The effects of cobalt chromium upon macrophages. J Biomed Mater Res 1998; 41: 468-473.

Horton JE, Raisz LG, Simmons HA, Oppenheim JJ, Mergenhagen SE: Bone resorbing activity in supernatant fluid from cultured human peripheral blood leukocytes. Science 1972; 177: 793-795.

Horton MA, Rimmer EF, Chambers TJ: Giant cell formation in rabbit long-term bone marrow cultures: immunological and functional studies. J Bone Miner Res 1986; 1: 5-14.

Horton MA, Rimmer EF, Lewis D, Pringle JA, Fuller K, Chambers TJ: Cell surface characterization of the human osteoclast: phenotypic relationship to other bone marrow-derived cell types. J Pathol 1984; 144: 281-294.

Hotokezaka H, Sakai E, Kanaoka K, Saito K, Matsuo K, Kitaura H, Yoshida N, Nakayama K: U0126 and PD98059, specific inhibitors of MEK, accelerate differentiation of RAW264.7 cells into osteoclast-like cells. J Biol Chem 2002; 277: 47366-47372.

Hou WS, Li W, Keyszer G, Weber E, Levy R, Klein MJ, Gravallese EM, Goldring SR, Bromme D: Comparison of cathepsins K and S expression within the rheumatoid and osteoarthritic synovium. Arthritis Rheum 2002; 46: 663-674.

Howie DW, Vernon-Roberts B, Oakeshott R, Manthey B: A rat model of resorption of bone at the cement-bone interface in the presence of polyethylene wear particles. J Bone Joint Surg [Am] 1988; 70: 257-263.

Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan HL, Elliott G, Kelley MJ, Sarosi I, Wang L, Xia XZ, Elliott R, Chiu L, Black T, Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ: Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci U S A 1999; 96: 3540-3545.

Hummel KM, Petrow PK, Franz JK, Muller-Ladner U, Aicher WK, Gay RE, Bromme D, Gay S: Cysteine proteinase cathepsin K mRNA is expressed in synovium of patients with rheumatoid arthritis and is detected at sites of synovial bone destruction. J Rheumatol 1998; 25: 1887-1894.

Hungerford DS, Jones LC: The rationale for cementless total hip replacement. Orthop Clin North Am 1993; 24: 617-626.

Iba K, Albrechtsen R, Gilpin B, Frohlich C, Loechel F, Zolkiewska A, Ishiguro K, Kojima T, Liu W, Langford JK, Sanderson RD, Brakebusch C, Fassler R, Wewer UM: The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to beta1 integrin-dependent cell spreading. J Cell Biol 2000; 149: 1143-1156.

Inaoka T, Bilbe G, Ishibashi O, Tezuka K, Kumegawa M, Kokubo T: Molecular cloning of human cDNA for cathepsin K: novel cysteine proteinase predominantly expressed in bone. Biochem Biophys Res Commun 1995; 206: 89-96.

Inomoto M, Miyakawa S, Mishima H, Ochiai N: Elevated interleukin-12 in pseudosynovial fluid in patients with aseptic loosening of hip prosthesis. J Orthop Sci 2000; 5: 369-373.

Ishiguro N, Kojima T, Ito T, Saga S, Anma H, Kurokouchi K, Iwahori Y, Iwase T, Iwata H: Macrophage activation and migration in interface tissue around loosening total hip arthroplasty components. J Biomed Mater Res 1997; 35: 399-406.

Jahn R, Lang T, Sudhof TC: Membrane fusion. Cell 2003; 112: 519-533.

Jalkanen S, Jalkanen M: Lymphocyte CD44 binds the COOH-terminal heparin-binding domain of fibronectin. J Cell Biol 1992; 116: 817-825.

Jasty M, Bragdon C, Burke D, O'Connor D, Lowenstein J, Harris WH: In vivo skeletal responses to porous-surfaced implants subjected to small induced motions. J Bone Joint Surg [Am] 1997; 79: 707-714.

Jasty MJ, Floyd WE, III, Schiller AL, Goldring SR, Harris WH: Localized osteolysis in stable, non-septic total hip

replacement. J Bone Joint Surg [Am] 1986; 68: 912-919.

Jiang P, Lagenaur CF, Narayanan V: Integrin-associated protein is a ligand for the P84 neural adhesion molecule. J Biol Chem 1999; 274: 559-562.

Jimi E, Akiyama S, Tsurukai T, Okahashi N, Kobayashi K, Udagawa N, Nishihara T, Takahashi N, Suda T: Osteoclast differentiation factor acts as a multifunctional regulator in murine osteoclast differentiation and function. J Immunol 1999; 163: 434-442.

Jimi E, Nakamura I, Amano H, Taguchi Y, Tsurukai T, Tamura M, Takahashi N, Suda T: Osteoclast function is activated by osteoblastic cells through a mechanism involving cell-to-cell contact. Endocrinology 1996; 137: 2187-2190.

Jiranek WA, Machado M, Jasty M, Jevsevar D, Wolfe HJ, Goldring SR, Goldberg MJ, Harris WH: Production of cytokines around loosened cemented acetabular components. Analysis with immunohistochemical techniques and in situ hybridization. J Bone Joint Surg [Am] 1993; 75: 863-879.

Jones LC, Frondoza C, Hungerford DS: Immunohistochemical evaluation of interface membranes from failed cemented and uncemented acetabular components. J Biomed Mater Res 1999; 48: 889-898.

