Danger signals and inflammaging in osteoarthritis

M. Millerand[1], F. Berenbaum[1,2], C. Jacques[1]

[1]Sorbonne University, INSERM (UMR S938) and Labex Transimmunom, Paris, France; [2]Department of Rheumatology, AP-HP, APHP-Sorbonne University, Paris, France.

Marion Millerand
Francis Berenbaum, MD, PhD
Claire Jacques, PhD

Please address correspondence to:
Dr Francis Berenbaum,
UMR S938, CDR Saint-Antoine,
INSERM, Sorbonne Université,
184 Rue du Faubourg Saint-Antoine,
75012 Paris, France.
E-mail: francis.berenbaum@sat.aphp.fr

Received on July 18, 2019; accepted on September 4, 2019.

Clin Exp Rheumatol 2019; 37 (Suppl. 120): S48-S56.

Key words: DAMPs, inflammaging, osteoarthritis, innate immunity, TLR

Funding: this work was supported by grants from INSERM, Sorbonne University, French Society of Rheumatology (Société Française de rhumatologie) and Fondation Arthritis - Courtin.
M. Millerand was supported by a doctoral fellowship from Ministère del'Enseignement Supérieur et de la Recherche.

Competing interests: none declared

ABSTRACT

Osteoarthritis (OA) is the most common age-related chronic and disabling joint disease. Long considered to be a "wear and tear" disease, OA is now seen as a low-grade inflammation disease that affects all tissues of the joint, involving cartilage degradation, bone remodelling, osteophytes, and synovitis. The process, called inflammaging, is characterised by the association of low-grade inflammation, profound changes in intra-cellular mechanisms, and the decreased efficiency of the immune system with ageing. The activation of innate immunity plays a critical role in the development and progression of OA. Innate immunity, including inflammasome activation, is triggered by small endogenous molecules called alarmins or damage-associated molecular patterns (DAMPs). These molecules are released in the extracellular media after cell stress or damage, bind to pathogen-recognition receptors (PRRs), such as Toll-like receptors (TLRs) and the receptor for advanced glycation end products (RAGE), and activate the secretion of pro-inflammatory factors, leading to joint inflammation. Moreover, such sterile inflammation triggers cell senescence, characterised by a senescence-associated secretory phenotype (SASP). Understanding the substantial age-related changes of joint tissues that influence the pathogenesis of OA is critical to improving the quality of life of elderly people in the context of increased life expectancy. This review will focus on age-related sterile inflammation in OA and highlight the various innovative and promising therapies targeting the mechanisms of aging.

Inflammaging in osteoarthritis

Osteoarthritis (OA) is the most common and disabling joint disease worldwide and aging is the most important risk factor for its development (1). Typically considered to be mechanically induced, it has taken many years to accept that low-grade inflammation may also play a role in the OA process. This discovery came from the observation that an inflammatory state, although lower than in rheumatoid arthritis (RA), is present and is detectable both locally and systemically (2). The levels of several inflammatory mediators, such as IL6, IL-1β, and MCP1, are higher in OA than healthy sera (3, 4). In addition, the aging process is also associated with chronic, low-grade inflammation, also known as inflammaging (5). Aging is a multifactorial process, during which many alterations and damage appear at the molecular, cellular, and tissue level (6). Aging cells undergo alterations of their properties, such as genomic instability, telomere attrition, epigenetic alterations, and the loss of proteostasis, resulting in a senescent-cell phenotype. Consequently, impaired regulation of oxidative stress in some types of aging cells causes the pro-inflammatory production of various mediators, shifting their phenotype to a so-called "senescence-associated secretory phenotype" (SASP) (7-9). It is noteworthy that aging shares similar mechanisms with those found in OA, including low-grade, chronic, sterile inflammation (10, 11). In aging, inflammation is partially due to the dysregulation and reduced efficiency of the immune system.

In both OA and inflammaging, inflammation is mostly driven by damage-associated molecular patterns (DAMPs), also called alarmins, a term proposed by Oppenheim *et al*. (12). Throughout aging, the body is challenged by a myriad of antigens and stressors. The resulting oxidative stress causes, at least in part, the release of DAMPs. In OA, the main hypothesis is that cartilage matrix fragments are released into the synovial cavity and stimulate the synovium. The synovial cells (macrophages and fibroblasts) react by producing inflammatory mediators, such as cytokines, chemokines, lipid mediators, and

DAMPs, into the synovial fluid (SF). These SF mediators then activate chondrocytes to produce metalloproteinases, leading to a vicious circle (13). As DAMPs trigger the immune system, they likely contribute to the pathological association of ageing and OA (6, 14). Indeed, the local and systemic effects of DAMPs are numerous and largely contribute to the aging process and the progression of OA. They interact with pathogen-recognition receptors (PRRs), such as Toll-like receptors (TLRs) and the receptor for advanced glycation end products (RAGE). They also trigger the intracellular NLRP3 inflammasome (15). Overall, DAMPs exhibit pro-inflammatory effects that increase during aging, as well as during OA progression. As molecular targets, they constitute interesting therapeutic leads. Further research on alarmins needs to be conducted to understand their involvement in OA and inflammaging.

Age related-DAMPs involved in osteoarthritis

Throughout aging, the body is challenged by a multitude of antigens and stressors. The resulting oxidative stress causes the release of DAMPs. Whether due to aging or OA, the release of DAMPs increases and sustains inflammation, creating a state of chronic low-grade inflammation.

Physiologically, these molecules have many intracellular roles but can also be released into the extracellular media, both passively by stressed, damaged, or necrotic cells or actively through exosomes by immune cells (13). They trigger PRRs to alert the innate immune system and increase local inflammation and cartilage degradation (16). Age-related accumulation of reactive oxygen species from mitochondria has been demonstrated to be a major source of DAMP release (17). A large number of intracellular molecules have been shown to be alarmins, such as high mobility box 1 (HMGB1), uric acid (UA), ATP, S100 proteins, and heat shock proteins (HSPs). In addition, extracellular matrix fragments (polysaccharides and proteoglycans) cleaved during pathological conditions, such as OA, are able to activate PRRs (14, 16). The list of alarmins is still growing and their structural characteristics vary greatly. Several alarmins are specifically involved in aging and OA, such as HMGB1, S100A8/A9, UA, and hyaluronan fragments (15) (Fig. 1).

HMGB1 is a member of a group of non-histone nuclear proteins. This DAMP of 30 kDA is highly conserved throughout evolution and exerts essential functions inside and outside the cell (6). Oxidative stress and injury are highly involved in aging (17) and induce the active release of HMGB1 by immune cells or passive release by necrotic cells. Moreover, HMGB1 levels are elevated in aged mouse kidney and liver and associated with macrophage infiltration (18). HMGB1 levels are higher in the SF of OA patients than that of controls (19) and have an extracellular pro-inflammatory role. This alarmin is able to bind to various receptors, such as RAGE, TLR2, and TLR4 (20). The interaction between HMGB1 and RAGE induces cytokine expression (IL-17) (15). Although the release of HMGB1 increases during aging, mainly due to its release by immune cells and damaged cells, its expression decreases in aged tissues (21).

