Role of Cysteine Cathepsins in Joint Inflammation and Destruction in Human Rheumatoid Arthritis and Associated Animal Models

Uta Schurigt

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1. Introduction

Destruction of bone and articular cartilage during pathogenesis of rheumatoid arthritis (RA) is caused by increased activity of a huge panel of proteases, which are secreted by several cell types of arthritic joint. Besides matrix metalloproteases (MMPs), the papain-like cysteine proteases (clan CA, family C1) have been identified as proteases potentially involved in cartilage and bone destruction as well as in immune response during inflammatory arthritis. Several clinical studies demonstrated that expression and activity of different cysteine cathepsins have been increased frequently in synovial membranes and fluids from RA patients. However, the exact roles of papain-like cysteine proteases have not been fully understood yet. Therefore, their contribution to joint inflammation and destruction has been investigated by *in vivo* and *in vitro* experiments in the last decades of arthritis research. This chapter focuses on cysteine cathepsins K, B, L, and S - the best-studied members of the papain-like protease family in arthritic diseases - in order to understand better their impact on inflammatory arthritis in respect to their collagenolytic activities as well as to their contributions to immune response. Latest results about the impact of cysteine cathepsins in different animal models for RA are discussed comprehensively. Furthermore, a short excursion to cathepsin V (= cathepsin L2) - an exclusively human cathepsin L-like cysteine cathepsin - and its impact on autoimmune disease progression is included in this review. The chapter clarifies that cathepsins K and S are attractive targets for the development of new highly specific anti-arthritis drugs.

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2. Cysteine cathepsins

Cathepsins are a heterogeneous group of proteases. Originally, the name cathepsin was used for proteases with the highest activity in a slightly acidic environment as found in the lysosomes. The name cathepsin originates from greek "kathepsein" (= to digest). Today, the cathepsin family consists of at least 15 members and can be subdivided by their catalytic mechanism into three distinct groups: serine proteases (cathepsin A and G), aspartat proteases (cathepsin D and E), and cysteine proteases (cathepsins B, C, F, H, K, L, O, S, V, W, and X). Most cathepsins reside in endosomal/lysosomal compartment and are thus termed lysosomal cathepsins (except cathepsins E and G). Caused by this localization, cathepsins were initially considered as intracellularly active enzymes responsible for the non-specific bulk proteolysis in the acidic environment of the endosomal/lysosomal compartment, where they degrade intracellular and endocytosed extracellular proteins. However, this view has changed rapidly in the last years and there is a strong experimental evidence that cathepsins have huge panel of highly specialized functions [1, 2]. The cysteine cathepsins are characterized by the presence of a cysteine residue at their active site and are highly homologue to papain - a cysteine protease isolated originally from papaya fruit (*Carica papaya*). Therefore they are termed papain-like cysteine proteases and together with the parent protease papain they are classified in clan CA family C1 in "MEROPS – the peptidase database" [3]. Cysteine cathepsins are expressed by viruses, plants, primitive parasites, invertebrates, and vertebrates [4]. They play pivotal roles in chronic diseases (e.g. RA, cancer) as well as in infectious diseases (e.g. malaria, leishmaniasis) [2, 4, 5, 6]. Cysteine cathepsins are transported to the lysosomes via a specific mannose-6-phosphate receptor pathway, which explains the primary lysosomal localization [7]. Mature proteolytically active cathepsins are released after activation by removal of the N-terminal propeptide at the low pH of the lysosomes. The papain-like cysteine protease family contains both enzymes with endo- and exopeptidase activities. Cathepsin B is an endo- and an exopeptidase [3, 8]. It also acts as a peptidyl-dipeptidase [9]. Cysteine cathepsins K, L (= L1), S, and V (= L2) are endopeptidases [3]. The stability and activity of papain-like cysteine cathepsins depend on the acidic pH prevailing in lysosomes [2]. The functions of these enzymes may be altered with changes in pH and their cellular localization [2].

3. Cell types and tissues in arthritic joints

RA is an autoimmune disease with unknown etiology. The immune system of RA patients produces autoantibodies against components of their own extracellular matrix (ECM) in diarthrodial synovial joints (e.g. against collagens) [10]. This effectively leads the immune system to attack and finally to destroy - together with synovium-/pannus-associated cells - the articular cartilage and the bone in arthritic joints during disease progression. The diarthrodial synovial joint consists of highly specialized connective tissues (bone, hyaline cartilage, synovial tissue etc.) and a fibrous capsule (Figure 1). Bone is composed approximately to 70% of inorganic, mainly mineral compound called hydroxyapatite, 20% of organic material,

mainly type I collagen, and 10% water [11]. Morphologically two types of bone can be distinguished: porous trabecular bone, also known as spongy bone, and dense cortical bone, also known as compact bone. Osteoclasts are bone-demineralizing and -degrading cells, which are also responsible for bone resorption and type I collagen degradation during normal physiological bone turnover (Figure 1). They are large multinucleated cells that express tartrate-resistant phosphatase (TRAP), calcitonin receptors, and cathepsin K [12]. Osteoclasts are able to acidify an isolated area between the cell and bone matrix, which is named resorption lacuna. Active acidification of bone by osteoclasts results in demineralization of bone, solubilization of mineral components, and finally an uncovering/liberalization of matrix collagens. In addition, it provides an acidic environment for secreted cathepsin K for optimal proteolytic activity. Bone resorption occurs at the contact site between the osteoclast and the bone, the so called ruffled border. Minerals of bone are solubilized due to the secretion of acids, which depends on the activity of carbonic anhydrase and proton pumps of osteoclasts. The degradation of organic matrix of bone (mainly type I collagens) occurs probably due to the activity of lysosomal cysteine proteases, other lysosomal hydrolases, and collagenases of MMP family secreted by osteoclasts. So far cathepsins B, K, and L could also be detected in osteoclasts [13, 14, 15, 16, 17, 18]. Articular cartilage (= hyaline cartilage) covers articulating bone surfaces in diarthrodial joints. Cartilage is composed of water (65 - 85%) and a solid phase, consisting of 15 - 20% type II collagen, 3 - 10% large aggregating molecules of proteoglycan, which are called aggrecans, and various other types of collagen [19]. The synovial membrane (or synovium) is the soft tissue between the articular capsule and the joint cavity of diarthrodial synovial joints. The word "synovium" is related to the word "synovial" (= synovial fluid), which is the clear, viscid, lubricating fluid secreted by synovial fibroblasts of synovial membrane (Figure 1). Continuous inflammation of synovium during RA pathogenesis leads to membrane expansion by hyperproliferation of activated synovial fibroblasts. Such arthritic synovial fibroblasts are infiltrated by mononuclear cells (e.g. T helper (Th) cells, B cells, macrophages) and form finally, together with these infiltrates, the so called invasive pannus tissue, which is characterized by an increased protease expression.

