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# The protease systems and their pathogenic role in juvenile idiopathic arthritis

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ABSTRACT

Numerous proteases produced by synovial cells of arthritic joints, chondrocytes, macrophages and polymorphonuclear cells have been identified as responsible for the joint damage in rheumatoid arthritis. There are few scientific contributions aimed to identify similar mechanisms in the joints of patients with juvenile idiopathic arthritis. Recently, some mechanisms emerged, triggered by the TH17 and TH1/TH17 lymphocytes, which could shed new light on unexpected pathogenic pathways of joint damage in the JIA, mainly regarding the RANK-RANKL pathway. Other novelties are linked to the mechanisms of acidification of the synovial fluid, which create a microenvironment suitable for the extracellular activity of lysosomal enzymes. Some biological drugs currently used in the therapy of JIA can interfere with these mechanisms.

## 1. Introduction

Juvenile idiopathic arthritis (JIA) is an autoimmune inflammatory disease [1], sometimes included in a poly-autoimmune clinical setting [2], whose pathogenesis may be related to the detrimental role of Th17, Th1, and Th17/Th1 lymphocytes [3,4] which trigger a chain of events that eventuate into invalidating joint lesions. It is therefore of fundamental importance the task of delineating the molecular effectors through which such classes of CD4+ lymphocytes cause joint damage in the JIA. Obviously, the aspect linked to the proliferation of synovial fibroblasts, their infiltration and destruction of the articular cartilage, together with the recruitment of granulocytes and macrophages, plays a decisive role in determining joint damage and in the identification of possible therapeutic interventions. Actually, in inflammatory autoimmune joint pathologies, such as RA and JIA, Th17 have been supposed to induce an activated form of synovial fibroblasts, to activate osteoclasts and to recruit macrophages and granulocytes, enabling them to release extracellular matrix-destroying enzymes.

The enzymes involved in the lesions typical of inflammatory arthritis have as substrate the complex set of molecules that make up the structural part of the cartilage and of the articular bone heads.

## 2. Molecular composition of cartilage and bones

Two thirds of the dry mass of adult cartilage is collagen: the type II collagen is the most represented, but the fibrillar network is also composed of collagens III, VI, IX, X, XI, XII and XIV. The most important proteoglycan of cartilage is the aggrecan (chondroitin sulfate proteoglycan-1), which is distributed in particular in the internal areas of the cartilage, while in the external areas decorin and β-glycan are prevalent. Other important proteoglycans with less diffusion are the versican, which is combined with hyaluronic acid and the lumican, which determines the thickness of the collagen fibers. During embryonic life, the predominant glycosaminoglycan in the formation of proteoglycans is chondroitin sulphate, while in the adult ketaran sulphate predominates [5].

In the bone the mineral component constitutes about 65% of the dry weight and is formed by the crystals of hydroxyapatite [Ca10 (PO4) 6 (OH) 2], while the organic fraction of the bone matrix is formed by proteoglycans (poorly represented) and Type I collagen, which accounts for about 20% of bone dry weight. The most abundant glycoproteins are fibronectin, thrombospondin, osteopontin, osteocalcin and osteonectin [6].

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## 3. Enzymes involved in arthritic joint damage in JIA

More than half a century of studies have clearly shown that tissue infiltration and degradation in pathologies ranging from tumors to inflammatory states, including inflammatory joint diseases, are related to the activity of the following classes of enzymes: the matrix-metallo proteinases (MMPs), the cell-associated plasminogen activation system, cathepsins and acid-hydrolases.

### 3.1. Matrix-metallo-proteinases (MMPs)

MMPs belong to a complex set of enzymes with different and sometimes overlapping substrates. However, the main specificities make it possible to divide the MMPs into the following categories.