The S100 family is comprised of low molecular weight proteins. They share common features with calmodulin and have two calcium-binding motifs. S100 proteins are involved in many intracellular processes, such as calcium homeostasis, cell growth, and proliferation. Like all alarmins, S100 proteins have pathophysiological roles when released outside of cells. For example, S100A8, A9, A12, and S100B have extracellular pro-inflammatory effects (15). Their expression varies throughout aging. They bind both to TLR and RAGE (22) but the main receptor of S100 is TLR4. The binding of S100A9 to TLR4 induces nuclear factor NF-κB activation. TLR4 appears to be crucial for the induction of most cytokines, whereas RAGE is involved mainly in IL-1β secretion (23).

UA is an intracellular chemical product that results from the degradation and excretion of purines and is excreted through urine in humans. UA is constitutively produced in normal cells and can be found both in the nucleus and cytoplasm. When its concentration exceeds the limit of solubility (6.8 mg/dL), UA forms crystals, which activate the inflammasome (24). UA levels increase in injured cells and it is released by dying cells (25). UA is a danger signal and a marker of OA severity. A clinical study suggested a possible role of elevated serum UA in the multifactorial aetiology of generalised OA (26). Increasing evidence shows that UA may be a factor that promotes the pathological process of OA through activation of the inflammasome (27). A correlation has been found between the levels of cytokines (IL-18 and IL-1β) produced by UA-activated inflammasomes and UA, showing the importance of this DAMP in the activation of the innate immune system in OA.

Overall, these observations show that DAMPs are highly involved in the age-related progression of OA.

DAMPs trigger innate immunity through RAGE and TLRs

The immune system undergoes profound changes during aging in a process called immunosenescence. Immunosenescence is primarily caused by clonal expansion among T and B cells and the exhaustion of the pool of naïve T cells. This process thus decreases the body's ability to respond to new antigens (28). PRRs are critical for the activation of the immune system of the host due to their ability to recognise both pathogen-associated molecular patterns (PAMPs) and DAMPs. TLRs and RAGE are the most well know receptors involved in the activation of immunity. They share many ligands and can physically interact (29).

TLRs are a family of PRRs crucial for the activation of innate immunity in response to the detection of pathogens and cell/tissue damage (30). The 10 isoforms of TLRs expressed by mammals participate in both PAMP and DAMP recognition. Many cell types express TLRs, but they are mostly expressed by innate immune cells, including macrophages. Elevated TLR4 expression and pro-inflammatory signalling have been observed in the muscles of older individuals (31). Studies have shown

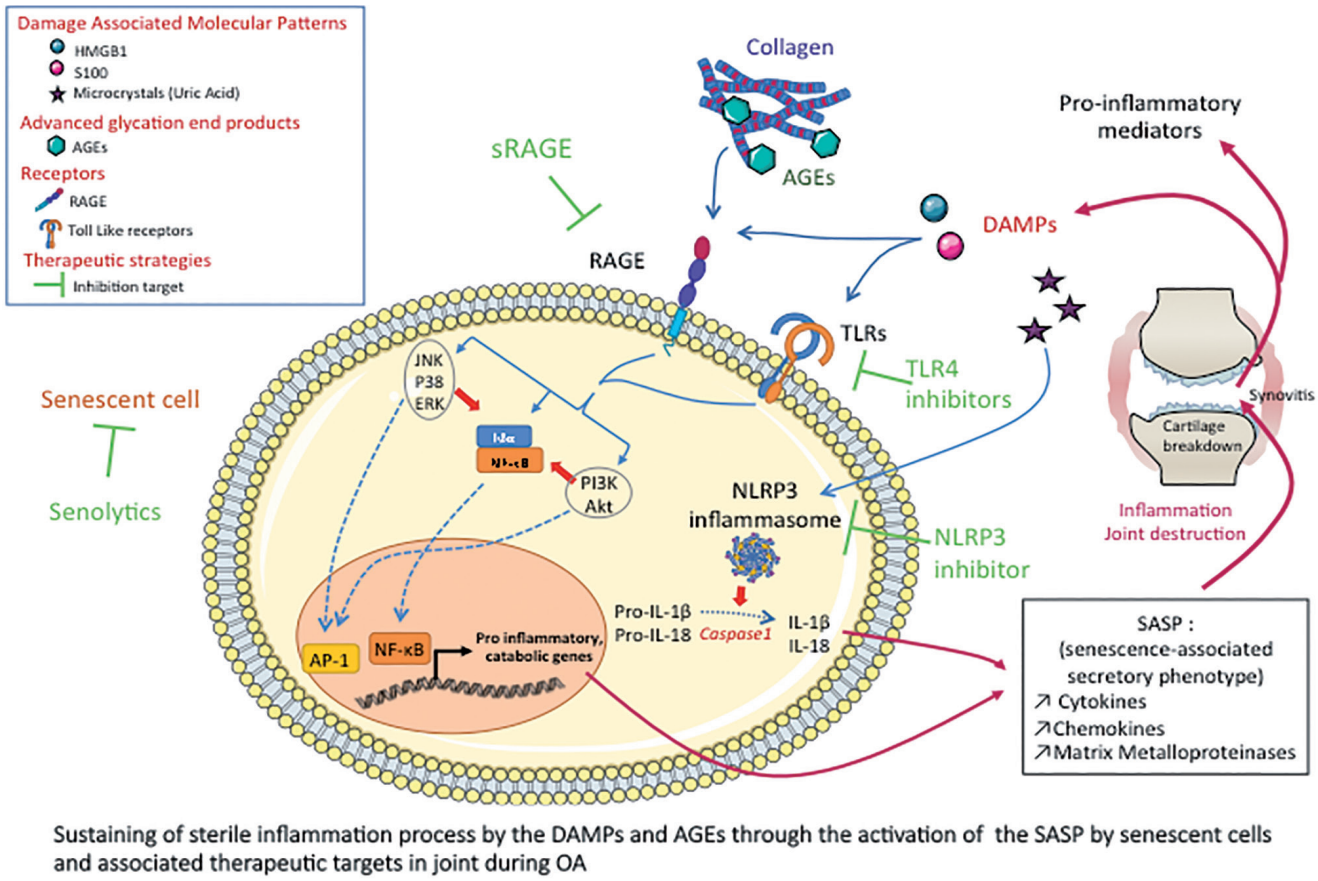

Fig. 1. Sustaining of sterile inflammation process by the DAMPS and AGEs through the activation of the SASP by senescent cells and associated therapeutic targets in joint during OA.
Osteoarthritis (OA) and aging share a common characteristic: a chronic, sterile low grade inflammation. The resulting synovitis provokes the release of damage signals by activated synovial cells, the DAMPS (HMGB1, S100, Uric acic). Moreover, the presence of AGEs on collagen fibrils increases through aging. AGEs and DAMPS accumulation in the joint induce MAPK and NF-κB inflammatory pathways in senescent cells through the activation of 2 main types of receptors, RAGE and the TLRs as well as the NLRP3 inflammasome. Then, senescent cells of the joint release pro-inflammatory and pro-catabolic factors (cytokines, chemokines, Matrix Metalloproteinases), which is called "senescence-associated secretory phenotype" (SASP). These mediators released in the synovial fluid worsen the inflammation of the synovium and the cartilage breakdown, leading to a positive-feedback loop. Taken together this phenomenon enhances and sustains a vicious circle of inflammation in the joint. Few emerging therapeutic strategies are under investigations and focus on inhibiting RAGE with soluble RAGE, TLR4 and NLRP3 with specific inhibitors and kill specifically the senescent cell called "senolyse".

that human TLR function is impaired during the aging process. TLR signalling, and NF-κB signalling differ in aged macrophages relative to young macrophages (32) Moreover, there is evidence of the inappropriate persistence of TLR activation associated with aging (33).