In advanced RA, arthritic synovial fibroblasts are the main source of destructive proteinases (e.g. MMPs and cathepsins) mediating pannus invasion of bone and articular cartilage. Additionally pannus-infiltrating macrophages contribute after their activation to joint degradation by increased cytokine and protease expression. Expression of cathepsins B, K, L, and S by different cell types of synovium of RA patients was detected [20, 21, 22]. Professional antigen presenting cells (APC) in arthritic joints are dendritic cells, B cells, and macrophages. Cathepsins B, L, and S contribute to antigen presentation in APCs [23]. Furthermore, B cells are responsible for producing autoantibodies. Studies of Th cell-secreted cytokine spectrum led to the classification of RA as a Th1-like disease [24]. This cell population, predominantly producing gamma interferon (IFNγ) and interleukin-2 (IL-2), stimulates protease overexpression in synovial fibroblasts and macrophages in pannus tissue. In contrast, Th2 cells, predominantly producing IL-4 and IL-10, are rarely found in arthritic joints. Anyway, both Th1 and Th2 cells can stimulate MMP expression in arthritic synovial fibroblasts by secretion of macrophage migration inhibitory factor [25]. Tumor necrosis factor alpha (TNFα) is considered as the main proinflammatory cytokine in the pathogenesis of RA [26]. It is pro-

duced by Th1 cells, synovial monocytes/macrophages, synovial fibroblasts, lymphocytes, and osteoblasts. TNFα can stimulate osteoclast formation in pannus tissue. Furthermore, TNFα appears to influence the distribution of osteoclast precursor cells in the body by increasing their influx from the bone marrow into synovium. TNFα also had a stimulating effect on secretion of procathepsin B by human arthritic synovial fibroblasts [27].
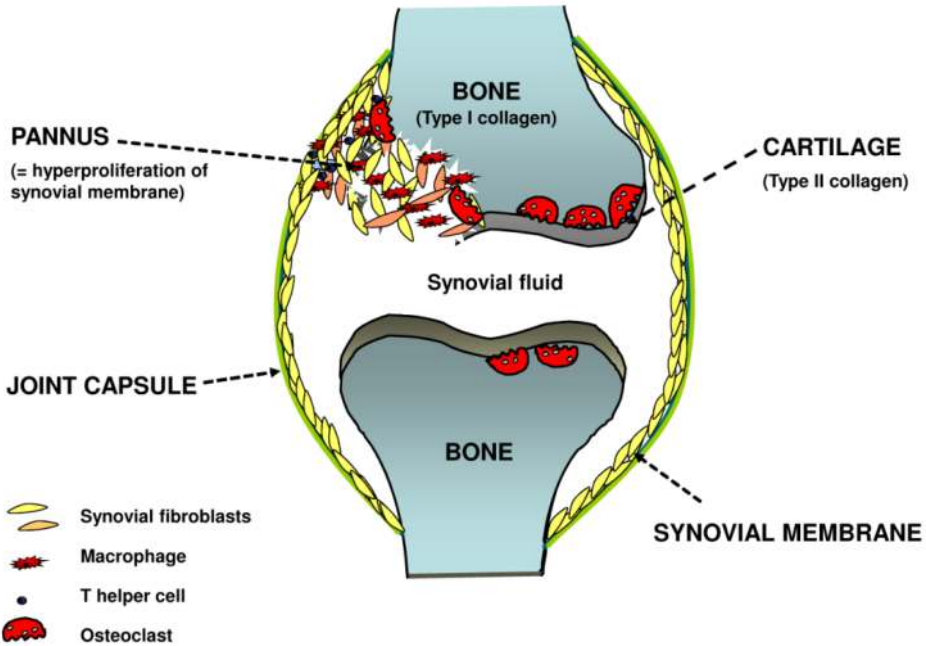

Figure 1. Organization of a diarthrodial synovial joint

4. Type I and type II collagens

One hallmark of human RA is the proteolytic degradation of collagens in ECM of affected joints. The ECM is the material between the cells in tissues of multicellular organisms. It provides structural framework of bone and articular cartilage of joints and is responsible for their resistance to pressure, torsion, and tension. Articular cartilage and bone contain specialized ECM components (collagens, elastin, proteoglycans etc.), which give diarthrodial joints strength and structural qualities. Collagens - the structural main components in joints - are extracellular matrix molecules used by cells for structural integrity and with a variety of other functions. About 28 different collagens have been identified in mammals and humans [28]. The typical mature collagen molecule consists of three single collagen polypep-

tide chains, so called alpha (α) chains, which coil into a helical molecule [28]. The different types of collagen are formed from a combination of more than 45 distinct collagen α polypeptide chains [28]. In the triple helical regions of collagens, termed Col domains, every third animo acid is glycine (gly) organized in as repeating peptide triplets of gly-X-Y [28]. In this triplet, X often is proline, and Y frequently is 4-hydroxyproline [28]. Col domains of each α chain are flanked by non-helical (non-gly-X-Y) regions, termed NC domains [28, 29]. In contrast, the telopeptides - the NC domains - of collagens have not the repeating gly-X-Y structure and do not adopt triple helical conformation. Telopeptides account for 2% of the collagen α chain and are essential for fibril formation [29]. Triple helical molecules aggregate spontaneously and form covalent cross-links among themselves to form collagen fibrils [29]. Both, the Col and the NC domains of collagen molecules are immunogenic [30]. Bone organic matrix contains predominantly type I collagen (90%). Type II collagen is the molecular principal compound of mammalian and human articular collagen, but additionally collagens III, VI, IX, X, XI, XII, and XIV contribute to composition of ECM of cartilage [31]. Type I and type II collagens, together with the other extracellular matrix molecules, are degraded during physiological processes (e.g. morphogenesis, growth, wound healing, physiological bone turnover) but also during pathological processes (e.g. cancer, RA).

5. Collagenolytic activities of papain-like cysteine proteases

Native collagens are highly resistant to proteolytic degradation due to their rigid and compact structure. However, hydrolysis of non-helical collagen telopeptides by proteases leads to depolymerization of the fibrillar collagen network, whereas cleavage within the triple helix results in depolymerization and denaturation of native triple helical collagen molecule. Only few proteases with collagenase activity have the capacity to initiate the cleavage of native triple helical collagens. Collagenases are enzymes that catalyze the hydrolysis of peptide bonds in triple helical regions of collagen. In contrast, denatured collagens (= gelatin) lost the triple helical structure and they are readily degraded by multiple proteinases (= gelatinases). Gelatinases are proteolytic enzymes hydrolyzing denatured collagen (= gelatin).

The exact mechanisms of collagen degradation have been not completely understood yet. Historically, MMPs have been considered as the main players of ECM degradation. This was justified by their membrane association or extracellular localization, their neutral pH optimum, and their ability to degrade structural extracellular proteins such as collagens, elastin, and proteoglycans. MMPs are members of a subfamily of proteases, which includes collagenases (MMP-1, -8, -13, and -18), stromelysins (MMP-3, -7, -10, -11, and -12), gelatinases (MMP-2 and -9), and membrane type MMPs (MT-MMPs: MMP-14, -15, -16, and -17). The collagenases among the MMPs are able to initiate degradation of native triple helical collagens. However, results of various studies have suggested that also other proteases must degrade ECM components. Especially, the papain-like cysteine cathepsins were supposed to contribute to collagen cleavage that occurs at acidic pH, in particular in collagen cleavage mediated by osteoclasts.

The investigation of tissue-degrading enzyme expression in synovial membrane, synovial fluid, and serum of RA patients is of particular interest in arthritis research, because elevations of analysed protease imply an impact on RA pathogenesis. The contribution of papain-like cysteine proteases to bone and cartilage destruction in RA was supposed, because several clinical studies showed that cysteine cathepsins were increasingly expressed and highly active in clinical samples from RA patients. Elevated levels of cysteine cathepsins B, L, S were detected in synovial fluids and in different cell types from patients with RA [32, 33, 34, 35]. Furthermore, it was shown, that cathepsins B and L were expressed in the synovial membrane shortly after symptom onset what implies that the potential for joint destruction exists at a very early stage in the course of the disease [36]. An enhanced transcription of cathepsin B in synovial cells from RA patients was detected [37]. Cathepsin B and L activities were detected in synovial membranes of RA patients [38]. Macrophages abundant in chronic RA subchondral bone lesions were characterized by high cathepsin L expression and an involvement of this protease in bone and cartilage destruction was supposed [39]. Furthermore, it was suggested that cathepsins B and L expressed by chondrocytes are involved in cartilage destruction during arthritis [40]. Cathepsin K was elevated in the serum of RA patients [41].