1. Gelatinases,directly secreted by the cell [MMP2 (Gelatinase-A, 72 kDa gelatinase), whose substrates include gelatin (denatured collagen), collagens I, II, III, IV, VII, and X; MMP9 (Gelatinase-B, 92 kDa gelatinase), whose substrates include gelatin, Col IV, V]. Both gelatinases degrade other important ECM proteins, such as elastin, fibronectin, laminin, tenascin, vitronectin [7,8].Upon secretion both gelatinases may bind to different cellular binding sites. The integrin αvβ3 is a receptor for MMP2, while MMP9 binds to the cell surface proteoglycan CD44 [9]. Actually, MMP-2 and MMP-9 are synthesized as latent enzymes (pro-MMP2, pro-MMP9), which undergo a complex process of activation by plasmin and by other membrane-type MMPs (see below), when cells activate tissue-invasive programs [10].Following activation of the pro-enzyme, the catalytic site of activated gelatinases initiates breakdown of extracellular macromolecules, a feature thatconnotes a degradative and invasive phenotype of the cell, including synovial fibroblasts [10–12].This is the only MMP family provided of binding sites on the cell surface.
2. Collagenases, directly secreted by the cell [MMP1 (interstitial collagenase, or collagenase-1), MMP8 (collagenase-2, or neutrophil collagenase), MMP13 (collagenase-3)]. Such MMPs cleave triple helical regions of interstitial collagen types I, II, and III, but also gelatin [12].
3. Stromelysins, directly secreted by the cell (MMP3, MMP10, MMP11), whose substrates include Col II, IV, IX, X, XI, gelatin, laminin, fibronectin, elastin, aggrecan [13].
4. Matrilysins, directly secreted by the cell(MMP7, which becomes cell surface-associated through binding to cholesterol sulfate in cell membranes), whose substrates include: fibronectin, laminin, Col IV, gelatin; MMP26. Its binding to the membrane has not been demonstrated) [13].
5. The macrophage metalloelastase, secreted by the cell(MMP12, whose substrates include elastin, fibronectin, Col IV) [13].
6. The membrane-type MMPs (MMP14/15/16/17/24/25), a series of transmembrane MMPs, whose substrates include gelatin, fibronectin, laminin [13].
7. The ADAMs (short for “a disintegrin and metallo-proteases”), transmembrane and secreted metalloendopeptidases that are also provided with a “disintegrin RTD sequence” that can also inhibit interaction between cell surface integrins and extracellular-matrix macromolecules [13].

MMPs are inhibited by specific endogenous inhibitors (TIMPS, tissue inhibitors of MMPs). Four TIMPS have been described: TIMP1,2,3,4. Thus, the final activity of MMPs results from the balance between active enzymes and inhibitors, even if the gelatinases (MMP2 and MMP9) can form complexes with TIMPs even when the enzymes are in their latent form [14].

A considerable amount of observations has indicated, for many years now, that MMPs can be produced by cells resident in the joints and by inflammatory cells, such as fibroblasts, chondrocytes, osteoclasts, macrophages and neutrophils [15]. In rheumatoid arhtritis (RA) an evident over-production of MMPs has been demonstrated by rheumatoid synovial tissue [16,17].

Only recently in the synovial membrane of patients with JIA an over-expression of the collagenase MMP1 and of stromelysin MMP3has been observed, accompanied by an insufficient production of TIMP-1, leading the authors to hypothesize a possible role of MMPs in the pathogenesis of synovitis which characterizes this group of idiopathic pediatric arthropathies [18,19].

The levels of MMP1, MMP3 and TIMP-1 in paired sera and in synovial fluids of JIA patients were then studied in a longitudinal study [20]. The authors concluded that MMP-1 is up-regulated in synovial fluids in parallel with the inflammatory activity of JIA in all patients, in all subtypes of JIA and age groups, throughout the course of the disease. Furthermore, they hypothesize that MMP3 may be important for the activation of MMP1 and suggest that serum MMP3 levels may represent a useful measurable index and a specific marker of disease activity in the JIA.

