Chapter 13

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.

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 peptidyldipeptidase [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,

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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].

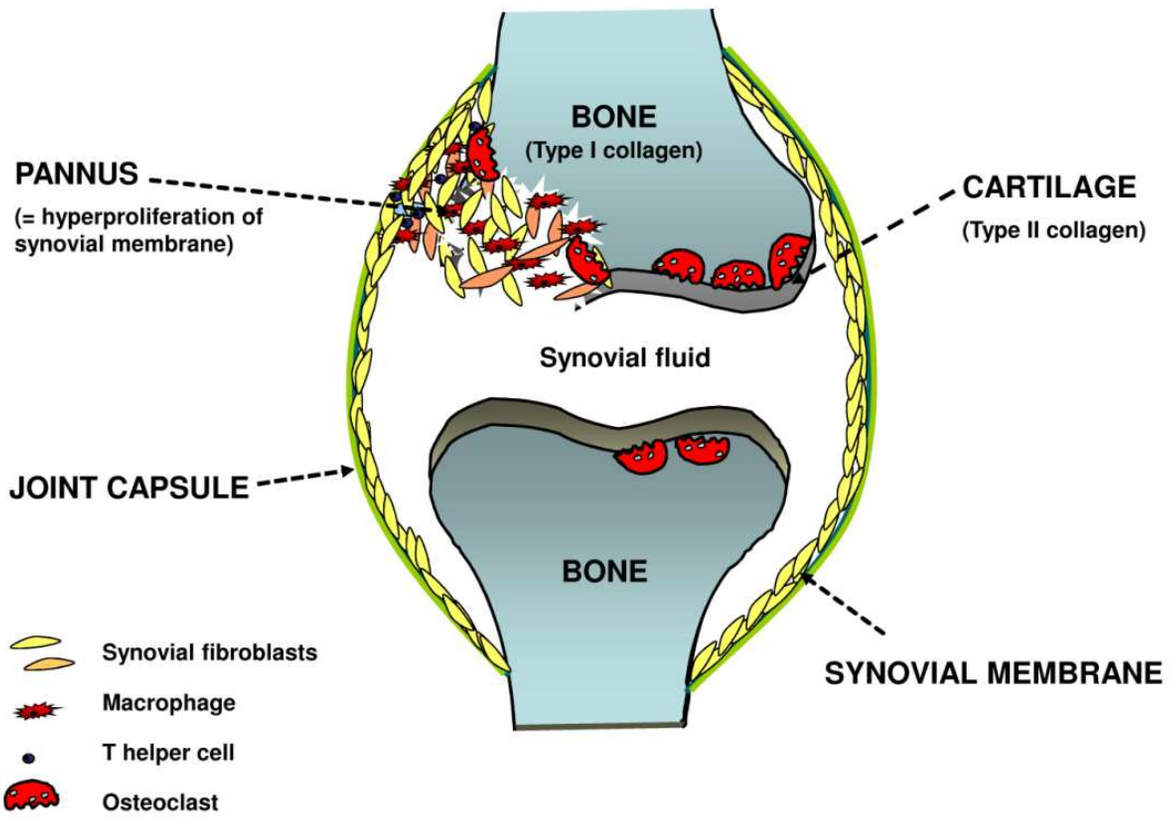

Figure 1. Organization of a diarthrodial synovial joint

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-

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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].

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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

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