RAGE is a multi-ligand receptor of the immunoglobulin superfamily of cell surface molecules expressed in mammals. This receptor is broadly expressed in all organs (lung, liver, kidney, skeletal muscle, and heart (34)). Immune cells of both the innate and adaptative immune response express this receptor (35-37). It is composed of two terminal domains, a "V"-type domain, which recognises extracellular ligands and a "C"-type domain, which mediates signalling through the MAPK and NF-κB pathways (13). RAGE expression increases with aging (38). The activation of RAGE leads to increased stimulation of chondrocytes and synoviocytes in OA (39). RAGE was first studied as the receptor of advanced glycation end products (AGEs) which are elevated in aging tissues, especially cartilage (40). The accumulation of AGEs in collagen during aging alters the mechanical features of the cartilage extracellular matrix (41). Aside from binding by AGEs, RAGE interacts with multiple PAMPs and DAMPs. RAGE deletion in mice protects against age-related diseases, showing its role in aging (42).

TLRs and RAGE trigger inflammatory intra-cellular pathways using various adaptor proteins or effectors, such as TIRAP, MyD88, or mDia1 (43, 44). These intermediates activate intracellular pathways, including mainly the NF-κB pathway and the MAPK pathway via the ERK, JNK, and p38 kinase signalling cascade. Activation of these inflammatory pathways induces the expression of catabolic and inflammatory genes that participate in the induction of matrix metalloproteinase (MMP)-13, a collagenase with high selectivity towards collagen-II (14) (Fig. 1).

Contribution of the inflammasome to inflammaging in osteoarthritis

The inflammasome, first described by Martinon in 2002 (45), has been identified as an important driver of sterile inflammation and is a key contributor to the innate immune response seen in

the aging population (46-48). There is accumulating evidence that the inflammasome plays an important role during inflammaging (10, 49).

The inflammasome functions as a molecular platform for caspase-1-dependent proteolytic maturation and the secretion of IL-1β and IL-18 (50). It is composed of a nucleotide-binding and oligomerisation domain-like receptor-containing protein (NLRP) as a sensor, an adapter protein called apoptosis-associated speck-like protein (ASC), containing an N-terminal caspase recruitment domain, and procaspase-1 (51). The best characterised member is NLRP3, which is highly expressed in macrophages, chondrocytes, synoviocytes, and osteoblasts (52). The NLRP3 inflammasome is activated by two steps; the priming step (signal 1) is provided by inflammatory stimuli, such as TLR4 agonists, and the activation step (signal 2) is triggered by PAMPs and DAMPs (53, 54) Interestingly, active caspase-1 has been associated with the release of HMGB1 (55). Regulation of the NLRP3 inflammasome, including by post-translational modifications (56, 57), is essential for maintaining adequate immune protection while preventing tissue damage from the overproduction of cytokines, which can cause chronic inflammation and excessive cell death (11, 56, 58).

In OA, the principal DAMPs that activate NLRP3 are crystals, such as those containing basic calcium phosphate (BCP), calcium pyrophosphate dihydrate (CPPD), or UA (52). These microcrystals are interpreted as 'danger signals' by the innate immune system and contribute to inflammation (59). Various types of phosphate crystals are found in the joints of OA patients in association with radiographic lesions (60). Both *in vitro* and *in vivo* data demonstrate that NLRP3 is activated by hydroxyapatite crystals, suggesting that NLRP3 is responsible for the pathogenic effects of hydroxyapatite in OA, driving cartilage degeneration and synovitis through the production of IL-1β, IL-18, and matrix-degrading enzymes (61).

UA is released from dying cells as a danger signal. In knee OA, SF UA levels correlate with disease severity and SF IL-1β and IL-18 levels, suggesting that it is a driver of OA pathology through the activation of NLRP3 (27) (Fig. 1). Indeed, UA may be used as a marker of disease severity.

Moreover, other studies have shown that OA cartilage can be degraded independently of inflammasome activity and that the IL-1β involved in OA cartilage degradation may not be produced by the cartilage itself but rather by synovial tissue. Thus, synovial tissue may have an important impact on cartilage degradation via NLRP3 activation (62).

Cell senescence and the SASP in osteoarthritis

One of the mechanisms by which aging promotes the chronic inflammation that may be important in OA is cell senescence, a concept first described by Hayflick and Moorhead (63). Cellular senescence is characterised by the accumulation of senescent cells (SnCs) in degenerated tissues (64) and the increase in the expression of certain senescence markers. SnCs contribute to aging and shorten health span (24). They accumulate in OA joints near the OA lesion and their quantity positively correlates with OA severity (65). Such an accumulation of SnCs may predispose joints to OA development (66). The accumulation of SnCs in degenerative OA cartilage was first described by Price *et al*. in 2002 (67). The term "chondrosenescence" refers to all age-dependent deterioration of chondrocytes as a consequence of replicative and stress-induced factors. There is a strong correlation between inflammaging, chondrosenescence, the presence of the inflammasome, and autophagy. Recent data demonstrate that SnCs also accumulate in the synovium, as well as at the cartilage surface, during experimental OA (68).

SnCs are characterised by the association of multiple markers, including SAβ-gal staining (senescent-associated-β-galactosidase) (69) and the upregulated expression of p16INK4A (70). Moreover, SnCs have an important role in aging through the secretion of matrix-degrading proteins and proinflammatory cytokines, which is called a "senescence-associated secretory phenotype" or SASP (7, 41, 71, 72) (Fig. 1).

Studies have highlighted SAβ-gal staining in OA cartilage (73). Indeed, it is the most commonly used senescence marker, due to its easy detection (74).

The selective inhibitor of cyclin-dependent kinases CDK4 and CDK6, p16INK4A, is the second most widely used senescence marker after SAβ-gal. The expression of p16INK4A in SnCs is elevated and participates in cell-cycle arrest (75). Aging organisms accumulate SnCs expressing p16INK4A in various tissues (76). During the processes of cartilage aging, it may be partially responsible for the senescence of chondrocytes, such as that seen in OA (76). Indeed, significantly higher p16INK4a levels have been detected in OA chondrocytes than in age-matched healthy chondrocytes (77). Moreover, P16INK4 silencing results in increased proliferation and decreased SAβ-gal staining of OA chondrocytes (76). Finally, p16INK4A has been detected in multiple tissues in arthritic joints, including cartilage, subchondral bone, and synovium (67-68, 78-79).