Kadoya Y, al Saffar N, Kobayashi A, Revell PA: The expression of osteoclast markers on foreign body giant cells. Bone Miner 1994; 27: 85-96.

Kaji K, Katogi R, Azuma Y, Naito A, Inoue JI, Kudo A: Tumor necrosis factor alpha-induced osteoclastogenesis requires tumor necrosis factor receptor-associated factor 6. J Bone Miner Res 2001; 16: 1593-1599.

Kamiya T, Kobayashi Y, Kanaoka K, Nakashima T, Kato Y, Mizuno A, Sakai H: Fluorescence microscopic demonstration of cathepsin K activity as the major lysosomal cysteine proteinase in osteoclasts. J Biochem (Tokyo) 1998; 123: 752-759.

Kamolmatyakul S, Chen W, Li YP: Interferon-gamma down-regulates gene expression of cathepsin K in osteoclasts and inhibits osteoclast formation. J Dent Res 2001; 80: 351-355.

Kaneko H, Arakawa T, Mano H, Kaneda T, Ogasawara A, Nakagawa M, Toyama Y, Yabe Y, Kumegawa M, Hakeda Y: Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. Bone 2000; 27: 479-486.

Kania JR, Kehat-Stadler T, Kupfer SR: CD44 antibodies inhibit osteoclast formation. J Bone Miner Res 1997; 12: 1155-1164.

Kanzaki H, Chiba M, Shimizu Y, Mitani H: Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor kappaB ligand up-regulation via prostaglandin E2 synthesis. J Bone Miner Res 2002; 17: 210-220.

Karrholm J, Borssen B, Lowenhielm G, Snorrason F: Does early micromotion of femoral stem prostheses matter? 4-7-year stereoradiographic follow-up of 84 cemented prostheses. J Bone Joint Surg [Br] 1994; 76: 912-917.

Karsdal MA, Hjorth P, Henriksen K, Kirkegaard T, Nielsen KL, Lou H, Delaisse JM, Foged NT: Transforming Growth Factor-{beta} Controls Human Osteoclastogenesis through the p38 MAPK and Regulation of RANK Expression. J Biol Chem 2003; 278: 44975-44987.

Kataoka M, Torisu T, Tsumura H, Hirayama T, Fujikawa Y: Role of multinuclear cells in granulation tissue in osteomyelitis: immunohistochemistry in 66 patients. Acta Orthop Scand 2000; 71: 414-418.

Katunuma N, Matsui A, Inubushi T, Murata E, Kakegawa H, Ohba Y, Turk D, Turk V, Tada Y, Asao T: Structure-based development of pyridoxal propionate derivatives as specific inhibitors of cathepsin K in vitro and in vivo. Biochem Biophys Res Commun 2000; 267: 850-854.

Kawaguchi N, Sundberg C, Kveiborg M, Moghadaszadeh B, Asmar M, Dietrich N, Thodeti CK, Nielsen FC, Moller P, Mercurio AM, Albrechtsen R, Wewer UM: ADAM12 induces actin cytoskeleton and extracellular matrix reorganization during early adipocyte differentiation by regulating beta1 integrin function. J Cell Sci 2003; 116: 3893-3904.

Kim KJ, Hijikata H, Itoh T, Kumegawa M: Joint fluid from patients with failed total hip arthroplasty stimulates pit formation by mouse osteoclasts on dentin slices. J Biomed Mater Res 1998a; 43: 234-240.

Kim KJ, Kobayashi Y, Itoh T: Osteolysis model with continuous infusion of polyethylene particles. Clin Orthop 1998b; 46-52.

Kim KJ, Kotake S, Udagawa N, Ida H, Ishii M, Takei I, Kubo T, Takagi M: Osteoprotegerin inhibits in vitro mouse osteoclast formation induced by joint fluid from failed total hip arthroplasty. J Biomed Mater Res 2001; 58: 393-400.

Kitazawa S, Kitazawa R: Epigenetic control of mouse receptor activator of NF-kappa B ligand gene expression. Biochem Biophys Res Commun 2002; 293: 126-131.

Kiviranta R, Morko J, Uusitalo H, Aro HT, Vuorio E, Rantakokko J: Accelerated turnover of metaphyseal trabecular bone in mice overexpressing cathepsin K. J Bone Miner Res 2001; 16: 1444-1452.

Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Morinaga T, Higashio K, Martin TJ, Suda T: Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000; 191: 275-286.

Kobayashi N, Kadono Y, Naito A, Matsumoto K, Yamamoto T, Tanaka S, Inoue J: Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. EMBO J 2001; 20: 1271-1280.

Kodama H, Yamasaki A, Nose M, Niida S, Ohgame Y, Abe M, Kumegawa M, Suda T: Congenital osteoclast deficiency in osteopetrotic (op/op) mice is cured by injections of macrophage colony-stimulating factor. J Exp Med 1991; 173: 269-272.

Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira dSA, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM: OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999; 397: 315-323.

Konttinen YT, Li TF, Mandelin J, Ainola M, Lassus J, Virtanen I, Santavirta S, Tammi M, Tammi R: Hyaluronan synthases, hyaluronan, and its CD44 receptor in tissue around loosened total hip prostheses. J Pathol 2001; 194: 384-390.

Kornak U, Kasper D, Bosl MR, Kaiser E, Schweizer M, Schulz A, Friedrich W, Delling G, Jentsch TJ: Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell 2001; 104: 205-215.