Increased serum MMP3 levels were also a good disease marker in the enthesitis-related arthritis category of JIA [21], similar to what has been reported in ankylosing spondylitis and rheumatoid arthritis [22–24].Studies that indicated an important pathogenic role of IL17 in JIA have stimulated research on the ability of IL17 to stimulate enzymatic activities in synovial fibroblasts of JIA. It has been observed that IL-17 induced synovial fibroblasts from enthesitis-related JIA to produce IL-6, IL-8, MMP3, and MMP1 [25]. The same authors also showed that IL6, IL8, MMP3 and MMP1 were produced by synovial fibroblasts of JIA following stimulation with specific ligands for TLR2/3/4/5, but not for TLR7 and 9. The same ligands did not produce TIMPs overproduction, therefore the final balance is in favor of protease activity after TLR stimulation [26].

The genetic investigation has provided interesting indications both on susceptibility to contracting the disease [27] and on the predisposition to over-expressing metalloproteases: a study of the influence of MMP1 and MMP3 genetic variations on susceptibility to JIA in Egyptian population has shown that, among MMP1 1G/2G and MMP3 5A/6A polymorphisms,the aplotype 2G for MMP-1 and 6A for MMP3 were significantly associated with oligoarticular RF+ polyarthritis and systemic JIA [28].

In view of their powerful degradative activity, also the gelatinases (MMP2 and MMP9) have been studied in the synovial fluid and in the serum of patients with JIA [29]. In both fluid specimens the gelatinolytic activities of both MMP2 and MMP9 are very high in JIA during active disease and are associated with inflammatory activity, regardless of the duration of the disease and the age of the patients. These data suggest that the gelatinases may be active from the beginning of the symptoms of the disease and may constitute specific markers of inflammatory joint destruction. In the same work the authors hypothesize that MMP9 can be produced by neutrophils and macrophages, but also by synovial fibroblasts and chondrocytes [30], under the effect of lymphocyte cytokines [31]. Furthermore, both MMP9 and MMP2 are produced as zymogens and are activated by MMP3, which is found in high concentrations in synovial fluid of patients with JIA. Another paper on gelatinases activity in JIA ha confirmed a pathogenic role of gelatinases and TIMP-1 in JIA, reporting also a decrease of serum MMP9 concentration following a good response to therapy [32].

A recent paper shows that tocilizumab, a humanized monoclonal antibody which neutralizes IL-6-mediated signaling, inhibits MMP9 production by immortalized human chondrocyte lines [33]. This observation is of particular importance in view of the significantly improved outcome of JIA patients subjected to cytokine-targeted therapies, mainly targeting IL-1 and IL-6 [34].

Further, the anti-TNF therapies (Infliximab and Etanercept) have been shown to reduce the joint degradation process in JIA by inhibiting the activity of MMP2, MMP9 and MMP3, as shown by enzyme determination in saliva samples [35,36].

The Th17 and Th17/Th1 lymphocytes are currently of great interest due to recent reports of their pathogenic role in the JIA [3,37]. There is evidence of their activity in the induction of IL-6 by synovial fibroblasts, inducing an activated fibroblastic phenotype [38]. Moreover, IL17 induces osteoclastogenesis by stimulating the differentiation of monocytes / macrophages into osteoclasts [39] and stimulates osteoblasts to produce RANKL, thus inducing osteoclastogenesis [40]. Moreover RANKL, expressed on the surface of Th17, stimulates the activation of silent osteoclasts to resorptive osteoclasts [41].It is therefore evident that Th17 are important activators of osteoclastogenesis and bone resorption. The most important osteoclast bone-resorption enzymes are cathepsin K (see below) and MMP9. Actually, there is evidence of synovial fluid RANKL and MMPs increase in enthesitis-related subtype of JIA [42].