SnCs promote low-level local chronic inflammation through SASP factors, such as inflammatory cytokines and MMPs (80, 81). The SASP is a bioactive secretome produced by SnCs, which remain metabolically active, despite their not being proliferative (7). The SASP is associated with the production of proinflammatory cytokines (IL-6, IL-1α/β), chemokines (MCP-1, IL-8, GROa), growth factors (TGF-β, VEGF, IGFBP7), and proteases (MMP-3) (82). The specific secretory profiles of SnCs are highly cell- and context-dependent. SnCs may have distinct effects, depending on the inducing stress and/or the local tissue environment (83-86). Indeed, the SASP propagates senescence from cell to cell through paracrine mediators, thus exacerbating the pro-aging effects of SnCs. SASP regulators converge on the NF-κB signalling pathway (87). NF-κB activation and subsequent SASP production may also be initiated by oxidative stress and increased reactive oxygen species (88). Moreover, AGEs, as well as HMGB1, also activate the NF-κB pathway through their interaction with RAGE (89, 90).

Numerous SASP components described in SnCs are also present in OA tissues or SF (91). The proinflammatory cytokines IL-1β, TNF-α, IL-6, and IL-8 are particularly relevant to OA. These cytokines have been shown to promote OA progression, notably by inducing synovitis and by altering chondrocyte function and viability (91). IL-1β and TNF-α drive chondrocytes toward a senescent phenotype (78), inducing the secretion of a SASP containing IL-6, IL-8, MMP-13, MMP-1, MMP-9, MMP-3, MMP-14, and ADAMTS 4, 5, and 9, as well as PGE2 and NO, which participate in the pathogenesis of OA. IL-1β and TNF-α are the most widely used cytokines to induce an OA-like phenotype in chondrocytes *in vitro* (92, 93) and are now recognised to be inducers of senescence (94, 95). IL-6 and IL-8 levels are elevated in the SF of OA patients (96-98). Moreover, MMP-13 and MMP-1 are secreted by IL-1β-induced senescent chondrocytes and degrade aggrecan and type II collagen (78). Many chemokines are produced in OA, but MCP-1 is considered to be a major component of the SASP. The level of MCP-1 in SF positively correlates with OA severity and pain (99, 100). There are multiple possible connections between the synovium, SnCs, and OA. Indeed, SnCs in other joint tissues can affect the synovium through their SASPs, which can attract and modulate resident and migrating immune cells in the synovium through the action of multiple chemokines and cytokines (101). Cells in the synovium are activated and proliferate (102) and may themselves become more susceptible to undergoing senescence. In the ACLT-induced OA model, p16INK4A-positive SnCs are present in the synovium (68). Moreover, subchondral bone also appears to be involved in senescence. VEGF is a common component of the SASP and can participate in the dysregulation of bone remodelling through promoting osteophyte formation. Osteocytes retrieved from bone tissue of old animals have been found to be senescent. Moreover, these SnCs have been shown to be involved in age-related bone loss (103). Thus, SnCs in bone can have a significant physiological impact and may produce the subchondral bone changes and bone remodelling that are relevant to OA progression.

In addition to the increase of these classical markers of senescence, the decrease or loss of certain other markers may also be useful for the identification of senescent cells. Among such markers, HMGB1, which is localised to the nucleus in non-senescent cells, is secreted by SnCs, making the loss of nuclear staining for HMGB1 a sign of senescence (86). Thus, alarmins and DAMPs connect the presence of SnCs with tissue damage and repair responses, such as those in joint trauma. HMGB-1 is secreted by SnCs into the extracellular space (86) and stimulates the SASP through its binding to various receptors, such as RAGE or TLR4. A correlation between the total number of HMGB-1-positive chondrocytes and the OARSI score has been reported (104). Immunohistochemistry analysis of synovial membranes has shown that HMGB1 expression shifts from strictly nuclear localisation in healthy individuals to nuclear and cytoplasmic localisation in OA patients (19). Moreover, HMGB-1 levels in both the synovium and SF of OA patients are significantly higher than in that of healthy individuals (19). In addition the clearance of SnCs was found to reestablish the nuclear staining of HMGB1 in post-traumatic OA models (68). S100A8, another alarmin involved in OA, which increases with age, has recently been shown to be capable of inducing cellular senescence-like changes in bovine oviduct epithelial cells. Thus, the role of S100A8 in the inflammaging of OA needs to be explored in joint tissues (105).

Finally, recent findings suggest that SnCs can transmit limited senescent phenotypes to nearby cells, called secondary or paracrine senescence (106, 107). SnCs may exert central effector functions in the local tissue environment through extracellular vesicles (EVs), which are potential cellular communication tools. Indeed, SnCs secrete more EVs than their non-senescent counterparts (108, 109). In a recent study, senescent chondrocytes isolated from OA patients were found to secrete more EVs than non-senescent chondrocytes. These EVs are able to inhibit cartilage extra-cellular matrix deposition by healthy chondrocytes and can induce a senescent state in nearby cells (110).

The differences in SnC phenotypes and local environmental cues may determine which drugs are required for SnC clearance. Thus, tissue-specific SASP profiles should be studied to elucidate the detailed mechanistic roles of SnCs in the various joint structures (Fig. 1).

Future therapeutic targets in the inflammaging of OA

There are currently no effective treatments to stop or prevent OA, although many advances have been made in understanding the pathophysiological processes of this disease. Our current understanding of inflammaging puts innate immunity at the centre of this process. PRRs, which recognise DAMPs and induce inflammation, are key elements of innate immunity. The accumulation of DAMPs with age highlights the importance of studying PRRs (in particular TLRs and RAGE) as potential therapeutic targets in OA.

Several important ligands of RAGE increase or accumulate with age, such as HMGB1, S100s, and AGEs (18, 111), thus promoting increased low-grade, sterile inflammation with age. Contrary to full-length RAGE, sRAGE is thought to be beneficial by acting as a decoy for RAGE ligands and/or by interacting with RAGE at the membrane, thus preventing RAGE signalling. A newly generated sRAGE murine model which overexpresses circulating sRAGE able to block RAGE signalling and/or serve as a decoy for RAGE ligands could help to untangle the relationship between RAGE, inflammaging, and ageing (112). Such sRAGE studies in mice have the potential to help improve our understanding of OA in humans and open new therapeutic strategies (Fig. 1).

DAMPs are able to further perpetuate TLR activation. A TLR4 monoclonal antibody has recently demonstrated an adequate safety profile, and a phase II clinical trial in patients with rheumatoid arthritis has been launched (113). However, no small molecules or other TLR inhibitors have yet been tested in OA

(114). Some of these drugs, targeting MyD88-mediated inflammatory pathways, are now being tested in phase I studies for several human diseases, but none on OA (115, 116). Moreover, specific blockade of TLR4 by natural compounds, such as ginsenosides or ginger-derivatives, have been shown to exert anti-inflammatory actions (117-119) (Fig. 1).