Kukita A, Kukita T, Shin JH, Kohashi O: Induction of mononuclear precursor cells with osteoclastic phenotypes in a rat bone marrow culture system depleted of stromal cells. Biochem Biophys Res Commun 1993; 196: 1383-1389.

Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93: 165-176.

Lakkakorpi PT, Horton MA, Helfrich MH, Karhukorpi EK, Vaananen HK: Vitronectin receptor has a role in bone resorption but does not mediate tight sealing zone attachment of osteoclasts to the bone surface. J Cell Biol 1991; 115: 1179-1186.

Lakkakorpi PT, Vaananen HK: Kinetics of the osteoclast cytoskeleton during the resorption cycle in vitro. J Bone Miner Res 1991; 6: 817-826.

Lam J, Nelson CA, Ross FP, Teitelbaum SL, Fremont DH: Crystal structure of the TRANCE/RANKL cytokine reveals determinants of receptor-ligand specificity. J Clin Invest 2001; 108: 971-979.

Lassus J, Waris V, Xu JW, Li TF, Hao J, Nietosvaara Y, Santavirta S, Konttinen YT: Increased interleukin-8 (IL-8) expression is related to aseptic loosening of total hip replacement. Arch Orthop Trauma Surg 2000; 120: 328-332.

Lee ZH, Kwack K, Kim KK, Lee SH, Kim HH: Activation of c-Jun N-terminal kinase and activator protein 1 by receptor activator of nuclear factor kappaB. Mol Pharmacol 2000; 58: 1536-1545.

Lehenkari P, Hentunen TA, Laitala-Leinonen T, Tuukkanen J, Vaananen HK: Carbonic anhydrase II plays a major role in osteoclast differentiation and bone resorption by effecting the steady state intracellular pH and Ca2+. Exp Cell Res 1998; 242: 128-137.

Lehto VP, Hovi T, Vartio T, Badley RA, Virtanen I: Reorganization of cytoskeletal and contractile elements during transition of human monocytes into adherent macrophages. Lab Invest 1982; 47: 391-399.

Lemann J, Jr., Litzow JR, Lennon EJ: The effects of chronic acid loads in normal man: further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. J Clin Invest 1966; 45: 1608-1614.

Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Scully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik P, Hsu H, Fletcher F, Dunstan CR, Lacey DL, Boyle WJ: RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci U S A 2000; 97: 1566-1571.

Li X, Udagawa N, Itoh K, Suda K, Murase Y, Nishihara T, Suda T, Takahashi N: p38 MAPK-mediated signals are required for inducing osteoclast differentiation but not for osteoclast function. Endocrinology 2002; 143: 3105-3113.

Li YP, Alexander M, Wucherpfennig AL, Yelick P, Chen W, Stashenko P: Cloning and complete coding sequence of a novel human cathepsin expressed in giant cells of osteoclastomas. J Bone Miner Res 1995; 10: 1197-1202.

Li YP, Chen W, Liang Y, Li E, Stashenko P: Atp6i-deficient mice exhibit severe osteopetrosis due to loss of osteoclast-mediated extracellular acidification. Nat Genet 1999; 23: 447-451.

Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW: TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev 1999; 13: 1015-1024.

Lowe C, Yoneda T, Boyce BF, Chen H, Mundy GR, Soriano P: Osteopetrosis in Src-deficient mice is due to an autonomous defect of osteoclasts. Proc Natl Acad Sci U S A 1993; 90: 4485-4489.

Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, North R, Gerard C, Rollins BJ: Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. J Exp Med 1998; 187: 601-608.

Mackay CR: Chemokines: immunology's high impact factors. Nat Immunol 2001; 2: 95-101.

Mano H, Yuasa T, Kameda T, Miyazawa K, Nakamaru Y, Shiokawa M, Mori, Yamada T, Miyata K, Shindo H, Azuma H, Hakeda Y, Kumegawa M: Mammalian mature osteoclasts as estrogen target cells. Biochemical & Biophysical Research Communications 1996; 223: 637-642.

Mansky KC, Sankar U, Han J, Ostrowski MC: Microphthalmia transcription factor is a target of the p38 MAPK pathway in response to receptor activator of NF-kappa B ligand signaling. J Biol Chem 2002; 277: 11077-11083.

Marks SC, Jr., Chambers TJ: The giant cells recruited by subcutaneous implants of mineralized bone particles and slices in rabbits are not osteoclasts. J Bone Miner Res 1991; 6: 395-400.

Matsumoto M, Sudo T, Saito T, Osada H, Tsujimoto M: Involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-kappa B ligand (RANKL). J Biol Chem 2000; 275: 31155-31161.

Matsuo K, Owens JM, Tonko M, Elliott C, Chambers TJ, Wagner EF: Fosl1 is a transcriptional target of c-Fos during osteoclast differentiation. Nat Genet 2000; 24: 184-187.

McHugh KP, Hodivala-Dilke K, Zheng MH, Namba N, Lam J, Novack D, Feng X, Ross FP, Hynes RO, Teitelbaum SL: Mice lacking beta3 integrins are osteosclerotic because of dysfunctional osteoclasts. J Clin Invest 2000; 105: 433-440.

McQueney MS, Amegadzie BY, D'Alessio K, Hanning CR, McLaughlin MM, McNulty D, Carr SA, Ijames C, Kurdyla J, Jones CS: Autocatalytic activation of human cathepsin K. J Biol Chem 1997; 272: 13955-13960.