However, first direct experimental evidence supporting the role of papain-like cysteine proteases in bone resorption was provided by showing that specific inhibitors for different cysteine cathepsins and broad spectrum cysteine cathepsin inhibitors decreased bone resorption by osteoclasts [14, 16, 42, 43, 44]. The inhibition of lysosomes with cathepsin K-specific inhibitors led to an accumulation of undigested material within the endosomal/lysosomal compartment of osteoclasts [45]. Additionally, invasiveness of synovial fibroblasts from RA patients into cartilage both *in vitro* and *in vivo* in the SCID mouse coimplantation model was reduced after treatment with ribozymes cleaving specifically cathepsin L mRNA and therefore decreasing the synthesis of this cysteine protease [46].

Finally, *in vitro* analyses of collagenolytic activities helped to clarify the contribution of these individual cysteine cathepsins to physiological and pathological cartilage and bone degradation. Cleavage of soluble type I and type II collagen *in vitro* has been reported for cathepsins B, K, L, S, and V [47, 48, 49, 51, 59, 63] (Table 1). However, it is notable that latter proteases have only gelatinolytic activities and additionally contribute to unspecific cleavage of telopeptides of collagens [50]. Native triple helical type I and type II collagens are resistant to proteolysis by cathepsins B, L, S, and V. Although these cathepsins have not the capacity to cleave triple helical collagen, they attack their telopeptides, which are involved in intra- and intermolecular links [29]. This attack by cysteine cathepsin - similar to MMP-9 highly expressed in osteoclast - may destabilize the fibril collagen helices and therefore may contribute to joint destruction. Cathepsin L is the cysteine protease hitherto considered to have the highest telopeptidase and gelatinase activity among the papain-like cysteine proteases. Despite its own limited proteolytic activity, cathepsin B is able to proteolytically activate collagenase that mediates triple helical collagen cleavage [64].

Protease	Proteolytic activities		Cartilage- and bone-related phenotypes of protease-deficient mice	Investigation of protease-deficient mice in animal models for RA
	Collagenase activity	Gelatinase activity		
Cathepsin B	No	Yes [47, 48, 49]	No phenotypes reported	Antigen-induced arthritis: [personal communication by author] No differences to arthritic wild-type mice
Cathepsin K	Yes [50]	Yes [51]	Osteopetrotic phenotype in long bones, trabelular and cortical bone mass is increased, higher brittleness of bone [52, 53, 54, 55, 56]	hTNFtg mice: [57] Reduction of osteoclast-dependent cartilage and bone destruction Adjuvant arthritis: [58] Reduction in pro-inflammatory Th17 cells number by suppression of toll-like receptor 9 signaling in dendritic cells is responsible for attenuated arthritis
Cathepsin L	No	Yes [47, 48, 49, 51, 59]	Decrease in trabecular bone volume [60]	Antigen-induced arthritis: [61] Impairment of Th cell response, reconstitution by expression of human cathepsin V in thymus
Cathepsin S	No	Yes [47]	No phenotypes reported	Collagen-induced arthritis: [62] Milder arthritis by impairment of antigen-presentation
Cathepsin V	No	Yes [63]	Not expressed in mice	

Table 1. Summary of proteolytic activities of individual cysteine cathepsins, the resulting phenotypes of protease-deficient mice, and the clinical outcome of these mice in animal models for human RA

However, only cathepsin K is able to cleave native type I collagen within the triple helical domain [50] (Table 1). This unique proteolytic activity is caused by the formation of an oligomeric complex between cathepsin K molecules and extracellular matrix-resident glycosaminoglycans [65]. However, in the absence of this complex, monomeric cathepsin K exhibits only the telopeptide cleavage capability and lacks this collagenase activity like the other papain-like cysteine cathepsins [50, 66]. To control the collagenase activity of cathepsin K by disruption of the glycosaminoglycan/cathepsin K complex or by prevention of its formation may open possibilities to develop new drugs to reduce bone destruction in RA. Cathepsin K

was originally identified as an osteoclast-specific lysosomal protease. It is highly expressed and active in osteoclasts associated with bone surface and is secreted in resorption lacuna [15, 67]. The importance of cathepsin K for bone resorption has been demonstrated by cathepsin K inhibition studies with cathepsin K antisense oligodeoxynucleotides [68]. It has been shown that cathepsin K is capable to cleave type II collagen within the helical region of N-terminus, a unique capacity of this protease among papain-like cysteine proteases [69]. Therefore, inhibition of cathepsin K has been suggested to also play a pivotal role in protection of cartilage degradation during RA. Furthermore, cathepsin K is a critical protease in synovial fibroblast-mediated collagen degradation [70]. In contrast to MMPs with neutral or near-neutral pH optimum, cathepsin K is able to degrade the organic matrix in an acidic microenviroment. This acidic "collagenase" cleaves both triple-helical type I and type I collagen, the major structural components of the extracellular matrix of articular cartilage and bone. In contrast to collagenases (MMPs -1, -8, -13, -18), which cleave collagen creating typical ¼ C-terminal and ¾ N-terminal fragment, cathepsin K can cleave triple helical type I collagen at multiple sites resulting in a more complex degradation pattern [50, 69].

6. Phenotypes of cysteine cathepsin-deficient mice

Phenotyping is one of the first analytical steps after generation of gene knock out mice. Cartilage and bone phenotypes would be expected in cysteine cathepsin-deficient mice, if these proteases would contribute to physiological cartilage and bone turnover. Mice deficient in cysteine cathepsins B, K, L, and S were generated in the last years [52, 53, 54, 55, 62, 71, 72]. Cathepsin V is expressed exclusively in humans. No phenotypes of the bone or articular cartilage have been reported so far for cathepsin B-, and S-deficient mice (Table 1). In contrast, the bone phenotype in cathepsin K-deficient mice is very strong [52, 53, 54, 55, 56] (Table 1). Therefore, cathepsin K is possibly the most important proteolytic enzyme of osteoclasts in the papain-like cysteine protease family. Cathepsin K-deficient mice partially reflect the phenotype of pycnodysostosis, a human hereditary disease [52, 73]. The name "pycnodysostosis" appropriately describes this disease as formation of abnormally dense (greek: pykno) bone. The late 19th century French poster artist Henri de Toulouse-Lautrec (1864 - 1901) was the most prominent pycnodysostosis patient [74]. Therefore, this disease is sometimes referred as Toulouse-Lautrec syndrome. Cathepsin K mutations in patients with pycnodysostosis result in a total loss or inactivity of cathepsin K, which causes abnormal degradation of bone matrix proteins such as type I collagen [75]. Pycnodysostosis is characterized by a variable clinical appearance that includes short stature, open fontanelles, partial or total aplasia of the terminal phalanges, a predisposition to bone fractures, osteopetrosis, and an increased roentgenographic density of the entire skeleton [73, 74, 76, 77]. Cathepsin K-deficient mice are phenotypically characterized by an osteopetrotic phenotype in long bones - especially in distal femur - and lumbar vertebrae [52, 53, 54, 55, 56]. The trabecular and cortical bone mass is increased in cathepsin K-deficient mice compared with their wild-type littermates [55]. The bones of cathepsin K-deficient mice show a higher brittleness [53]. However, the osteopetrosis of pycnodysostosis patients seems to be more severe than that of cathepsin K-

deficient mice and some of the skeletal changes seen in pycnodysostosis patients, such as retardation, phalangeal deformities, or delayed suture closure in the skull, have not been reported in cathepsin K-deficient mice [52, 53, 54, 55]. However, other clinical symptoms of pycnodysostosis as for instance the accumulation of undigested collagen fibrils in lysosomes of osteoclasts and fibroblasts are described for cathepsin K-deficient mice [45, 70, 73]. The lack of cathepsin K decreases the rate of osteoclast-mediated bone resorption but does not completely inhibit this process [52, 55]. The number of osteoclasts was significantly increased in trabecular bone of cathepsin K-deficient mice compared to wild-type controls, probably to compensate the inefficient bone degradation [54]. A cartilage phenotype of cathepsin K-deficient mice has not been reported. Furthermore, and in strong contrast to cathepsin K-deficient mice, cathepsin L knock out mice revealed a decrease in trabecular bone volume [60] (Table 1). This reduction in bone mass may suggest that cathepsin L is involved in endochondral ossification [60]. This effect was reduced after ostrogen withdrawal by ovariectomy [60].