The NLRP3 inflammasome is an attractive therapeutic target due to its involvement in the pathogenesis of OA. The identification of inhibitory agents that are specific for NLRP3 is expected to provide the most potent therapeutic strategies (120). Several approaches are currently being investigated to target various components of the inflammasome directly or through involved pathways (52, 121). MCC950, a small-molecule inhibitor, specifically inhibits NLRP3 inflammasome activation and IL-1β secretion by preventing NLRP3-induced ASC oligomerisation. Additional research is needed to clarify the use of NLRP3 as a biomarker to assist clinical decisions and it could also be a useful biomarker for the diagnosis and monitoring of OA (52) (Fig. 1).

Another emerging therapeutic strategy for age-related diseases is to specifically kill SnCs to prevent detrimental secretion of SASP-related factors. Selective removal of senescent chondrocytes from OA patients, which decreases the expression of senescence and inflammatory biomarkers and increases the expression of ECM proteins, shows promise (68). Preclinical data support the concept of selective targeting of senescent chondrocytes as a novel therapeutic strategy for OA. Direct targeting and killing of SnCs provides a potential opportunity to eliminate the source of OA disease. Several senolytics have been identified and new and/or more selective drugs are continuing to be developed. Senolysis of SnCs has been achieved by the inhibition of antiapoptotic proteins, including the BCL-2 family members BCL-2, BCL-XL, and BCL-W (122, 123). For example, the senolytic molecule ABT-263 counteracts their antiapoptotic functions, permitting SnCs to initiate apoptosis. Small molecules that inhibit antiapoptotic pathways have been shown to eliminate SnCs that develop after joint trauma and those present in cultured human OA chondrocytes (68) (Fig. 1).

Another example of a selective SnC killing strategy is the inhibition of HSP90, a ubiquitously expressed molecular chaperone (124). HSP90 inhibitors, such as geldanamycin and tanespimycin, can induce apoptosis of SnCs by multiple mechanisms (125). Directly targeting mechanisms that induce SnCs in OA, and cartilage in particular, may also lead to new therapeutic targets, although there is always the danger of promoting cancer in such strategies. SIRT6 depletion can induce senescence in human chondrocytes, suggesting that the upregulation of this protein deacetylase could suppress senescence and the SASP (125). Finally, the interactions between SnCs and stem cells have attracted recent attention, given the prominent role of regenerative medicine in age-related diseases (126).

Conclusion

In conclusion, OA is a complex multifaceted disease associated with aging and inflammation, making inflammaging a pivotal process in its pathogenesis. As a result, research and development focusing on multiple therapeutic targets, as well as many diverse treatment modalities based on senescence processes, is now ongoing, with the hope of curing OA.

References

1. JOHNSON VL, HUNTER DJ: The epidemiology of osteoarthritis. *Best Pract Res Clin Rheumatol 2014*; 28: 5-15.
2. SCANZELLO CR, LOESER RF: Inflammatory activity in symptomatic knee osteoarthritis: not all inflammation is local. *Arthritis Rheumatol* 2015; 67: 2797-800.
3. SOHN DH, SOKOLOVE J, SHARPE O *et al*.: Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. *Arthritis Res Ther* 2012; 14: R7.
4. ATTUR M, STATNIKOV A, ALIFERIS CF *et al*.: Inflammatory genomic and plasma biomarkers predict progression of symptomatic knee OA (SKOA). *Osteoarthritis Cartilage* 2012; 20: S34-S35.
5. FRANCESCHI C, BONAFÈ M, VALENSIN S *et al*.: Inflamm-aging: an evolutionary perspective on immunosenescence. *Ann NY Acad Sci* 2000; 908: 244-54.
6. HUANG J, XIE Y, SUN X *et al*.: DAMPs, ageing, and cancer: The "DAMP Hypothesis." *Ageing Res Rev* 2015; 24(Pt. A): 3-16.
7. COPPE J-P, PATIL CK, RODIER F *et al*.: Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 2008; 6: e301.
8. WANG S: Characterization Of IGFBP-3, PAI-1 and SPARC mRNA expression in senescent fibroblasts. Mechanisms of Ageing and Development 1996; 92: 121-32.
9. KUMAR S, MILLIS AJ, BAGLIONI C: Expression of interleukin 1-inducible genes and production of interleukin 1 by aging human fibroblasts. *Proc Natl Acad Sci USA* 1992; 89: 4683-87.
10. FRANCESCHI C, ZAIKIN A, GORDLEEVA S *et al*.: Inflammaging 2018: An update and a model. *Semin Immunol* 2018; 40:1-5.
11. FELDMAN N, ROTTER-MASKOWITZ A, OKUN E: DAMPs as mediators of sterile inflammation in aging-related pathologies. *Ageing Res Rev* 2015; 24: 29-39.
12. OPPENHEIM JJ, YANG D: Alarmins: chemotactic activators of immune responses. *Curr Opin Immunol* 2005; 17: 359-365.
13. NEFLA M, HOLZINGER D, BERENBAUM F, JACQUES C: The danger from within: alarmins in arthritis. *Nat Rev Rheumatol* 2016; 12: 669-83.
14. ROSENBERG JH, RAI V, DILISIO MF, AGRAWAL DK: Damage associated molecular patterns in the pathogenesis of osteoarthritis: potentially novel therapeutic targets. *Mol Cell Biochem* 2017; 434: 171-79.
15. CHUNG HY, KIM DH, LEE EK *et al*.: Redefining chronic inflammation in aging and age-related diseases: proposal of the senoinflammation concept. *Aging Dis* 2019; 10: 367.
16. VAN DEN BOSCH MHJ: Inflammation in osteoarthritis: is it time to dampen the alarm(in) in this debilitating disease?: The importance of alarmins in OA. *Clin Exp Immunol* 2019; 195: 153-66.
17. TANG D, KANG R, ZEH HJ, LOTZE MT: High-Mobility Group Box 1, Oxidative Stress, and Disease. *Antioxid Redox Signal* 2011; 14: 1315-35.
18. SON M, CHUNG W-J, OH S *et al*.: Age dependent accumulation patterns of advanced glycation end product receptor (RAGE) ligands and binding intensities between RAGE and its ligands differ in the liver, kidney, and skeletal muscle. *Immun Ageing* 2017; 14: 12.
19. KE X, JIN G, YANG Y *et al*.: Synovial fluid HMGB-1 levels are associated with osteoarthritis severity. *Clin Lab* 2015; 61: 809-18.
20. VAN BEIJNUM JR, BUURMAN WA, GRIFFIOEN AW: Convergence and amplification of toll-like receptor (TLR) and receptor for advanced glycation end products (RAGE) signaling pathways via high mobility group B1 (HMGB1). *Angiogenesis* 2008; 11: 91-99.
21. ENOKIDO Y, YOSHITAKE A, ITO H, OKAZAWA H: Age-dependent change of HMGB1 and DNA double-strand break accumulation in mouse brain. *Biochem Biophys Res Commun* 2008; 376: 128-33.
22. SUNAHORI K, YAMAMURA M, YAMANA J *et al*.: The S100A8/A9 heterodimer amplifies proinflammatory cytokine production by