Meghji S, Morrison MS, Henderson B, Arnett TR: pH dependence of bone resorption: mouse calvarial osteoclasts are activated by acidosis. Am J Physiol Endocrinol Metab 2001; 280: E112-E119.

Merkel KD, Erdmann JM, McHugh KP, Abu-Amer Y, Ross FP, Teitelbaum SL: Tumor necrosis factor-alpha mediates orthopedic implant osteolysis. Am J Pathol 1999; 154: 203-210.

Miyake K, Underhill CB, Lesley J, Kincade PW: Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. J Exp Med 1990; 172: 69-75.

Moreschini O, Fiorito S, Magrini L, Margheritini F, Romanini L: Markers of connective tissue activation in aseptic hip prosthetic loosening. J Arthroplasty 1997; 12: 695-703.

Motyckova G, Weilbaecher KN, Horstmann M, Rieman DJ, Fisher DZ, Fisher DE: Linking osteopetrosis and pycnodysostosis: regulation of cathepsin K expression by the microphthalmia transcription factor family. Proc Natl Acad Sci U S A 2001; 98: 5798-5803.

Mukohyama H, Ransjo M, Taniguchi H, Ohyama T, Lerner UH: The inhibitory effects of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide on osteoclast formation are associated with upregulation of osteoprotegerin and downregulation of RANKL and RANK. Biochem Biophys Res Commun 2000; 271: 158-163.

Muller WA: New mechanisms and pathways for monocyte recruitment. J Exp Med 2001; 194: F47-F51.

Mundy GR: Cytokines and growth factors in the regulation of bone remodeling. J Bone Miner Res 1993; 8 Suppl 2: S505-S510.

Murray DW, Rushton N: Macrophages stimulate bone resorption when they phagocytose particles. J Bone Joint Surg [Br] 1990; 72: 988-992.

Nagai M, Sato N: Reciprocal gene expression of osteoclastogenesis inhibitory factor and osteoclast differentiation factor regulates osteoclast formation. Biochem Biophys Res Commun 1999; 257: 719-723.

Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T, Inoue J: Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. Genes Cells 1999; 4: 353-362.

Naito M, Umeda S, Yamamoto T, Moriyama H, Umezu H, Hasegawa G, Usuda H, Shultz LD, Takahashi K: Development, differentiation, and phenotypic heterogeneity of murine tissue macrophages. J Leukoc Biol 1996; 59: 133-138.

Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Yano K, Morinaga T, Higashio K: RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun 1998; 253: 395-400.

Nakase T, Takeuchi E, Sugamoto K, Kaneko M, Tomita T, Myoui A, Uchiyama Y, Ochi T, Yoshikawa H: Involvement of multinucleated giant cells synthesizing cathepsin K in calcified tendinitis of the rotator cuff tendons. Rheumatology (Oxford) 2000; 39: 1074-1077.

Nakashima T, Kobayashi Y, Yamasaki S, Kawakami A, Eguchi K, Sasaki H, Sakai H: Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. Biochem Biophys Res Commun 2000; 275: 768-775.

Nakashima Y, Sun DH, Trindade MC, Chun LE, Song Y, Goodman SB, Schurman DJ, Maloney WJ, Smith RL: Induction of macrophage C-C chemokine expression by titanium alloy and bone cement particles. J Bone Joint Surg Br 1999; 81: 155-162.

Neale SD, Kristelly R, Gundle R, Quinn JM, Athanasou NA: Giant cells in pigmented villo nodular synovitis express an osteoclast phenotype. J Clin Pathol 1997; 50: 605-608.

Nesbitt SA, Horton MA: Trafficking of matrix collagens through bone-resorbing osteoclasts. Science 1997; 276: 266-269.

Niissalo S, Li TF, Santavirta S, Takagi M, Hietanen J, Konttinen YT: Dense innervation in pseudocapsular tissue compared to aneural interface tissue in loose totally replaced hips. J Rheumatol 2002; 29: 796-803.

O'Brien CA, Gubrij I, Lin SC, Saylors RL, Manolagas SC: STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF-kappaB ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. J Biol Chem 1999; 274: 19301-19308.

Palokangas H, Mulari M, Vaananen HK: Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts. J Cell Sci 1997; 110: 1767-1780.

Pfaff M, Jurdic P: Podosomes in osteoclast-like cells: structural analysis and cooperative roles of paxillin, proline-rich tyrosine kinase 2 (Pyk2) and integrin alphaVbeta3. J Cell Sci 2001; 114: 2775-2786.

Quinn JM, Elliott J, Gillespie MT, Martin TJ: A combination of osteoclast differentiation factor and macrophage-colony stimulating factor is sufficient for both human and mouse osteoclast formation in vitro. Endocrinology 1998; 139: 4424-4427.

Quinn JM, McGee JO, Athanasou NA: Cellular and hormonal factors influencing monocyte differentiation to osteoclastic bone-resorbing cells. Endocrinology 1994; 134: 2416-2423.

Reponen P, Sahlberg C, Munaut C, Thesleff I, Tryggvason K: High expression of 92-kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. J Cell Biol 1994; 124: 1091-1102.

Rieman DJ, McClung HA, Dodds RA, Hwang SM, Holmes MW, James IE, Drake FH, Gowen M: Biosynthesis and processing of cathepsin K in cultured human osteoclasts. Bone 2001; 28: 282-289.

Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM: Mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest 2003; 111: 821-831.

Robertsson O, Wingstrand H, Kesteris U, Jonsson K, Onnerfalt R: Intracapsular pressure and loosening of hip prostheses. Preoperative measurements in 18 hips. Acta Orthop Scand 1997; 68: 231-234.

Rodan GA, Martin TJ: Role of osteoblasts in hormonal control of bone resorption--a hypothesis. Calcif Tissue Int 1981; 33: 349-351.

Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G, Pacifici R: Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci U S A 2001; %20;98: 13960-13965.

Rood JA, Van HS, Drake FH, Gowen M, Debouck C: Genomic organization and chromosome localization of the human cathepsin K gene (CTSK). Genomics 1997; 41: 169-176.

Rousselle AV, Heymann D: Osteoclastic acidification pathways during bone resorption. Bone 2002; 30: 533-540.

Rubash HE, Sinha RK, Shanbhag AS, Kim SY: Pathogenesis of bone loss after total hip arthroplasty. Orthop Clin North Am 1998; 29: 173-186.

Saari H, Santavirta S, Nordstrom D, Paavolainen P, Konttinen YT: Hyaluronate in total hip replacement. Journal of Rheumatology 1993; 20: 87-90.

Sabokbar A, Fujikawa Y, Neale S, Murray DW, Athanasou NA: Human arthroplasty derived macrophages differentiate into osteoclastic bone resorbing cells. Ann Rheum Dis 1997; 56: 414-420.

Sabokbar A, Kudo O, Athanasou NA: Two distinct cellular mechanisms of osteoclast formation and bone resorption in periprosthetic osteolysis. J Orthop Res 2003; 21: 73-80.

Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W, Moritz JD, Schu P, von Figura K: Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci

U S A 1998; 95: 13453-13458.

Saginario C, Sterling H, Beckers C, Kobayashi R, Solimena M, Ullu E, Vignery A: MFR, a putative receptor mediating the fusion of macrophages. Mol Cell Biol 1998; 18: 6213-6223.

Sakai H, Jingushi S, Shuto T, Urabe K, Ikenoue T, Okazaki K, Kukita T, Kukita A, Iwamoto Y: Fibroblasts from the inner granulation tissue of the pseudocapsule in hips at revision arthroplasty induce osteoclast differentiation, as do stromal cells. Ann Rheum Dis 2002; 61: 103-109.

Salo J, Lehenkari P, Mulari M, Metsikko K, Vaananen HK: Removal of osteoclast bone resorption products by transcytosis. Science 1997; 276: 270-273.

Santavirta S, Ceponis A, Solovieva SA, Hurri H, Jin J, Takagi M, Suda A, Konttinen YT: Periprosthetic microvasculature in loosening of total hip replacement. Arch Orthop Trauma Surg 1996; 115: 286-289.

Santavirta S, Gristina A, Konttinen YT: Cemented versus cementless hip arthroplasty. A review of prosthetic biocompatibility. Acta Orthop Scand 1992; 63: 225-232.

Santavirta S, Nordstrom D, Metsarinne K, Konttinen YT: Biocompatibility of polyethylene and host response to loosening of cementless total hip replacement. Clin Orthop 1993; 100-110.

Santavirta S, Xu JW, Hietanen J, Ceponis A, Sorsa T, Kontio R, Konttinen YT: Activation of periprosthetic connective tissue in aseptic loosening of total hip replacements. Clin Orthop 1998; 16-24.

Sapp JP: An ultrastructural study of nuclear and centriolar configurations in multinucleated giant cells. Lab Invest 1976; 34: 109-114.

Sato T, Foged NT, Delaisse JM: The migration of purified osteoclasts through collagen is inhibited by matrix metalloproteinase inhibitors. J Bone Miner Res 1998; 13: 59-66.

Schlomann U, Wildeboer D, Webster A, Antropova O, Zeuschner D, Knight CG, Docherty AJ, Lambert M, Skelton L, Jockusch H, Bartsch JW: The metalloprotease disintegrin ADAM8. Processing by autocatalysis is required for proteolytic activity and cell adhesion. J Biol Chem 2002; 277: 48210-48219.

Schlondorff J, Blobel CP: Metalloprotease-disintegrins: modular proteins capable of promoting cell-cell interactions and triggering signals by protein-ectodomain shedding. J Cell Sci 1999; 112: 3603-3617.

Schmalzried TP, Clarke IC, McKellop HA: Bearing Surfaces. In: Callaghan J.J., Rosenberg A.G., Rubash HE (eds) The Adult Hip, 1998, pp 247-264.

Schmalzried TP, Jasty M, Harris WH: Periprosthetic bone loss in total hip arthroplasty. Polyethylene wear debris and the concept of the effective joint space. J Bone Joint Surg [Am] 1992; 74: 849-863.

Schwartzberg PL, Xing L, Hoffmann O, Lowell CA, Garrett L, Boyce BF, Varmus HE: Rescue of osteoclast function by transgenic expression of kinase-deficient Src in src-/- mutant mice. Genes Dev 1997; 11: 2835-2844.

Seiffert M, Cant C, Chen Z, Rappold I, Brugger W, Kanz L, Brown EJ, Ullrich A, Buhring HJ: Human signal-regulatory protein is expressed on normal, but not on subsets of leukemic myeloid cells and mediates cellular adhesion involving its counterreceptor CD47. Blood 1999; 94: 3633-3643.