7. Animal models of RA

The use of animal models allows *in vivo* investigation of single aspects, as for instance inflammation, antigen presentation, and joint destruction during the complex pathogenesis of inflammatory arthritis. Additionally, animal models have been applied to evaluate potential anti-arthritis drugs for clinical use. RA models are relatively easy to use, produce reproducible results, and are of short duration [78, 79, 80, 81]. They feature many of the clinical symptoms of the human disease. The most important difference between animal models of RA and human RA is the disease progression rate. It is much faster in animal models of RA than in the human disease. Therefore, animal models of inflammatory arthritis are characterized primarily by an acute inflammatory response and only a weak chronification of disease. Anyway, investigation of inflammatory arthritis with test animals is important for the understanding of specific aspects in pathogenesis of human RA. Especially the investigation of cysteine cathepsin-deficient or -transgenic mice in such models as well as the application of specific inhibitors in arthritic animals enables the understanding of the contribution of individual proteases to the disease outcome. Animal models for human RA can be classified into induced and spontaneous models [82]. It is important to select the right animal model for RA to address a specific scientific question. The repertory of animal models of RA includes among others adjuvant arthritis, antigen-induced arthritis (AIA), collagen-induced arthritis (CIA), and human TNF-transgenic (hTNFtg) mice [78, 80, 81, 82]. Each of these animal models only reflects a few of the clinical aspects of the human disease. Therefore, the exact knowledge of all clinical aspects, disease progression rate, and the contribution of individual cell types to inflamed joints to disease outcome is fundamental to understand the *in vivo* functions of investigated proteases or the *in vivo* effects of applied cysteine cathepsin-specific drugs. The latter is especially important because papain-like cysteine proteases not only directly contribute to ECM degradation in arthritic joints but also to local and systemic immune response. Several cysteine cathepsins are involved in antigen presentation and inflam-

matory pathways [23, 58]. First experimental results in animal models for RA with cysteine cathepsin-deficient and -transgenic mice have been helpful to understand the impact of these proteases on joint inflammation and destruction *in vivo*.

TNFα plays a central role in pathophysiology of RA [26, 83]. This was confirmed by the development of transgenic mice that overexpress human TNFα [81, 84]. The phenotype of hTNFtg mice validated the theory that TNFα is the apex of pro-inflammatory cascade in RA. In this simple mouse model for RA the investigators utilized a targeting vector that contained a genomic fragment encoding the entire human TNFα gene in which the ARE-containing 3`UTR was replaced with the 3`UTR from β-globin gene [81, 84]. This mutation resulted in a chronic overexpression of TNFα mRNA. hTNFtg mice develop spontaneously an erosive symmetrical polyarthritis with histopathological features of inflammation and bone destruction similar to human RA [81, 84]. Early symptoms of disease in hTNFtg mice after spontaneous onset are infiltration with polymorphonuclear cells, lymphocytes, and synovial hyperplasia [81]. Pannus formation, destruction of fibrous tissue, as well as massive articular cartilage and subchondral bone destruction are additional hallmarks of the late stage of arthritis in hTNFtg mice [81, 84]. The bone surface of hTNFtg mice is covered by multinucleated TRAP+ osteoclasts, interposed between the bone surface and the "erosive" front of the synovium [81, 84]. The process of bone destruction is mediated exclusively by osteoclasts because c fos-deficient hTNFtg mice completely lacking osteoclasts were fully protected against bone destruction [85]. This absence of osteoclasts alters TNF-mediated arthritis from a destructive to a nondestructive arthritis [85]. Taken together, the hTNFtg mouse model is especially interesting to investigate the impact of an individual protease to osteoclast-dependent bone resorption during inflammatory arthritis. The investigation of cathepsin K-deficient hTNFtg mice for instance confirmed that cathepsin K is a protease secreted by osteoclasts that has a very high impact to bone destruction [57] (Table 1). Unexpectedly it was also demonstrated that cathepsin K is important but not essential for osteoclast-dependent bone resorption in hTNFtg mouse model for RA [57]. The bone destruction in cathepsin K-deficient hTNFtg mice was only reduced about 50% [57]. Therefore, other proteases, especially MMPs might contribute to subchondral bone destruction process. The MMP activity detected in cathepsin K-deficient osteoclasts might be a compensatory mechanism [57]. Consequently, strategies to prevent arthritic osteoclast-dependent bone destruction cannot be restricted to a selective inhibiton of cathepsin K activity. The detected impairment of synovium-derived osteoclast formation might be partially responsible for the significant reduction in the area of bone erosion in cathepsin K-deficient hTNFtg mice [57]. A clinical case of the onset of an erosive psoriatic arthritis in a "cathepsin K activity-deficient" pycnodysostosis patient was recently reported [86]. This "experiment of nature" supported the idea that cathepsin K in humans is also not essential for osteoclast-mediated bone degradation during inflammatory arthritis [86]. Nevertheless, cathepsin K plays a pivotal role in arthritis. Transgenic mice, overexpressing cathepsin K, become spontaneously susceptible to inflammatory arthritis characterized by synovitis, synovial hyperplasia, fibrosis, and subsequently in degradation of articular cartilage and bone [87].

Rat adjuvant arthritis is an experimental model of polyarthritis that has been widely used for preclinical drug testing. In rats it is induced by a single dosis of Freund`s adjuvant, containing *Mycobacterium tuberculosis* [79, 80]. Arthritis develops in around 10 - 45 days after induction and generally subsides after one month [80]. The hallmarks of this model are a reliable onset of robust polyarticular inflammation with infiltration of joints with mono- and polymorphonuclear cells, pannus formation, and marked bone resorption [79, 80]. The cartilage destruction is relatively mild in comparison to the observed inflammation and bone destruction [79]. The mechanism of arthritis development after immunization with complete Freund`s adjuvant is unknown. Activation of APCs was supposed to contribute to arthritis onset. The enzymatic activity of cathepsin B correlated positively with the severity of joint destruction and inflammation in rat adjuvant-induced arthritis [88]. Oral administration of a vinyl sulfone cysteine cathepsin-specific inhibitor reduced the signs of inflammation and tissue destruction in this animal model probably by direct local effects and attenuation of MHC-dependent antigen-presentation [88]. Oral administration of fluoromethyl ketones in rats with adjuvant-induced arthritis inhibited at least cysteine cathepsins B and L, and resulted in a reduction of articular cartilage and bone destruction [89]. Adjuvant arthritis can also be investigated in mice. Induction of adjuvant arthritis in cathepsin K-deficient mice demonstrated clearly that cathepsin K plays, besides its role in osteoclast-mediated bone destruction, a critical role in toll-like receptor 9 signaling in dendritic cells [58]. The suppression of this signal pathway by cathepsin K deficiency resulted in attenuated induction of pro-inflammatory Th17 cells, without affecting the antigen-presenting ability of dendritic cells [58] (Table 1). In addition, pharmacological inhibition using cathepsin K-specific inhibitors resulted in the reduction of inflammation in joints [58]. Furthermore, cathepsin B and L activities were strongly increased in chondrocytes and cells of the inflamed synovium of rats, which developed an arthritis induced by the synthetic adjuvant CP20961 [90].