macrophages via activation of nuclear factor kappa B and p38 mitogen-activated protein kinase in rheumatoid arthritis. *Arthritis Res Ther* 2006; 8: R69
23. RIVA M, KÄLLBERG E, BJÖRK P *et al*.: Induction of nuclear factor-κB responses by the S100A9 protein is Toll-like receptor-4-dependent. *Immunology* 2012; 137: 172-82.
24. MARTINON F, PÉTRILLI V, MAYOR A, TARDIVEL A, TSCHOPP J: Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006; 440: 237-41.
25. SHI Y, EVANS JE, ROCK KL: Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 2003; 425: 516-21.
26. SUN Y, BRENNER H, SAUERLAND S: Serum uric acid and patterns of radiographic osteoarthritis - the Ulm Osteoarthritis Study. *Scand J Rheumatol* 2000; 29: 380-86.
27. DENOBLE AE, HUFFMAN KM, STABLER TV *et al*.: Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation. *Proc Natl Acad Sci USA* 2011; 108: 2088-93.
28. FREUND A, ORJALO AV, DESPREZ P-Y, CAMPISI J: Inflammatory networks during cellular senescence: causes and consequences. *Trends Mol Med* 2010; 16: 238-46.
29. IBRAHIM ZA, ARMOUR CL, PHIPPS S, SUKKAR MB: RAGE and TLRs: Relatives, friends or neighbours? *Mol Immunol* 2013; 56: 739-44.
30. SABROE I, PARKER LC, DOWER SK, WHYTE MKB: The role of TLR activation in inflammation. *J Pathol* 2008; 214: 126-35.
31. GHOSH S, LERTWATTANARAK R, GARDUNO JDE J *et al*.: Elevated muscle TLR4 expression and metabolic endotoxemia in human aging. *J Gerontol A Biol Sci Med Sci* 2015; 70: 232-46.
32. PONNAPPAN S, PONNAPPAN U: Aging and immune function: molecular mechanisms to interventions. *Antioxid Redox Signal* 2011; 14: 1551-85.
33. SHAW AC, PANDA A, JOSHI SR, QIAN F, ALLORE HG, MONTGOMERY RR: Dysregulation of human Toll-like receptor function in aging. *Ageing Res Rev* 2011; 10: 346-53.
34. BRETT J, SCHMIDT AM, YAN SD *et al*.: Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues. *Am J Pathol* 1993; 143: 1699-712.
35. MANFREDI AA, CAPOBIANCO A, ESPOSITO A *et al*.: Maturing dendritic cells depend on RAGE for in vivo homing to lymph nodes. *J Immunol* 2008; 180: 2270-75.
36. CHEN Y, AKIRAV EM, CHEN W *et al*.: RAGE ligation affects T cell activation and controls T cell differentiation. *J Immunol* 2008; 181: 4272-78.
37. AKIRAV EM, PRESTON-HURLBURT P, GARYU J *et al*.: RAGE expression in human T cells: a link between environmental factors and adaptive immune responses. *PLoS One* 2012; 7.
38. CASSELMANN C, REIMANN A, FRIEDRICH I, SCHUBERT A, SILBER R-E, SIMM A: Age-dependent expression of advanced glycation end product receptor genes in the human heart. *Gerontology* 2004; 50: 127-34.
39. STEENVOORDEN MMC, HUIZINGA TWJ, VERZIJL N *et al*.: Activation of receptor for advanced glycation end products in osteoarthritis leads to increased stimulation of chondrocytes and synoviocytes. *Arthritis Rheum* 2006; 54: 253-63.
40. LOESER RF, YAMMANI RR, CARLSON CS *et al*.: Articular chondrocytes express the receptor for advanced glycation end products: Potential role in osteoarthritis. *Arthritis Rheum* 2005; 52: 2376-85.
41. LOESER RF, COLLINS JA, DIEKMAN BO: Ageing and the pathogenesis of osteoarthritis. *Nat Rev Rheumatol* 2016; 12: 412-20.
42. PARK S, YOON SJ, TAE HJ, SHIM CY: RAGE and cardiovascular disease. *Front Biosci* 2011; 16: 486-97.
43. SAKAGUCHI M, MURATA H, YAMAMOTO K *et al*.: TIRAP, an adaptor protein for TLR2/4, transduces a signal from RAGE phosphorylated upon ligand binding. *PLoS One* 2011; 6: e23132.
44. MANIGRASSO MB, PAN J, RAI V *et al*.: Small molecule inhibition of ligand-stimulated RAGE-DIAPH1 signal transduction. *Sci Rep* 2016; 6: 22450.
45. MARTINON F, BURNS K, TSCHOPP J: The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 2002; 10: 417-26.
46. YOUM Y-H, GRANT RW, MCCABE LR *et al*.: Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *cell metab* 2013; 18: 519-32.
47. BAUERNFEIND F, NIEPMANN S, KNOLLE PA, HORNUNG V: Aging-associated TNF production primes inflammasome activation and NLRP3-related metabolic disturbances. *J Immunol* 2016; 197: 2900-8.
48. CORDERO MD, WILLIAMS MR, RYFFEL B: AMP-activated protein kinase regulation of the NLRP3 Inflammasome during aging. *Trends Endocrinol Metab* 2018; 29: 8-17.
49. LATZ E, DUEWELL P: NLRP3 inflammasome activation in inflammaging. *Semin Immunol* 2018; 40: 61-73.
50. MIAO EA, LEAF IA, TREUTING PM *et al*.: Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat Immunol* 2010; 11: 1136-42.
51. ZHONG Y, KINIO A, SALEH M: Functions of NOD-like receptors in human diseases. *Front Immunol* 2013; 4: 333.
52. MCALLISTER MJ, CHEMALY M, EAKIN AJ, GIBSON DS, MCGILLIGAN VE: NLRP3 as a potentially novel biomarker for the management of osteoarthritis. *Osteoarthritis Cartilage* 2018; 26: 612-19.
53. KIM J-J, JO E-K: NLRP3 inflammasome and host protection against bacterial infection. *J Korean Med Sci* 2013; 28: 1415-23.
54. THORNBERRY NA, BULL HG, CALAYCAY JR *et al*.: A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* 1992; 356: 768-74.
55. WILLINGHAM SB, ALLEN IC, BERGSTRALH DT *et al*.: NLRP3 (NALP3, cryopyrin) facilitates *in vivo* caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol* 2009; 183: 2008-15.
56. YANG J, LIU Z, XIAO TS: Post-translational regulation of inflammasomes. *Cell Mol Immunol* 2017; 14: 65-79.
57. ZHONG Z, SANCHEZ-LOPEZ E, KARIN M: Autophagy, NLRP3 inflammasome and auto-inflammatory/immune diseases. *Clin Exp Rheumatol* 2016; 34 (Suppl. 98): S12-16.
58. STROWIG T, HENAO-MEJIA J, ELINAV E, FLAVELL R: Inflammasomes in health and disease. *Nature* 2012; 481: 278-86.
59. BUSSO N, SO A: Microcrystals as DAMPs and their role in joint inflammation. *Rheumatology* 2012; 51: 1154-60.
60. NALBANT S, MARTINEZ JAM, KITUMNUAYPONG T, CLAYBURNE G, SIECK M, SCHUMACHER HR: Synovial fluid features and their relations to osteoarthritis severity: new findings from sequential studies. *Osteoarthritis Cartilage* 2003; 11: 50-54.
61. JIN C, FRAYSSINET P, PELKER R *et al*.: NLRP3 inflammasome plays a critical role in the pathogenesis of hydroxyapatite-associated arthropathy. *Proc Natl Acad Sci USA* 2011; 108: 14867-72.
62. BOUGAULT C, GOSSET M, HOUARD X *et al*.: Stress-induced cartilage degradation does not depend on the NLRP3 inflammasome in human osteoarthritis and mouse models. *Arthritis Rheum* 2012; 64: 3972-81
63. HAYFLICK L, MOORHEAD PS: The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961; 25: 585-621.
64. BAKER DJ, PEREZ-TERZIC C, JIN F *et al*.: Opposing roles for p16Ink4a and p19Arf in senescence and ageing caused by BubR1 insufficiency. *Nat Cell Biol* 2008; 10: 825.
65. MCCULLOCH K, LITHERLAND GJ, RAI TS: Cellular senescence in osteoarthritis pathology. *Aging Cell* 2017; 16: 210-18.
66. KOZHEMYAKINA E, ZHANG M, IONESCU A *et al*.: Identification of a Prg4 -expressing articular cartilage progenitor cell population in mice. *Arthritis Rheumatol* 2015; 67: 1261-73.
67. PRICE JS, WATERS JG, DARRAH C *et al*.: The role of chondrocyte senescence in osteoarthritis. *Aging Cell* 2002; 1: 57-65.
68. JEON OH, KIM C, LABERGE R-M *et al*.: Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat Med* 2017; 23: 775-81.
69. EVANGELOU K, LOUGIAKIS N, RIZOU SV *et al*.: Robust, universal biomarker assay to detect senescent cells in biological specimens. *Aging Cell* 2017; 16: 192-97.
70. VINATIER C, DOMÍNGUEZ E, GUICHEUX J, CARAMÉS B: Role of the inflammation-autophagy-senescence integrative network in osteoarthritis. *Front Physiol* 2018; 9.
71. LOTZ MK, CARAMÉS B: Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA. *Nat Rev Rheumatol* 2011; 7: 579-87.
72. COPPÉ J-P, DESPREZ P-Y, KRTOLICA A, CAMPISI J: The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 2010; 5: 99-118.