Shi GP, Chapman HA, Bhairi SM, DeLeeuw C, Reddy VY, Weiss SJ: Molecular cloning of human cathepsin O, a novel endoproteinase and homologue of rabbit OC2. FEBS Lett 1995; 357: 129-134.

Shiina-Ishimi Y, Abe E, Tanaka H, Suda T: Synthesis of colony-stimulating factor (CSF) and differentiation-inducing factor (D-factor) by osteoblastic cells, clone MC3T3-E1. Biochem Biophys Res Commun 1986; 134: 400-406.

Silver IA, Murrills RJ, Etherington DJ: Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. Exp Cell Res 1988; 175: 266-276.

Silverton SF, Dodgson SJ, Fallon MD, Forster RE: Carbonic anhydrase activity of chick osteoclasts is increased by parathyroid hormone. Am J Physiol 1987; 253: E670-E674.

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone

T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Boyle WJ: Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997; 89: 309-319.

Sly WS, Hewett-Emmett D, Whyte MP, Yu YS, Tashian RE: Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc Natl Acad Sci U S A 1983; 80: 2752-2756.

Soriano P, Montgomery C, Geske R, Bradley A: Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991; 64: 693-702.

Spear PG, Longnecker R: Herpesvirus entry: an update. J Virol 2003; 77: 10179-10185.

Spessotto P, Rossi FM, Degan M, Di Francia R, Perris R, Colombatti A, Gattei V: Hyaluronan-CD44 interaction hampers migration of osteoclast-like cells by down-regulating MMP-9. J Cell Biol 2002; 158: 1133-1144.

Suda T, Takahashi N, Martin TJ: Modulation of osteoclast differentiation. Endocr Rev 1992; 13: 66-80.

Suzuki K, Zhu B, Rittling SR, Denhardt DT, Goldberg HA, McCulloch CA, Sodek J: Colocalization of intracellular osteopontin with CD44 is associated with migration, cell fusion, and resorption in osteoclasts. J Bone Miner Res 2002; 17: 1486-1497.

Takagi M: Neutral proteinases and their inhibitors in the loosening of total hip prostheses. Acta Orthop Scand Suppl 1996; 271: 3-29.

Takagi M, Santavirta S, Ida H, Ishii M, Takei I, Niissalo S, Ogino T, Konttinen YT: High-turnover periprosthetic bone remodeling and immature bone formation around loose cemented total hip joints. J Bone Miner Res 2001; 16: 79-88.

Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseley JM, Martin TJ, Suda T: Osteoblastic cells are involved in osteoclast formation. Endocrinology 1988; 123: 2600-2602.

Takahashi N, Udagawa N, Akatsu T, Tanaka H, Isogai Y, Suda T: Deficiency of osteoclasts in osteopetrotic mice is due to a defect in the local microenvironment provided by osteoblastic cells. Endocrinology 1991a; 128: 1792-1796.

Takahashi N, Udagawa N, Akatsu T, Tanaka H, Shionome M, Suda T: Role of colony-stimulating factors in osteoclast development. J Bone Miner Res 1991b; 6: 977-985.

Takai H, Kanematsu M, Yano K, Tsuda E, Higashio K, Ikeda K, Watanabe K, Yamada Y: Transforming growth factor-beta stimulates the production of osteoprotegerin/osteoclastogenesis inhibitory factor by bone marrow stromal cells. J Biol Chem 1998; 273: 27091-27096.

Takayanagi H, Iizuka H, Juji T, Nakagawa T, Yamamoto A, Miyazaki T, Koshihara Y, Oda H, Nakamura K, Tanaka S: Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 2000; 43: 259-269.

Takei I, Takagi M, Ida H, Ogino T, Santavirta S, Konttinen YT: High macrophage-colony stimulating factor levels in synovial fluid of loose artificial hip joints. J Rheumatol 2000; 27: 894-899.

Tallroth K, Eskola A, Santavirta S, Konttinen YT, Lindholm TS: Aggressive granulomatous lesions after hip arthroplasty. J Bone Joint Surg [Br] 1989; 71: 571-575.

Tanaka S, Takahashi N, Udagawa N, Tamura T, Akatsu T, Stanley ER, Kurokawa T, Suda T: Macrophage colony-stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. J Clin Invest 1993; 91: 257-263.

Thesingh CW, Scherft JP: Fusion disability of embryonic osteoclast precursor cells and macrophages in the microphthalmic osteopetrotic mouse. Bone 1985; 6: 43-52.

Thomas GP, Baker SU, Eisman JA, Gardiner EM: Changing RANKL/OPG mRNA expression in differentiating murine primary osteoblasts. J Endocrinol 2001; 170: 451-460.

Thompson SK, Halbert SM, Bossard MJ, Tomaszek TA, Levy MA, Zhao B, Smith WW, Abdel-Meguid SS, Janson

CA, D'Alessio KJ, McQueney MS, Amegadzie BY, Hanning CR, DesJarlais RL, Briand J, Sarkar SK, Huddleston MJ, Ijames CF, Carr SA, Garnes KT, Shu A, Heys JR, Bradbeer J, Zembryki D, Veber DF: Design of potent and selective human cathepsin K inhibitors that span the active site. Proc Natl Acad Sci U S A 1997; 94: 14249-14254.

Tondravi MM, McKercher SR, Anderson K, Erdmann JM, Quiroz M, Maki R, Teitelbaum SL: Osteopetrosis in mice lacking haematopoietic transcription factor PU.1. Nature 1997; 386: 81-84.