Collagen-induced arthritis (CIA) is an experimental autoimmune disease that can be elicited in susceptible strains of rodents (rat und mouse) and non-human primates by immunization with type II collagen of several species the major constituent of articular cartilage [78, 80]. Susceptibility to CIA is restricted to mouse strains with MHC class II types I-A^q and I-A^r [78, 80]. The immune response to type II collagen is characterized by the stimulation of collagen-specific T cells and the production of high titers of collagen-specific antibody [78]. Hallmarks of polyarthritic CIA are synovitis, infiltration of joint with polymorphonuclear and mononuclear cells, pannus formation, erosion of cartilage and bone, and fibrosis [78, 80]. In mice, immunization with bovine, chick or rat type II collagens usually leads to a relatively acute form of arthritis [80]. Papain-like cysteine proteases contribute to disease progression in the CIA arthritis model. Cathepsin K expression is upregulated in murine CIA [91]. Pharmacological inhibition of the proteolytic activity of cathepsin K in murine CIA reduced the destruction of bone and cartilage within arthritic joints [92]. Additionally, the severity of CIA in DBA/1 mice was decreased by fluoroketone inhibitors, which inhibit specifically cathepsin B and L [89]. Cathepsin S-deficient mice develop a diminished CIA probably caused by influences of cathepsin S to late stages of Li degradation in APCs and influencing the peptide repertoire displayed by MHC class II molecules [62] (Table 1). Therapeutic applications of a highly selective and oral available cathepsin S inhibitor reduced significantly

the disease score in arthritic CIA mice [93]. The development of further new cathepsin S-specific inhibitors may be useful in treatment of human RA and other autoimmune diseases. Interestingly, the development of highly selective activity-based probes to monitor cathepsin S activity and their successful application in murine zymosam-induced arthritis was reported [94]. These active site probes open the possibility to investigate the *in vivo* roles of cathepsin S in CIA and other RA models more precisely and to monitor the bioavailability of cathepsin S-specific inhibitors in therapeutical trials with arthritic animals.

The antigen-induced arthritis (AIA) can be induced in mice, rats, and rabbits following intra-articular injection of a protein antigen (e.g. methylated bovine serum albumin) into the knee joint of animals that have been previously immunized with the same antigen [80]. The histopathological appearance of AIA has similarities to human RA, including synovial lining layer hyperplasia, perivascular infiltration with lymphocytes and plasma cells, lymphoid follicles, pannus formation, and cartilage erosion [80]. Bone erosion in this arthritis model is relatively week [61, 95]. The AIA is strict Th cell-dependent as shown with depletion experiments with anti CD4 antibodies [96]. Depletion of CD25+ regulatory T cell resulted in an increase of disease severity [95]. In contrast to RA, the AIA is a monoarticular disease that affects only treated joints [80]. Anyway, susceptibility to AIA is not MHC class II-restricted and this makes this model useful for studies with transgenic and gene-deficient mice on different genetic backgrounds [80]. So far the investigated cysteine cathepsins play no or unexpected roles in this RA model. At least the contribution of these proteases to antigen presentation and therefore an alteration in disease outcome was expected because Th1/Th2 balance was influenced by cathepsin L- and B-specific inhibitors applied in *Leishmania*-infected and ovalbumine-immunized mice [43, 97, 98, 99]. However, cathepsin B-deficient mice did not show any difference in disease outcome compared to wild-type mice (unpublished data by author) (Table 1). In addition, no significant upregulation at mRNA level of cathepsin B was detected during time course of AIA [100]. The severity of AIA was decreased in cathepsin L-deficient mice [61]. Clinical outcome in this mice was characterized by decreased inflammation, reduction in cartilage and bone destruction, as well as diminished cellular and humoral immune responsiveness [61] (Tabel 1). Both, Th1 and Th2 cell responses were impaired in arthritic cathepsin L-deficient mice [61]. Interestingly this effect was not caused by local activity of cathepsin L in the arthritic joint, which correlated with only slight local upregulation of cathepsin L in arthritic knee joints in the acute phase and no increase in expression during chronic phase of AIA [100]. In fact the attenuation of AIA in cathepsin L-deficient mice was caused by an impaired positive selection of conventional disease promoting CD4+ Th cells in thymus and a unchanged development of the protective CD25+/FOXP3+ regulatory T cells compartment [61, 101]. Experimentally it could be further clearly demonstrated that transgenic expression of human cathepsin L-like protease cathepsin V in thymic epithelium of cathepsin L-deficient mice reconstituted all parameters by normalization of the ratio of regulatory to conventional T cells [61, 101] (Tabel 1). Therefore, human cathepsin V - the syntenic orthologous proteases of mouse cathepsin L - is clearly involved in Th cell positive selection in the thymus. This influence of cathepsin V on Th cell compartment development might further explain that genetic polymorphisms of cathepsin V are associated with human autoimmune diseases such as diabetes type 1 and myasthenia gravis [102]. In future studies it

would be highly attractive to investigate whether cathepsin V polymorphisms are associated with the incidence and clinical outcomes in patients with RA.

As described above cysteine cathepsin-specific inhibitors were applied successfully in several animal models of human RA [58, 88, 89, 92, 93]. The reduction of disease severity was observed. The proteolytic activities of cysteine cathepsins, which contribute directly to joint destruction by collagen degradation as well as indirectly by modulation of the immune response, were inhibited. However, the exact understanding of the contribution of cysteine cathepsins to immune response will be very critically to avoid severe side effects in patients. Potential consequences of systemic application of cathepsin S- and K-specific inhibitors for the outcome of other human chronic and infectious diseases must be critically discussed. Cell type-specific delivery of inhibitors should become a key aspect in arthritis research in future. Osteoclast-specific delivery of cathepsin K-specific inhibitors for instance could be an interesting strategy to avoid joint destruction by inhibition of the collagenolytic activities without interfering with systemic immune response.

8. Summary

Several papain-like cysteine cathepsins are able to cleave type I and type II collagen and therefore contribute to direct joint destruction. Additionally, they play roles in antigen presentation and development of Th cell compartment. Especially cathepsin K with its unique collagenase activity has a great impact to bone degradation in inflammatory arthritis and plays a crucial role in inflammatory processes. In addition, cathepsin S is a key player in antigen-presentation during arthritis. At least cathepsin K and S are attractive targets for the development of new anti-arthritic drugs.

Author details

Uta Schurigt

Address all correspondence to: uta.schurigt@uni-wuerzburg.de

Institute of Molecular Infection Biology (IMIB), University of Wuerzburg, Wuerzburg, Germany

References

[1] Reinheckel T, Deussing J, Roth W, Peters C. Towards specific functions of lysosomal cysteine peptidases: phenotypes of mice deficient for cathepsin B or cathepsin L. Biol Chem 2001; 382: 735-41.

[2]   Turk V, Stoka V, Vasiljeva O, Renko M, Sun T, Turk B, Turk D. Cysteine cathepsins: from structure, function and regulation to new frontiers. Biochim Biophys Acta 2012; 1824: 68-88.

[3]   MEROPS. The Peptidase Database. http://merops.sanger.ac.uk

[4]   Otto HH, Schirmeister T. Cysteine Proteases and Their Inhibitors. Chem Rev 1997; 97: 133-172.

[5]   Mottram JC, Coombs GH, Alexander J. Cysteine peptidases as virulence factors of *Leishmania*. Curr Opin Microbiol 2004; 7: 375-81.

[6]   Mason SD, Joyce JA. Proteolytic networks in cancer. Trends Cell Biol 2011; 21: 228-37.

[7]   Hasilik A, Wrocklage C, Schroder B. Intracellular trafficking of lysosomal proteins and lysosomes. Int J Clin Pharmacol Ther 2009; 47 Suppl 1: S18-33.