### 3.2. The cell-associated plasminogen activation system

This system is composed of the serine-proteinase urokinase plasminogen activator (uPA) and its receptor (uPAR). uPAR is not a classical receptor protein, as it is attached to the membrane by a GPI anchor. uPAR is made up of three homologous domains (DI, DII and DIII from the N-terminus). The binding site for uPA is in domain I, but the whole molecule is still required to have an efficient link of uPA. Also in this case, as for MMP2 and MMP9, the receptor binds the uPA zymogen (pro-uPA), which is activated to uPA by plasmin, in turn linked to numerous low-affinity cellular binding sites. Following the activation of uPA, the system triggers a series of proteolytic events (activation of plasminogen to plasmin, which activates the latent forms of MMP2 and MMP9 bound to their respective receptors) features which culminate with the degradation of the macromolecules of the extracellular matrixalso in synovial fibroblasts [43–45]. Malignant cells overexpress uPAR, that has become a marker of extracellular matrix destruction and of tumor invasiveness [44], and we have previously shown that RA synovial fibroblasts exhibit the fibrinolytic pattern of invasive tumor-like cells [46,47]For other properties of the cell surface plasminogen activation system, such as its signaling properties and roles in immunity, we refer to other focused reviews [48,49].

Serine-proteinases inhibitors (serpins) control the proteolytic activities of the uPA/uPAR system. Alpha-2-antiplasmin is responsible for inactivating plasmin [50], while alpha-2-macroglobulin, that does not belong to the serpin family, is a whole-range inhibitor of almost all the serine-proteinases [51]. The most important serpins that inhibit uPA activity are the Plasminogen Activator Inhibitor 1 and 2 (PAI-1 and PAI-2). PAI-1 is the main fibrinolysis inhibitorand controls uPA activity, while PAI-2 is mainly secreted by placenta and is normally present in very low concentrations [52]. Similarly to the regulation of the activity of the MMPs by the TIMPs, the final fibrinolytic activity of the invasive cells is regulated by the balance between uPA and PAI-1.

Given these premises, the cell membrane-associated fibrinolytic system has all the characteristics to account for a possible destructive activity of the synovial pannus in inflammatory arthritis. Indeed, the evidences of an important role of the uPA / uPAR system in the pathogenesis of RA are have been treated in a several reviews on the subject [53–55]. Nevertheless, research on this invasive system in the JIA is not present in the literature. The few available data indicate the presence of D-dimers in the serum of patients with JIA. In JIA the few available data indicate the presence of D-dimers in the serum of patients. An increase in D-dimers testifies to intense fibrinolysis, which is particularly pronounced in children with systemic JIA and may predict outcome over a short followup period [56]. Stimulated by the lack of information in the literature, in our laboratories we performed a study of synovial samples of healthy subjects and of patients with JIA and we found no significant differences in the amount of uPAR and uPA (unpublished results). We therefore believe that this could be an important signature that differentiates JIA from other inflammatory arthritides.

### 3.3. Cathepsins

Lysosomes contain approximately 50 degradative enzymes that are collectively grouped under the term “acid hydrolases”, which means enzymes that work best at acidic pH. Such enzymes include proteases (including cathepsins), lipases, glycosidases, nuclease, phospholipases, phosphatases, and sulfatases, and are therefore able to digest proteins, nucleic acids, carbohydrates, and lipids.

About fifteen different members of the cathepsin family are known, distinguished by structure, catalytic mechanism and substrates. The majority (cathepsins B, C, F, H, K, L, O, S, V, W and X) are cysteine proteases (which have a cysteine in the catalytic site), with the exception of cathepsins A and G, which are serine proteases, and of cathepsins D and E (aspartyl-proteases) [57]. Cysteine cathepsins K,B, L and S belong to the so called papain-like protease family and exhibit collagenolytic activity [57]. The harmful activity of cathepsins has been ascertained in tumors, neurological diseases (Alzheimer), and Rheumatoid Arthritis [58].Most cathepsins are normally confined within lysosomes [57], with some exceptions, among which the most important is cathepsin K, which is secreted by osteoclasts during the bone resorption process.