Udagawa N, Takahashi N, Akatsu T, Sasaki T, Yamaguchi A, Kodama H, Martin TJ, Suda T: The bone marrow-derived stromal cell lines MC3T3-G2/PA6 and ST2 support osteoclast-like cell differentiation in cocultures with mouse spleen cells. Endocrinology 1989; 125: 1805-1813.

Udagawa N, Takahashi N, Akatsu T, Tanaka H, Sasaki T, Nishihara T, Koga T, Martin TJ, Suda T: Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. Proc Natl Acad Sci U S A 1990; 87: 7260-7264.

Uy HL, Dallas M, Calland JW, Boyce BF, Mundy GR, Roodman GD: Use of an in vivo model to determine the effects of interleukin-1 on cells at different stages in the osteoclast lineage. J Bone Miner Res 1995; 10: 295-301.

Vaananen HK, Zhao H: Osteoclast Function. In: Bilezikian JP, Raisz LG, Rodan GA (eds) Principles of Bone Biology, Academic Press, San Diego, CA, 2002, pp 127-139.

Vaananen HK, Zhao H, Mulari M, Halleen JM: The cell biology of osteoclast function. J Cell Sci 2000; 113: 377-381.

Van Wesenbeeck L, Odgren PR, MacKay CA, D'Angelo M, Safadi FF, Popoff SN, van Hul W, Marks SC, Jr.: The osteopetrotic mutation toothless (tl) is a loss-of-function frameshift mutation in the rat Csf1 gene: Evidence of a crucial role for CSF-1 in osteoclastogenesis and endochondral ossification. Proc Natl Acad Sci U S A 2002; 99: 14303-14308.

Verrier S, Hogan A, McKie N, Horton M: ADAM gene expression and regulation during human osteoclast formation. Bone 2004; 35: 34-46.

Viereck V, Grundker C, Blaschke S, Siggelkow H, Emons G, Hofbauer LC: Phytoestrogen genistein stimulates the production of osteoprotegerin by human trabecular osteoblasts. J Cell Biochem 2002; 84: 725-735.

Vignery A: Osteoclasts and giant cells: macrophage-macrophage fusion mechanism. Int J Exp Pathol 2000; 81: 291-304.

Vignery A, Niven-Fairchild T, Ingbar DH, Caplan M: Polarized distribution of Na+,K+-ATPase in giant cells elicited in vivo and in vitro. J Histochem Cytochem 1989; 37: 1265-1271.

Vignery A, Raymond MJ, Qian HY, Wang F, Rosenzweig SA: Multinucleated rat alveolar macrophages express functional receptors for calcitonin. Am J Physiol 1991a; 261: F1026-F1032.

Vignery A, Wang F, Ganz MB: Macrophages express functional receptors for calcitonin-gene-related peptide. J Cell Physiol 1991b; 149: 301-306.

Vignery A, Wang F, Qian HY, Benz EJ, Jr., Gilmore-Hebert M: Detection of the Na(+)-K(+)-ATPase alpha 3-isoform in multinucleated macrophages. Am J Physiol 1991c; 260: F704-F709.

Walker DG: Bone resorption restored in osteopetrotic mice by transplants of normal bone marrow and spleen cells. Science 1975a; 190: 784-785.

Walker DG: Spleen cells transmit osteopetrosis in mice. Science 1975b; 190: 785-787.

Wang W, Ferguson DJ, Quinn JM, Simpson AH, Athanasou NA: Biomaterial particle phagocytosis by bone-resorbing osteoclasts. J Bone Joint Surg [Br] 1997a; 79: 849-856.

Wang W, Ferguson DJ, Quinn JM, Simpson AH, Athanasou NA: Osteoclasts are capable of particle phagocytosis and bone resorption. J Pathol 1997b; 182: 92-98.

Weber GF, Ashkar S, Glimcher MJ, Cantor H: Receptor-ligand interaction between CD44 and osteopontin (Eta-1). Science 1996; 271: 509-512.

Weilbaecher KN, Hershey CL, Takemoto CM, Horstmann MA, Hemesath TJ, Tashjian AH, Fisher DE: Age-resolving osteopetrosis: a rat model implicating microphthalmia and the related transcription factor TFE3. J Exp Med 1998; 187: 775-785.

Williams SR, Jiang Y, Cochran D, Dorsam G, Graves DT: Regulated expression of monocyte chemoattractant protein-1 in normal human osteoblastic cells. Am J Physiol 1992; 263: C194-C199.

Wittrant Y, Couillaud S, Theoleyre S, Dunstan C, Heymann D, Redini F: Osteoprotegerin differentially regulates protease expression in osteoclast cultures. Biochem Biophys Res Commun 2002; 293: 38-44.

Wittrant Y, Theoleyre S, Couillaud S, Dunstan C, Heymann D, Redini F: Regulation of osteoclast protease expression by RANKL. Biochem Biophys Res Commun 2003; 310: 774-778.

Wolfsberg TG, Bazan JF, Blobel CP, Myles DG, Primakoff P, White JM: The precursor region of a protein active in sperm-egg fusion contains a metalloprotease and a disintegrin domain: structural, functional, and evolutionary implications. Proc Natl Acad Sci U S A 1993; 90: 10783-10787.

Wong BR, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, Choi Y: TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. Mol Cell 1999; 4: 1041-1049.