[8]   Musil D, Zucic D, Turk D, Engh RA, Mayr I, Huber R, Popovic T, Turk V, Towatari T, Katunuma N, et al. The refined 2.15 A X-ray crystal structure of human liver cathepsin B: the structural basis for its specificity. EMBO J 1991; 10: 2321-30.

[9]   Aronson NN, Jr., Barrett AJ. The specificity of cathepsin B. Hydrolysis of glucagon at the C-terminus by a peptidyldipeptidase mechanism. Biochem J 1978; 171: 759-65.

[10] Rowley MJ, Nandakumar KS, Holmdahl R. The role of collagen antibodies in mediating arthritis. Mod Rheumatol 2008; 18: 429-41.

[11] Antoine SE, Child AM, Nicholson RA, Pollard AM. The biochemistry and microbiology of buried human bone, in ralation to dietary reconstruction. Circaea 1992; 9: 65-79.

[12] Faust J, Lacey DL, Hunt P, Burgess TL, Scully S, Van G, Eli A, Qian Y, Shalhoub V. Osteoclast markers accumulate on cells developing from human peripheral blood mononuclear precursors. J Cell Biochem 1999; 72: 67-80.

[13] Sazaki T, Ueno-Matsuda E. Cystein-proteinase localization in osteoclasts: An immunocytochemical study. Cell Tissue Res 1993; 271: 177-179.

[14] Rifkin BR, Vernillo AT, Kleckner AP, Auszmann JM, Rosenberg LR, Zimmerman M. Cathepsin B and L activities in isolated osteoclasts. Biochem Biophys Res Commun 1991; 179: 63-9.

[15] 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 1998; 123: 752-9.

[16] Kakegawa H, Nikawa T, Tagami K, Kamioka H, Sumitani K, Kawata T, Drobnic-Kosorok M, Lenarcic B, Turk V, Katunuma N. Participation of cathepsin L on bone resorption. FEBS Lett 1993; 321: 247-50.

[17] Goto T, Yamaza T, Tanaka T. Cathepsins in the osteoclast. J Electron Microsc (Tokyo) 2003; 52: 551-8.

[18] Goto T, Tsukuba T, Kiyoshima T, Nishimura Y, Kato K, Yamamoto K, Tanaka T. Immunohistochemical localization of cathepsins B, D and L in the rat osteoclast. Histochemistry 1993; 99: 411-4.

[19] Choi JA, Gold GE. MR imaging of articular cartilage physiology. Magn Reson Imaging Clin N Am 2011; 19: 249-82.

[20] 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: 663-74.

[21] Justen HP, Grunewald E, Totzke G, Gouni-Berthold I, Sachinidis A, Wessinghage D, Vetter H, Schulze-Osthoff K, Ko Y. Differential gene expression in synovium of rheumatoid arthritis and osteoarthritis. Mol Cell Biol Res Commun 2000; 3: 165-72.

[22] Kaneko M, Tomita T, Nakase T, Ohsawa Y, Seki H, Takeuchi E, Takano H, Shi K, Takahi K, Kominami E, Uchiyama Y, Yoshikawa H, Ochi T. Expression of proteinases and inflammatory cytokines in subchondral bone regions in the destructive joint of rheumatoid arthritis. Rheumatology (Oxford) 2001; 40: 247-55.

[23] Honey K, Rudensky AY. Lysosomal cysteine proteases regulate antigen presentation. Nat Rev Immunol 2003; 3: 472-82.

[24] Miossec P, van den Berg W. Th1/Th2 cytokine balance in arthritis. Arthritis Rheum 1997; 40: 2105-15.

[25] Schurigt U, Pfirschke C, Irmler IM, Huckel M, Gajda M, Janik T, Baumgrass R, Bernhagen J, Brauer R. Interactions of T helper cells with fibroblast-like synoviocytes: upregulation of matrix metalloproteinases by macrophage migration inhibitory factor from both Th1 and Th2 cells. Arthritis Rheum 2008; 58: 3030-40.

[26] Feldmann M. The cytokine network in rheumatoid arthritis: definition of TNF alpha as a therapeutic target. J R Coll Physicians Lond 1996; 30: 560-70.

[27] Huet G, Flipo RM, Colin C, Janin A, Hemon B, Collyn-d'Hooghe M, Lafyatis R, Duquesnoy B, Degand P. Stimulation of the secretion of latent cysteine proteinase activity by tumor necrosis factor alpha and interleukin-1. Arthritis Rheum 1993; 36: 772-80.

[28] Gordon MK, Hahn RA. Collagens. Cell Tissue Res 2010; 339: 247-57.

[29] Kadler KE, Holmes DF, Trotter JA, Chapman JA. Collagen fibril formation. Biochem J 1996; 316 (Pt 1): 1-11.

[30] Lynn AK, Yannas IV, Bonfield W. Antigenicity and immunogenicity of collagen. J Biomed Mater Res B Appl Biomater 2004; 71: 343-54.

[31] Eyre D. Collagen of articular cartilage. Arthritis Res 2002; 4: 30-5.

[32] Ikeda Y, Ikata T, Mishiro T, Nakano S, Ikebe M, Yasuoka S. Cathepsins B and L in synovial fluids from patients with rheumatoid arthritis and the effect of cathepsin B on the activation of pro-urokinase. J Med Invest 2000; 47: 61-75.

[33] Lenarcic B, Gabrijelcic D, Rozman B, Drobnic-Kosorok M, Turk V. Human cathepsin B and cysteine proteinase inhibitors (CPIs) in inflammatory and metabolic joint diseases. Biol Chem Hoppe Seyler 1988; 369 Suppl: 257-61.

[34] Gabrijelcic D, Annan-Prah A, Rodic B, Rozman B, Cotic V, Turk V. Determination of cathepsins B and H in sera and synovial fluids of patients with different joint diseases. J Clin Chem Clin Biochem 1990; 28: 149-53.

[35] Hashimoto Y, Kakegawa H, Narita Y, Hachiya Y, Hayakawa T, Kos J, Turk V, Katunuma N. Significance of cathepsin B accumulation in synovial fluid of rheumatoid arthritis. Biochem Biophys Res Commun 2001; 283: 334-9.

[36] Cunnane G, FitzGerald O, Hummel KM, Gay RE, Gay S, Bresnihan B. Collagenase, cathepsin B and cathepsin L gene expression in the synovial membrane of patients with early inflammatory arthritis. Rheumatology (Oxford) 1999; 38: 34-42.

[37] Trabandt A, Gay RE, Fassbender HG, Gay S. Cathepsin B in synovial cells at the site of joint destruction in rheumatoid arthritis. Arthritis Rheum 1991; 34: 1444-51.

[38] Solau-Gervais E, Zerimech F, Lemaire R, Fontaine C, Huet G, Flipo RM. Cysteine and serine proteases of synovial tissue in rheumatoid arthritis and osteoarthritis. Scand J Rheumatol 2007; 36: 373-7.

[39] Iwata Y, Mort JS, Tateishi H, Lee ER. Macrophage cathepsin L, a factor in the erosion of subchondral bone in rheumatoid arthritis. Arthritis Rheum 1997; 40: 499-509.

[40] Maciewicz RA, Wotton SF. Degradation of cartilage matrix components by the cysteine proteinases, cathepsins B and L. Biomed Biochim Acta 1991; 50: 561-4.

[41] Skoumal M, Haberhauer G, Kolarz G, Hawa G, Woloszczuk W, Klingler A. Serum cathepsin K levels of patients with longstanding rheumatoid arthritis: correlation with radiological destruction. Arthritis Res Ther 2005; 7: R65-70.

[42] Delaisse JM, Eeckhout Y, Vaes G. *In vivo* and *in vitro* evidence for the involvement of cysteine proteinases in bone resorption. Biochem Biophys Res Commun 1984; 125: 441-7.