Cathepsins potentially involved in the degradation of extracellular matrices are cathepsin D and K. In fact, cathepsin D can cut fibronectin and laminin, two important structural proteins of extracellular matrices, and high levels of this cathepsin have been associated with high degree of cellular invasiveness [59]. Cathepsin K is the most powerful mammalian collagenase. Its secretion is activated in osteoclasts by the RANK/RANK-ligand mechanism [39–42]. This enzyme degrades type 1 collagen, the major component of the non-mineral bone protein matrix, gelatin and elastin [60]. Its role has been ascertained in osteolytic tumor metastases [61] and arthritis [62].

In RA, cathepsins L and B have been shown to be produced by synovial fibroblasts [63] while macrophages produce cathepsin B [64].

Five cyclic peptides of the cystatin family show inhibitory activity towards human cathepsins L, B, H, and K [65,66]. Therefore, as for all the enzymes previously considered, the activity of cathepsins depends on the ratio between enzyme and inhibitors.

Synovial fibroblasts are the major source of production of MMPs [25,26,63] and cathepsins [67] in the advanced stages of RA.In turn, macrophages that are associated with the infiltration of the synovium into the joint structures overexpress joint destructive enzymes [68]. High levels of cysteine cathepsins B, L, S are present in synovial fluid of RA patients [69]. Cathepsin B has been identified in the synovial fibroblasts, in the sites of destruction and erosion of involved RA joints [70].

Few data are available on the role of lysosomal cathepsins in the joint damage of JIA. Immunohistology of the synovial membrane in different clinical subgroups of JIA patients has shown that marked macrophage populations are present in the lining layer and sublining layer of different subgroups (oligoarticular RF-, polyarticular RF+, persistent oligoarticular) of JIA patients, with no significant differences between subgroups [71].In the first study showing data on cathepsin expression in pannus tissue of JIA [72] there was no difference between patients with monoarticular and polyarticular JIA, and similarity of overexpression of cathepsins B, D and L in synovial tissues of RA and JIA suggests that both diseases may share similarmechanism of cartilage and bone destruction. A very old observation [73] reports the presence of variably dense PMN infiltrates in the pannus-free and apparently microscopical healthy surface of articular cartilage in RA and JIA (at the time defined JCA, “juvenile chronic arthritis”). Such infiltrates exhibited cathepsin G, elastase and esterase activity. We have shown that culture supernatants of both classic and non-classic Th1, but not of Th17, produce CD106 (VCAM-1) over-expression on the membrane of synovial fibroblasts from healthy subjects [74]. This feature, which is dependent on TNF-α, determines the adhesion of circulating monocytes and PMN on synovial fibroblasts. In the same work we have

also observed that synovial fibroblasts SF of JIA patients expressed CD106 levels higher than those from healthy donors, thus exhibiting the phenotype of synovial fibroblasts activated in vitro with supernatants of Th1-clones. Our observations suggest that classic and non-classic Th1 induce a TNF-α-dependent CD106 overexpression in synovial fibroblasts, an event that incudes leukocyte retention in the joints of rheumatic patients. We have also shown that these effects were inhibited by Etanercept. Taken together, such data indicate an important role of the inflammatory cellular exudate in the pathogenesis of RA and JIA joint lesions.

Proteolytic enzymes that destroy articular cartilage and subcondral bone must be present in an active form, allowing them to degrade bone and cartilage collagens and cartilage proteoglycans (such as aggrecan). The hydrogen concentration of the enzyme microenvironment is important to allow their activity. MMPs, identified both in synovial fluid and synovial pannus in RA and JIA work at neutral pH, while acid hydrolases (and cathepsins) perform better in an acid environment. Actually, very old measurements of pH in healthy and arthritic joints synovial fluids [75] reported values of about 7.4 in healthy subjects and of 7.1–7.2 in RA patients, and indicated that pH becomes more acidic as the intensity of the inflammatory reaction increases [76]. Recent data obtained by our group on the expression and activity of carbonic anhydrase (CA) have paved the way for a better understanding of the mechanisms of acidification of the synovial fluid, taking as a model the synovial fluid and fibroblasts of JIA patients [77] where we have shown the presence and the up-regulation of CA IX and XII protein and activity, with respect to synovial fluid and synovial fibroblasts of healthy subjects. Carbonic anhydrases (carbonate hydrolases: EC 4.2.1.1) are metalloenzymes that catalyze the conversion of CO2 to bicarbonate and protons [78]. Carbonic anhydrase Iis over-expressed in the synovium of the patients with ankylosing spondylitis [79],it is abiomarker in saliva of Sjogren syndrome patients [80] and their up-regulation lowers pH in the inflammatory microenvironment, thereby facilitating the harmful activity of cathepsins and of other acid hydrolases. Such results provide the first evidence of a role of these enzymes in rheumatic pathologies and suggest the therapeutic use of specific inhibitors to develop new therapies for arthritis, including JIA [78].