Wong BR, Josien R, Lee SY, Vologodskaia M, Steinman RM, Choi Y: The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor. J Biol Chem 1998; 273: 28355-28359.

Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Bartlett FS, Frankel WN, Lee SY, Choi Y: TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 1997; 272: 25190-25194.

Wong GL: Paracrine interactions in bone-secreted products of osteoblasts permit osteoclasts to respond to parathyroid hormone. J Biol Chem 1984; 259: 4019-4022.

Wright TM, Goodman SB: What are the local and systemic biologic reactions to wear debris? In: Wright TM, Goodman SB (eds) Implant Wear in Total Joint Replacement: Clinical and Biologic Issues, Material and Design Considerations, American Academy of Orthopaedic Surgeons, 2001a, pp 61-70.

Wright TM, Goodman SB: What is the clinical scope of implant wear in the hip and how has it changed since 1995? In: Wright TM, Goodman SB (eds) Implant Wear in Total Joint Replacement: Clinical and Biologic Issues, Material and Design Considerations, American Academy of Orthopaedic Surgeons, 2001b, pp 3-7.

Xing L, Bushnell TP, Carlson L, Tai Z, Tondravi M, Siebenlist U, Young F, Boyce BF: NF-kappaB p50 and p52 expression is not required for RANK-expressing osteoclast progenitor formation but is essential for RANK- and cytokine-mediated osteoclastogenesis. J Bone Miner Res 2002; 17: 1200-1210.

Xu JW, Konttinen YT, Waris V, Patiala H, Sorsa T, Santavirta S: Macrophage-colony stimulating factor (M-CSF) is increased in the synovial-like membrane of the periprosthetic tissues in the aseptic loosening of total hip replacement (THR). Clin Rheumatol 1997; 16: 243-248.

Yagami-Hiromasa T, Sato T, Kurisaki T, Kamijo K, Nabeshima Y, Fujisawa-Sehara A: A metalloprotease-disintegrin participating in myoblast fusion. Nature 1995; 19: 652-656.

Yamamoto A, Miyazaki T, Kadono Y, Takayanagi H, Miura T, Nishina H, Katada T, Wakabayashi K, Oda H, Nakamura K, Tanaka S: Possible involvement of IkappaB kinase 2 and MKK7 in osteoclastogenesis induced by receptor activator of nuclear factor kappaB ligand. J Bone Miner Res 2002; 17: 612-621.

Yamaza T, Goto T, Kamiya T, Kobayashi Y, Sakai H, Tanaka T: Study of immunoelectron microscopic localization of cathepsin K in osteoclasts and other bone cells in the mouse femur. Bone 1998; 23: 499-509.

Yamaza T, Tsuji Y, Goto T, Kido MA, Nishijima K, Moroi R, Akamine A, Tanaka T: Comparison in localization between cystatin C and cathepsin K in osteoclasts and other cells in mouse tibia epiphysis by immunolight and immunoelectron microscopy. Bone 2001; 29: 42-53.

Yan T, Riggs BL, Boyle WJ, Khosla S: Regulation of osteoclastogenesis and RANK expression by TGF-beta1. J Cell Biochem 2001; 83: 320-325.

Yang SY, Mayton L, Wu B, Goater JJ, Schwarz EM, Wooley PH: Adeno-associated virus-mediated osteoprotegerin gene transfer protects against particulate polyethylene-induced osteolysis in a murine model. Arthritis Rheum 2002; 46: 2514-2523.

Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K: Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology 1998a; 139: 1329-1337.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T: Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci U S A 1998b; 95: 3597-3602.

Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, Segal D, Dzivenu OK, Vologodskaia M, Yim M, Du K, Singh S, Pike JW, Darnay BG, Choi Y, Wu H: Distinct molecular mechanism for initiating TRAF6 signalling. Nature 2002; 418: 443-447.

Yoshida H, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okamura H, Sudo T, Shultz LD, Nishikawa S: The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature 1990; 345: 442-444.

Yu X, Huang Y, Collin-Osdoby P, Osdoby P: Stromal cell-derived factor-1 (SDF-1) recruits osteoclast precursors by inducing chemotaxis, matrix metalloproteinase-9 (MMP-9) activity, and collagen transmigration. J Bone Miner Res 2003; 18: 1404-1418.

Zhang XP, Kamata T, Yokoyama K, Puzon-McLaughlin W, Takada Y: Specific interaction of the recombinant disintegrin-like domain of MDC-15 (metargidin, ADAM-15) with integrin alphavbeta3. J Biol Chem 1998; 273: 7345-7350.

Zhang YH, Heulsmann A, Tondravi MM, Mukherjee A, Abu-Amer Y: Tumor necrosis factor-alpha (TNF) stimulates RANKL-induced osteoclastogenesis via coupling of TNF type 1 receptor and RANK signaling pathways. J Biol Chem 2001; 276: 563-568.

Zhao H, Laitala-Leinonen T, Parikka V, Vaananen HK: Downregulation of small GTPase Rab7 impairs osteoclast polarization and bone resorption. J Biol Chem 2001; 276: 39295-39302.

Zou W, Bar-Shavit Z: Dual modulation of osteoclast differentiation by lipopolysaccharide. J Bone Miner Res 2002; 17: 1211-1218.

Bilezikian JP, Raisz LG, Rodan GA (eds): Principles of Bone Biology. 2nd Ed., Academic Press, San Diego, CA, 2002.