[43] Katunuma N, Matsunaga Y, Matsui A, Kakegawa H, Endo K, Inubushi T, Saibara T, Ohba Y, Kakiuchi T. Novel physiological functions of cathepsin B and L on antigen processing and osteclastic bone resorption. Advan. Enzyme Regul. 1998; 38: 235-251.

[44] Hill PA, Buttle DJ, Jones SJ, Boyde A, Murata M, Reynolds JJ, Meikle MC. Inhibition of bone resorption by selective inactivators of cysteine proteinases. J Cell Biochem 1994; 56: 118-30.

[45] Everts V, Hou WS, Rialland X, Tigchelaar W, Saftig P, Bromme D, Gelb BD, Beertsen W. Cathepsin K deficiency in pycnodysostosis results in accumulation of non-digested phagocytosed collagen in fibroblasts. Calcif Tissue Int 2003; 73: 380-6.

[46] Schedel J, Seemayer CA, Pap T, Neidhart M, Kuchen S, Michel BA, Gay RE, Muller-Ladner U, Gay S, Zacharias W. Targeting cathepsin L (CL) by specific ribozymes de-

creases CL protein synthesis and cartilage destruction in rheumatoid arthritis. Gene Ther 2004; 11: 1040-7.

[47]   Maciewicz RA, Etherington DJ. A comparison of four cathepsins (B, L, N and S) with collagenolytic activity from rabbit spleen. Biochem J 1988; 256: 433-40.

[48]   Delaisse JM, Ledent P, Vaes G. Collagenolytic cysteine proteinases of bone tissue. Cathepsin B, (pro)cathepsin L and a cathepsin L-like 70 kDa proteinase. Biochem J 1991; 279 (Pt 1): 167-74.

[49]   Garnero P, Ferreras M, Karsdal MA, Nicamhlaoibh R, Risteli J, Borel O, Qvist P, Delmas PD, Foged NT, Delaisse JM. The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. J Bone Miner Res 2003; 18: 859-67.

[50]   Garnero P, Borel O, Byrjalsen I, Ferreras M, Drake FH, McQueney MS, Foged NT, Delmas PD, Delaisse JM. The collagenolytic activity of cathepsin K is unique among mammalian proteinases. J Biol Chem 1998; 273: 32347-52.

[51]   Nosaka AY, Kanaori K, Teno N, Togame H, Inaoka T, Takai M, Kokubo T. Conformational studies on the specific cleavage site of type I collagen (alpha-1) fragment (157-192) by cathepsins K and L by proton NMR spectroscopy. Bioorg Med Chem 1999; 7: 375-9.

[52]   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: 13453-8.

[53]   Li CY, Jepsen KJ, Majeska RJ, Zhang J, Ni R, Gelb BD, Schaffler MB. Mice lacking cathepsin K maintain bone remodeling but develop bone fragility despite high bone mass. J Bone Miner Res 2006; 21: 865-75.

[54]   Kiviranta R, Morko J, Alatalo SL, NicAmhlaoibh R, Risteli J, Laitala-Leinonen T, Vuorio E. Impaired bone resorption in cathepsin K-deficient mice is partially compensated for by enhanced osteoclastogenesis and increased expression of other proteases via an increased RANKL/OPG ratio. Bone 2005; 36: 159-72.

[55]   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: 1654-63.

[56]   Pennypacker B, Shea M, Liu Q, Masarachia P, Saftig P, Rodan S, Rodan G, Kimmel D. Bone density, strength, and formation in adult cathepsin K (-/-) mice. Bone 2009; 44: 199-207.

[57]   Schurigt U, Hummel KM, Petrow PK, Gajda M, Stockigt R, Middel P, Zwerina J, Janik T, Bernhardt R, Schuler S, Scharnweber D, Beckmann F, Saftig P, Kollias G, Schett G, Wiederanders B, Brauer R. Cathepsin K deficiency partially inhibits, but does not

prevent, bone destruction in human tumor necrosis factor-transgenic mice. Arthritis Rheum 2008; 58: 422-34.

[58]   Asagiri M, Hirai T, Kunigami T, Kamano S, Gober HJ, Okamoto K, Nishikawa K, Latz E, Golenbock DT, Aoki K, Ohya K, Imai Y, Morishita Y, Miyazono K, Kato S, Saftig P, Takayanagi H. Cathepsin K-dependent toll-like receptor 9 signaling revealed in experimental arthritis. Science 2008; 319: 624-7.

[59]   Kirschke H, Kembhavi AA, Bohley P, Barrett AJ. Action of rat liver cathepsin L on collagen and other substrates. Biochem J 1982; 201: 367-72.

[60]   Potts W, Bowyer J, Jones H, Tucker D, Freemont AJ, Millest A, Martin C, Vernon W, Neerunjun D, Slynn G, Harper F, Maciewicz R. Cathepsin L-deficient mice exhibit abnormal skin and bone development and show increased resistance to osteoporosis following ovariectomy. Int J Exp Pathol 2004; 85: 85-96.

[61]   Schurigt U, Eilenstein R, Gajda M, Leipner C, Sevenich L, Reinheckel T, Peters C, Wiederanders B, Brauer R. Decreased arthritis severity in cathepsin L-deficient mice is attributed to an impaired T helper cell compartment. Inflamm Res 2012; 61: 1021-9.

[62]   Nakagawa TY, Brissette WH, Lira PD, Griffiths RJ, Petrushova N, Stock J, McNeish JD, Eastman SE, Howard ED, Clarke SR, Rosloniec EF, Elliott EA, Rudensky AY. Impaired invariant chain degradation and antigen presentation and diminished collagen-induced arthritis in cathepsin S null mice. Immunity 1999; 10: 207-17.

[63]   BRENDA. The Comprehensive Enzyme Information System. http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.4.22.43

[64]   Eeckhout Y, Vaes G. Further studies on the activation of procollagenase, the latent precursor of bone collagenase. Effects of lysosomal cathepsin B, plasmin and kallikrein, and spontaneous activation. Biochem J 1977; 166: 21-31.

[65]   Li Z, Hou WS, Bromme D. Collagenolytic activity of cathepsin K is specifically modulated by cartilage-resident chondroitin sulfates. Biochemistry 2000; 39: 529-36.

[66]   Li Z, Hou WS, Escalante-Torres CR, Gelb BD, Bromme D. Collagenase activity of cathepsin K depends on complex formation with chondroitin sulfate. J Biol Chem 2002; 277: 28669-76.

[67]   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: 379-84.

[68]   Inui T, Ishibashi O, Inaoka T, Origane Y, Kumegawa M, Kokubo T, Yamamura T. Cathepsin K antisense oligodeoxynucleotide inhibits osteoclastic bone resorption. J Biol Chem 1997; 272: 8109-12.

[69]   Kafienah W, Bromme D, Buttle DJ, Croucher LJ, Hollander AP. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. Biochem J 1998; 331 (Pt 3): 727-32.

[70] Hou WS, Li Z, Gordon RE, Chan K, Klein MJ, Levy R, Keysser M, Keyszer G, Bromme D. Cathepsin K is a critical protease in synovial fibroblast-mediated colla‐gen degradation. Am J Pathol 2001; 159: 2167-77.

[71] Deussing J, Roth W, Saftig P, Peters C, Ploegh HL, Villadangos JA. Cathepsins B and D are dispensable for major histocompatibility complex class II-mediated antigen presentation. Proc Natl Acad Sci U S A 1998; 95: 4516-21.

[72] Roth W, Deussing J, Botchkarev VA, Pauly-Evers M, Saftig P, Hafner A, Schmidt P, Schmahl W, Scherer J, Anton-Lamprecht I, Von Figura K, Paus R, Peters C. Cathepsin L deficiency as molecular defect of furless: hyperproliferation of keratinocytes and pertubation of hair follicle cycling. FASEB J 2000; 14: 2075-86.