The enzymes contained in the lysosomes of leukocytes are designed to act best at the acidic pH present within these organelles, in order to digest harmful agents (PAMPs, DAMPs) or to perform an “intramolecular selection” (in the antigen-presenting cells, such as macrophages) to initiate an adaptive immune response. Although the fate and the kinetics of the monocytes/macrophages in the inflammatory sites is still an unsolved problem [81] the macrophages and the PMNs are destined to undergo cellular degeneration which, in the case of the PMNs, occurs after a few hours. These cells are therefore destined to discharge their enzymatic content into the inflammatory environment. The acidity of the inflamed joints (including those of the JIA), determined by the activity of the carbonic anhydrase of synovial fibroblasts, is likely to provide a suitable microenvironment to manifest the destructive activity of the lysosomal enzymes, also once they are in a space which is non-physiological.

In summary, synovial fibroblasts of JIA are infiltrated by mononuclear leukocytes (Th17 and non classic Th1, macrophages) and PMN, thus forming a conglomeration of different cell types which is called “the invasive synovial pannus”, characterized by expression of different proteases that degrade and digest joint structures.

## 4. Bone and cartilage degradation products in the plasma of JIA patients

In the absence of typical clinical signs of arthritis, the diagnosis of JIA poses serious difficulties, and therefore the study of reliable markers detectable in the blood assumes a clinical importance to accelerate the speed of therapeutic interventions. The problem of circulating markers in systemic-JIA (SJIA) has recently been addressed in a review study [82]. The authors note that of 68 proposed circulating markers, as many as 50 have been studied and proposed by a single group. They conclude that biomarkers of high interest for SJIA diagnosis are heme-oxygenase-1, interleukin-6 (IL-6), IL-12, IL-18, osteoprotegerin, S100 calcium-binding protein A12 (S100A12) and S100A8/A9, a series of molecules that underline the mainly inflammatory nature of SJIA subtype sustained by innate immune cells such as monocytes and macrophages. Serum levels of MMP3, that some studies reported to be increases in SJIA,were included in the study but did not fulfill the criteria required by authors.

The literature on circulating markers in non-systemic JIA forms is rather poor [83]. However, a proteomic study performed on synovial fluid of patients with recurrent oligoarticular JIA, has shown proteolytic fragments of collagen X, fibrin β-chain, and T cell receptor α-region [84]. Collagen X is produced by chondrocytes during endochondral ossification and the increase of its proteolytic fragments in synovial fluid may be indicative of cartilage remodeling or destruction, in line with studies on the presence of high levels of MMP1, MMP2, MMP3, MMP9 and cathepsins in the synovial fluid and in the serum of JIA patients that have been taken into consideration in previous chapters of this review.