73. MOBASHERI A, MATTA C, ZÁKÁNY R, MUSUMECI G: Chondrosenescence: Definition, hallmarks and potential role in the pathogenesis of osteoarthritis. *Maturitas* 2015; 80: 237-44.
74. DEBACQ-CHAINIAUX F, ERUSALIMSKY JD, CAMPISI J, TOUSSAINT O: Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and *in vivo*. *Nat Protoc* 2009; 4: 1798-806.
75. ALCORTA DA, XIONG Y, PHELPS D, HANNON G, BEACH D, BARRETT JC: Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc Natl Acad Sci USA* 1996; 93: 13742-47.
76. KRISHNAMURTHY J, TORRICE C, RAMSEY MR *et al*.: Ink4a/Arf expression is a biomarker of aging. *J Clin Invest* 2004; 114: 1299-307.
77. ZHOU HW, LOU SQ, ZHANG K: Recovery of function in osteoarthritic chondrocytes induced by p16INK4a-specific siRNA *in vitro*. *Rheumatology* 2004; 43: 555-68.
78. PHILIPOT D, GUÉRIT D, PLATANO D *et al*.: p16INK4a and its regulator miR-24 link senescence and chondrocyte terminal differentiation-associated matrix remodeling in osteoarthritis. *Arthritis Res Ther* 2014; 16: R58.
79. MARTIN JA, BROWN T, HEINER A, BUCKWALTER JA: Post-traumatic osteoarthritis: The role of accelerated chondrocyte senescence. *Biorheology* 2004; 41: 479-91.
80. DE KEIZER PLJ: The fountain of youth by targeting senescent cells? *Trends Mol Med* 2017; 23: 6-17.
81. FRANCESCHI C, CAMPISI J: Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014; 69 (Suppl. 1): S4-9.
82. SOTO-GAMEZ A, DEMARIA M: Therapeutic interventions for aging: the case of cellular senescence. *Drug Discov Today* 2017; 22: 786-95.
83. WILEY CD, VELARDE MC, LECOT P *et al*.: Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab* 2016; 23: 303-14.
84. ORJALO AV, BHAUMIK D, GENGLER BK, SCOTT GK, CAMPISI J: Cell surface-bound IL-1α is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. *Proc Natl Acad Sci USA* 2009; 106: 17031-36.
85. COPPÉ J-P, RODIER F, PATIL CK, FREUND A, DESPREZ P-Y, CAMPISI J: Tumor suppressor and aging biomarker p16INK4a induces cellular senescence without the associated inflammatory secretory phenotype. *J Biol Chem* 2011; 286: 36396-403.
86. DAVALOS AR, KAWAHARA M, MALHOTRA GK *et al*.: p53-dependent release of Alarmin HMGB1 is a central mediator of senescent phenotypes. *J Cell Biol* 2013; 201: 613-29.
87. CHIEN Y, SCUOPPO C, WANG X *et al*.: Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. *Genes Dev* 2011; 25: 2125-36.
88. CHANDRASEKARAN A, IDELCHIK MDPS, MELENDEZ JA: Redox control of senescence and age-related disease. *Redox Biol* 2017; 11: 91-102.
89. ORLOVA VV, CHOI EY, XIE C *et al*.: A novel pathway of HMGB1-mediated inflammatory cell recruitment that requires Mac-1-integrin. *EMBO* 2007; 26: 1129-39.
90. LI J, SCHMIDT AM: Characterization and functional analysis of the promoter of RAGE, the receptor for advanced glycation end products. *J Biol Chem* 1997; 272: 16498-506.
91. KAPOOR M, MARTEL-PELLETIER J, LAJEUNESSE D, PELLETIER J-P, FAHMI H: Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2010; 7: 33.
92. FUKUI N, ZHU Y, MALONEY W, CLOHISY J, SANDELL L: Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF-α in normal and osteoarthritic chondrocytes. *J Bone Joint Surg Am* 2003; 85-A: 59-66.
93. CARAMÉS B, LÓPEZ-ARMADA MJ, CILLERO-PASTOR B *et al*.: Differential effects of tumor necrosis factor-α and interleukin-1β on cell death in human articular chondrocytes. *Osteoarthritis Cartilage* 2008; 16: 715-22.
94. PLATAS J, GUILLÉN MI, DEL CAZ MDP *et al*.: Paracrine effects of human adipose-derived mesenchymal stem cells in inflammatory stress-induced senescence features of osteoarthritic chondrocytes. *Aging* (Albany NY) 2016; 8: 1703-17.
95. SONG W, ZHANG Y, WANG J, MA T, HAO L, WANG K: Antagonism of cysteinyl leukotriene receptor 1 (cysLTR1) by montelukast suppresses cell senescence of chondrocytes. *Cytokine* 2018; 103: 83-89.
96. KANEKO S, SATOH T, CHIBA J, JU C, INOUE K, KAGAWA J: Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. *Cytokines Cell Mol Ther* 2000; 6: 71-79.
97. GUERNE P, BLANCO F, KAELIN A, DESGEORGES A, LOTZ M: Growth factor responsiveness of human articular chondrocytes in aging and development. *Arthritis Rheum* 1995; 38: 960-68.
98. TAKAHASHI A, DE ANDRÉS MC, HASHIMOTO K, ITOI E, OREFFO ROC: Epigenetic regulation of interleukin-8, an inflammatory chemokine, in osteoarthritis. *Osteoarthritis Cartilage* 2015; 23: 1946-54.
99. MILLER RE, TRAN PB, DAS R *et al*.: CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis. *Proc Natl Acad Sci USA* 2012; 109: 20602-7.
100. LI L, JIANG B-E: Serum and synovial fluid chemokine ligand 2/monocyte chemoattractant protein 1 concentrations correlates with symptomatic severity in patients with knee osteoarthritis. *Ann Clin Biochem* 2015; 52 (Pt 2): 276-82.
101. JEON OH, DAVID N, CAMPISI J, ELISSEEFF JH: Senescent cells and osteoarthritis: a painful connection. *J Clin Invest* 2018; 128: 1229-37.
102. SMITH MD: The Normal Synovium. *Open Rheumatol J* 2011; 5: 100-6.
103. FARR JN, FRASER DG, WANG H *et al*.: Identification of Senescent Cells in the Bone Microenvironment. *J Bone Miner Res* 2016; 31: 1920-29.
104. TERADA C, YOSHIDA A, NASU Y *et al*.: Gene expression and localization of high-mobility group box chromosomal protein-1 (HMGB-1) in human osteoarthritic cartilage. *Acta Med Okayama* 2011; 65: 9.
105. NAKAMURA Y, IWATA H, KUWAYAMA T, SHIRASUNA K: S100A8, which increases with age, induces cellular senescence-like changes in bovine oviduct epithelial cells. *Am J Reprod Immunol* 2019; 25: e13163
106. FERREIRA-GONZALEZ S, LU W-Y, RAVEN A *et al*.: Paracrine cellular senescence exacerbates biliary injury and impairs regeneration. *Nat Commun* 2018; 9: 1020.
107. ACOSTA JC, BANITO A, WUESTEFELD T *et al*.: A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol* 2013; 15: 978-90.
108. LEHMANN BD, PAINE MS, BROOKS AM *et al*.: Senescence-associated exosome release from human prostate cancer cells. *Cancer Res* 2008; 68: 7864-71.
109. EFFENBERGER T, VON DER HEYDE J, BARTSCH K *et al*.: Senescence-associated release of transmembrane proteins involves proteolytic processing by ADAM17 and microvesicle shedding. *FASEB J* 2014; 28: 4847-56.
110. JEON OH, WILSON DR, CLEMENT CC *et al*.: Senescence cell-associated extracellular vesicles serve as osteoarthritis disease and therapeutic markers. *JCI Insight* 2019; 4.
111. SELL DR, LANE MA, JOHNSON WA *et al*.: Longevity and the genetic determination of collagen glycoxidation kinetics in mammalian senescence. *Proc Natl Acad Sci USA* 1996; 93: 485-90.
112. PENG Y, PARK H-S, TANG LA, HORWITZ N, LIN L: Generation of sRAGEhigh transgenic mice to study inflammaging. *Front Biosci* (Landmark Ed) 2019; 24: 555-53.
113. MONNET E, LAPEYRE G, POELGEEST EV *et al*.: Evidence of NI-0101 pharmacological activity, an anti-TLR4 antibody, in a randomized phase I dose escalation study in healthy volunteers receiving LPS. *Clin Pharmacol Ther* 2017; 101: 200-8.
114. HERRERO-BEAUMONT G, PÉREZ-BAOS S, SÁNCHEZ-PERNAUTE O, ROMAN-BLAS JA, LAMUEDRA A, LARGO R: Targeting chronic innate inflammatory pathways, the main road to prevention of osteoarthritis progression. *Biochem Pharmacol* 2019; 165: 24-32.
115. SINGER JW, FLEISCHMAN A, AL-FAYOUMI S, MASCARENHAS JO, YU Q, AGARWAL A: Inhibition of interleukin-1 receptor-associated kinase 1 (IRAK1) as a therapeutic strategy. *Oncotarget* 2018; 9: 33416-39.
116. SEGANISH WM: Inhibitors of interleukin-1 receptor-associated kinase 4 (IRAK4): a patent review (2012-2015). *Expert Opin Ther Pat* 2016; 26: 917-32.
117. JOH E-H, LEE I-A, JUNG I-H, KIM D-H: Ginsenoside Rb1 and its metabolite compound K inhibit IRAK-1 activation--the key step of inflammation. *Biochem Pharmacol* 2011; 82: 278-86.
118. GRATAL P, MEDIERO A, LAMUEDRA A *et al*.:

The TLR-4 inhibitor 6-shogaol as a treatment in osteoarthritis trough the modulation of chondrocyte hypertrophy and matrix calcification. ACR Meeting Abstracts. 2018

119. LEE I-A, HYAM SR, JANG S-E, HAN MJ, KIM D-H: Ginsenoside Re ameliorates inflammation by inhibiting the binding of lipopolysaccharide to TLR4 on macrophages. *J Agric Food Chem* 2012; 60: 9595-602.
120. YANG Y, WANG H, KOUADIR M *et al*.: Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. *Cell Death Dis* 2019; 10: 128.
121. BROZ P, VON MOLTKE J, JONES JW, VANCE RE, MONACK DM: Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe* 2010; 8: 471-83.
122. CHANG J, WANG Y, SHAO L *et al*.: Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med* 2016; 22: 78-83.
123. YOSEF R, PILPEL N, TOKARSKY-AMIEL R *et al*.: Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. *Nat Commun* 2016; 7: 11190.
124. FUHRMANN-STROISSNIGG H, LING YY, ZHAO J *et al*.: Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun* 2017; 8: 422.
125. NAGAI K, MATSUSHITA T, MATSUZAKI T *et al*.: Depletion of SIRT6 causes cellular senescence, DNA damage, and telomere dysfunction in human chondrocytes. *Osteoarthritis Cartilage* 2014; 22: S137.
126. LOESER RF, GOLDRING SR, SCANZELLO CR, GOLDRING MB: Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum* 2012; 64: 1697-707.