[73] Gelb BD, Shi GP, Chapman HA, Desnick RJ. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. Science 1996; 273: 1236-8.

[74] McKiernan M. Henri de Toulouse-Lautrec medical examination, Rue des Moulins (1894): North wall fresco, lower panel 5.398 m x 13.716 m. Detroit Institute of Arts, Detroit, USA. Occup Med (Lond) 2009; 59: 366-8.

[75] Xue Y, Cai T, Shi S, Wang W, Zhang Y, Mao T, Duan X. Clinical and animal research findings in pycnodysostosis and gene mutations of cathepsin K from 1996 to 2011. Orphanet J Rare Dis 2011; 6: 20.

[76] Edelson JG, Obad S, Geiger R, On A, Artul HJ. Pycnodysostosis. Orthopedic aspects with a description of 14 new cases. Clin Orthop Relat Res 1992; 263-76.

[77] Fratzl-Zelman N, Valenta A, Roschger P, Nader A, Gelb BD, Fratzl P, Klaushofer K. Decreased bone turnover and deterioration of bone structure in two cases of pycno‐dysostosis. J Clin Endocrinol Metab 2004; 89: 1538-47.

[78] Brand DD, Kang AH, Rosloniec EF. The mouse model of collagen-induced arthritis. Methods Mol Med 2004; 102: 295-312.

[79] Bendele A, McComb J, Gould T, McAbee T, Sennello G, Chlipala E, Guy M. Animal models of arthritis: relevance to human disease. Toxicol Pathol 1999; 27: 134-42.

[80] Williams RO. Rodent models of arthritis: relevance for human disease. Clin Exp Im‐munol 1998; 114: 330-2.

[81] Li P, Schwarz EM. The TNF-alpha transgenic mouse model of inflammatory arthritis. Springer Semin Immunopathol 2003; 25: 19-33.

[82] Cuzzocrea S. Characterization of a novel and spontaneous mouse model of inflam‐matory arthritis. Arthritis Res Ther 2011; 13: 126.

[83] Geiler J, Buch M, McDermott MF. Anti-TNF treatment in rheumatoid arthritis. Curr Pharm Des 2011; 17: 3141-54.

[84] Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J 1991; 10: 4025-31.

[85] Redlich K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias G, Steiner G, Smolen JS, Wagner EF, Schett G. Osteoclasts are essential for TNF-alpha-mediated joint destruction. J Clin Invest 2002; 110: 1419-27.

[86] Ainola M, Valleala H, Nykanen P, Risteli J, Hanemaaijer R, Konttinen YT. Erosive arthritis in a patient with pycnodysostosis: an experiment of nature. Arthritis Rheum 2008; 58: 3394-401.

[87] Morko J, Kiviranta R, Joronen K, Saamanen AM, Vuorio E, Salminen-Mankonen H. Spontaneous development of synovitis and cartilage degeneration in transgenic mice overexpressing cathepsin K. Arthritis Rheum 2005; 52: 3713-7.

[88] Biroc SL, Gay S, Hummel K, Magill C, Palmer JT, Spencer DR, Sa S, Klaus JL, Michel BA, Rasnick D, Gay RE. Cysteine protease activity is up-regulated in inflamed ankle joints of rats with adjuvant-induced arthritis and decreases with *in vivo* administration of a vinyl sulfone cysteine protease inhibitor. Arthritis Rheum 2001; 44: 703-11.

[89] Esser RE, Angelo RA, Murphey MD, Watts LM, Thornburg LP, Palmer JT, Talhouk JW, Smith RE. Cysteine proteinase inhibitors decrease articular cartilage and bone destruction in chronic inflammatory arthritis. Arthritis Rheum 1994; 37: 236-47.

[90] Meijers MH, Koopdonk-Kool J, Meacock SC, Van Noorden CJ, Bunning RA, Billingham ME. Cysteine proteinase activity in the development of arthritis in an adjuvant model of the rat. Agents Actions 1993; 39 Spec No: C219-21.

[91] Ibrahim SM, Koczan D, Thiesen HJ. Gene-expression profile of collagen-induced arthritis. J Autoimmun 2002; 18: 159-67.

[92] Svelander L, Erlandsson-Harris H, Astner L, Grabowska U, Klareskog L, Lindstrom E, Hewitt E. Inhibition of cathepsin K reduces bone erosion, cartilage degradation and inflammation evoked by collagen-induced arthritis in mice. Eur J Pharmacol 2009; 613: 155-62.

[93] Baugh M, Black D, Westwood P, Kinghorn E, McGregor K, Bruin J, Hamilton W, Dempster M, Claxton C, Cai J, Bennett J, Long C, McKinnon H, Vink P, den Hoed L, Gorecka M, Vora K, Grant E, Percival MD, Boots AM, van Lierop MJ. Therapeutic dosing of an orally active, selective cathepsin S inhibitor suppresses disease in models of autoimmunity. J Autoimmun 2011; 36: 201-9.

[94] Caglic D, Globisch A, Kindermann M, Lim NH, Jeske V, Juretschke HP, Bartnik E, Weithmann KU, Nagase H, Turk B, Wendt KU. Functional *in vivo* imaging of cysteine cathepsin activity in murine model of inflammation. Bioorg Med Chem 2011; 19: 1055-61.

[95] Frey O, Petrow PK, Gajda M, Siegmund K, Huehn J, Scheffold A, Hamann A, Radbruch A, Brauer R. The role of regulatory T cells in antigen-induced arthritis: aggra-

vation of arthritis after depletion and amelioration after transfer of CD4+CD25+ T cells. Arthritis Res Ther 2005; 7: R291-301.

[96] Pohlers D, Nissler K, Frey O, Simon J, Petrow PK, Kinne RW, Brauer R. Anti-CD4 monoclonal antibody treatment in acute and early chronic antigen-induced arthritis: influence on T helper cell activation. Clin Exp Immunol 2004; 135: 409-15.

[97] Maekawa Y, Himeno K, Katunuma N. Cathepsin B-inhibitor promotes the development of Th1 type protective T cells in mice infected with *Leishmania major*. J Med Invest 1997; 44: 33-9.

[98] Onishi K, Li Y, Ishii K, Hisaeda H, Tang L, Duan X, Dainichi T, Maekawa Y, Katunuma N, Himeno K. Cathepsin L is crucial for a Th1-type immune response during Leishmania major infection. Microbes Infect 2004; 6: 468-74.

[99] Zhang T, Maekawa Y, Sakai T, Nakano Y, Ishii K, Hisaeda H, Dainichi T, Asao T, Katunuma N, Himeno K. Treatment with cathepsin L inhibitor potentiates Th2-type immune response in *Leishmania major*-infected BALB/c mice. Int Immunol 2001; 13: 975-82.

[100] Schurigt U, Stopfel N, Huckel M, Pfirschke C, Wiederanders B, Brauer R. Local expression of matrix metalloproteinases, cathepsins, and their inhibitors during the development of murine antigen-induced arthritis. Arthritis Res Ther 2005; 7: R174-88.

[101] Sevenich L, Hagemann S, Stoeckle C, Tolosa E, Peters C, Reinheckel T. Expression of human cathepsin L or human cathepsin V in mouse thymus mediates positive selection of T helper cells in cathepsin L knock-out mice. Biochimie 2010; 92: 1674-80.

[102] Viken MK, Sollid HD, Joner G, Dahl-Jorgensen K, Ronningen KS, Undlien DE, Flato B, Selvaag AM, Forre O, Kvien TK, Thorsby E, Melms A, Tolosa E, Lie BA. Polymorphisms in the cathepsin L2 (CTSL2) gene show association with type 1 diabetes and early-onset myasthenia gravis. Hum Immunol 2007; 68: 748-55.