An interesting relationship was identified between the increase in plasma of JIA patients of keratan sulfate (KS), a degradation productof the aggrecan present in cartilage, related to increase of MMP3 and an enzyme of the ADAM family, and their reduction following therapy [85].The destruction of articular cartilage in the JIA and other forms of arthritis proceeds from the articular surface to the sub-chondral bone, thus involving articular cartilage. This destruction implies an alteration of the cartilage and bone extracellular matrix, including aggrecan and collagen, whose destruction is irreversible [86]. The aggrecan is a proteoglycan synthesized by chondrocytes, formed by a protein nucleus and a glycosaminoglycan chain (formed by alternating dimers of uronic acids and exosamines). In particular, glycosaminoglycans (mucopolysaccharides) of aggrecan are chondroitin sulfate (CS) (about 90%) and keratan sulphate (KS) [87]. Any imbalance between the rate of production and degradation of the aggrecan manifests itself with a modification of the plasma and urinary concentration of the glycosaminoglycan components [86].The same authors also report a modification of the plasma concentration of the CS moiety of aggrecan in JIA patients [88]. The increase in CS is accompanied by a decrease in chondroitin-sulfate 846 epitope, a biomarker of CS synthesis, a data which suggest that in JIA there is an imbalance between the demolition of the molecule and its biosynthesis.

However, all the studies on release in the circulation of products deriving from the degradation of joint structural molecules in JIA patients require broader case studies based on a stratification of patients allowing an exact correlation between markers and subtype of JIA.

## 5. Conclusions

In this review we focused on the various classes of proteases that can cause joint damage in the JIA, noting that the literature on the subject has identified the role of some MMPs that can be produced by resident cells such as synovial fibroblasts, chondrocytes and osteoclasts, and from inflammatory cells such as macrophages and neutrophils. Biological anti-IL6 and anti-TNF drugs inhibit the production of MMPs in JIA joints. The TH17 and TH17/TH1 lymphocytes, whose role in the pathogenesis of JIA is established, regulate the production of MMPs by synovial cells and chondrocytes, and therefore it is likely that the use of biological drugs capable of inhibiting specific interleukins may be able to control the production of MMPs in turn. Cathepsins are enzymes with a strong destructive potential in JIA joints.Cathepsin K, produced by the chondrocytes, has a powerful collagenase activity. The macrophage and granulocytic cathepsins, normally confined in the acidic environment of the lysosomes, can be active also in the synovial fluid of the JIA joints thanks to the strong overexpression of the carbonic anhydrase IX and

XII induced in the synovial fibroblasts by the TH17 and T17/TH1 lymphocytes. Such enzymes extrude H+ ions from synovial fibroblasts, thus creating an extracellular microenvironment suitable for the activity of acid hydrolases. Recent evidence on the targeted and timely use of biological drugs in the treatment of JIA [89] may therefore apply also to the control of MMPs and cathepsins activity.

There are a number of inhibitors of MMPs, previously entered into clinical trials for the treatment of tumors, which control the activity of MMPs [marimastat, batimastat, BAY 12–9566, BMS-275291, Andecaliximab (GS-5745) and bisphosphonates]. One of these inhibitors (GS-5745) has recently been used successfully in patients with RA [90].Furthermore, the RANK / RANKL / OPG pathway plays a key role in modulating the release of MMPs by osteoclasts. There is a fully humanized monoclonal antibody (Denosumab, also known as AMG 162), which has the ability to bind to RANKL similarly to osteoprotegerin, thus inhibiting the action of RANKL, which has been used in RA [91]. In view of the pathogenesis of osteoarticular lesions in RA and JIA, both types of drugs could be used for intra-articular injection in inflammatory joint diseases.There is the hope of a possible use of protease inhibitors also as a palliative cure of the macrophage activation syndrome associated with juvenile idiopathic systemic-onset arthritis [92], in which a massive release of macrophage proteases occurs that floods the plasma of patients, inducing activation of clotting and fibrinolytic factors, thereby leading to disseminated intravascular coagulation syndrome and to the fatal multiple organ failure typical of the disease.

## Take-home messages

- Th17 and Th17/Th1 lymphocytes regulate the production of MMPs by residents joint cells in JIA
- Various classes of proteases released by resident and inflammatory cells have a pathogenic role in JIA joint damage
- JIA synovial cells overexpression of carbonic-anhydrases IX and XII lowers the inflammatory microenviroment pH, promoting the activity of cathepsins and other acid hydrolases activity
- The use of targeted biological drugs reduce MMPs and cathepsins activity in synovial fluid, plasma and joints of JIA patients.

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