Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology REVIEW ARTICLE doi: 10.1111/cei.13237

Inflammation in osteoarthritis: is it time to dampen the alarm(in) in this debilitating disease?

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Accepted for publication 2 November 2018
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Summary

Osteoarthritis (OA) is the most common joint disease that strongly reduces the quality of life in patients; However, no disease-modifying therapy is available. For a long time, OA was considered a non-inflammatory disease that was the result of 'wear-and-tear' and abnormal mechanics, and therefore many considered the term 'osteoarthritis' a misnomer. However, during the last decades the notion arose that inflammation is not only present in the majority of OA patients but, rather, actively involved in the progression of the disease. Influx of immune cells is observed in the synovium and a plethora of inflammatory mediators is present in tissues and fluids from OA patients. These mediators cause the production of degrading enzymes that break down the cartilage matrix, which is the main hallmark of OA. Alarmins, which belong to the group of danger signals, have been implicated in many inflammatory diseases. They are among the first factors to be released upon cell stress due to, for example, infection, damage and inflammation. They attract and activate cells of the immune system and therefore lie at the base of the inflammatory reaction. In this narrative review, an overview of the history of OA, the evolving concept of inflammation as important factor in the OA pathogenesis, and particularly the central role that alarmins play in the initiation and maintenance of the low-grade inflammatory response in OA, is provided. Moreover, the targeting of alarmins as a promising approach to dampen the inflammation in OA is highlighted.

Keywords: alarmins, inflammation, osteoarthritis, pathology, S100A8/A9

Osteoarthritis

Osteoarthritis (OA) is a highly complex and the most prevalent joint disorder. Worldwide, 9·6% of men and 18·0% of women aged more than 60 years suffer from symptomatic OA, and a total of 242 million people are affected globally [1,2].

Clinical symptoms include severe pain, joint stiffness and strongly reduced mobility, which together seriously decrease the quality of life [3,4]. Pathologically, OA is characterized by changes in all joint tissues caused by coinciding catabolic and anabolic processes. Cartilage degeneration, ectopic bone formation, subchondral bone sclerosis, damage to ligaments and menisci and fibrosis and inflammation in the synovial membrane that lines the joint cavity are the main disease hallmarks [5].

However, no disease-modifying osteoarthritis drugs (DMOADs) are available to date. This limits options for therapy to treating symptoms such as pain and often leads to joint-replacement surgery at end-stage disease.

The following is a brief narrative overview of the history of OA, and how the view on the disease shifted from being a relatively simple and inevitable 'wear-and-tear' process towards an active disease of the joint as an organ where inflammation plays an important role in the aetiopathology.

History of osteoarthritis

It is often stated that OA might be the oldest 'known' disease, with signs of pathology present in dinosaur skeletons and ancient human skeletons, possibly because bones

carrying evidence of OA have withstood the sands of time better than other tissues [6,7]. However, the age of recognition of the disease nowadays known as OA is more debatable, mainly because of ambiguous nomenclature. From the time of Hippocrates until the 18th century all rheumatic complaints were considered to be gout. A first separation came with the description of *digitorum nodi* by Heberden [8]. Although OA of the hip was clinically recognized in the early 1800s, it was put on a par with rheumatoid arthritis (RA), together referred to as arthritis deformans. This term was introduced by Charcot and Trastour and later widely publicized by Virchow in the mid-19th century, but was used well into the 20th century [9–11]. The term osteo arthritis was most probably introduced by the German orthopaedic surgeon von Volkmann in the 1850s. He was also the first to anatomically and pathologically differentiate OA from RA lesions [9]. However, this met with fierce protest, because the term suggests the presence of inflammation, a view that was not endorsed by many at the time.

Osteoarthritis as a mechanical disease

OA has been considered by many a relatively simple 'wear-and-tear' disease leading to the loss of cartilage. In this view, OA was considered the sole consequence of fragility of the cartilage matrix due to, for example, ageing, which should not be classified as a disease. However, the majority of physicians supported Garrod's view that processes such as cartilage erosion, osteophyte formation and changes in bone could be the result of a disease process, provided that it was present for a long time [12]. As a result, because inflammation was not considered part of the aetiology, many believed OA to be solely driven by mechanical events, which would mean that the disease is caused by or related to physical forces or motion. For this reason the term osteoarthritis, implying inflammation as the major cause, was considered a misnomer. The mechanical origin of OA was thought to be underlined by the evidence that ancient skeletons mainly had signs of OA in the lower back and shoulders, whereas the knees were less affected [13,14]. This is in contrast to that observed in patients nowadays, and could be attributed to differences in physical activity (e.g. due to differences in professions). Furthermore, our current sedentary lifestyle has increased the prevalence of obesity, which strongly associates with knee OA [15,16]. Other clues that abnormal mechanics cause OA came from studies that showed that traumatic event, such as tears of the anterior cruciate ligament and meniscus and varus malalignment, greatly increase the risk of OA development [17–20], whereas individuals with focal high stress due to femoroacetabular impingement experience excess rates of OA [21,22].

However, the concept of OA as a mechanical disease implies that cartilage breakdown is accelerated when the cartilage is increasingly loaded. Nevertheless, running, which greatly increases stresses on hip and knee joints, does not aggravate OA incidence and reduced OA pain and hip replacement surgery [23,24].

Moreover, the notion arose that not all OA could be attributed to mechanical factors. Why is obesity not associated with OA in the hip, whereas this is also a weight-bearing joint; and why do obese individuals have higher rates of hand OA, whereas they do not experience greater stresses in those joints [25]?

The evolving concept of inflammation as driver of pathology in osteoarthritis

Although inflammation has been incidentally mentioned in association with OA since the mid-19th century, it was not until the last decades that inflammation was increasingly considered to be present and important in the development of OA. In fact, tissue and fluid samples from OA patients have long been used as negative, non-inflammatory controls in studies investigating rheumatoid arthritis and spondyloarthritis. This concealed the raised levels of proinflammatory factors present in OA tissues and fluids compared to healthy controls and arguably reinforced the idea of OA as a non-inflammatory disease.

A big step towards the recognition of inflammation in OA was taken in the 1990s, when molecular biology took a leap forwards and showed that soluble mediators, released by various tissues in the joint, could stimulate the production of matrix-degrading enzymes, such as matrix metalloproteinases (MMPs) and aggrecanases (ADAMTS4/5), which are closely involved in the breakdown of the articular cartilage although, even with these findings, it still took more than 10 years before inflammation was broadly accepted as a critical feature of OA pathogenesis.

Multiple studies have shown clear signs of synovial inflammation that correlate with disease severity, progression and pain sensitization using arthroscopy, ultrasonography or magnetic resonance imaging (MRI) [26–30]. Debate remains about whether synovial inflammation is causative for OA or rather a secondary effect of joint failure and products of matrix degradation. The most broadly accepted view is that synovial inflammation is the result of cartilage fragments, released due to, for example, a traumatic event in the joint, that activate the synovial cells to produce proinflammatory factors and MMPs, thereby further increasing cartilage degeneration [31]. A vicious inflammatory circle ensues in this way. An overview of processes that contribute to inflammation in OA is given in Fig. 1.

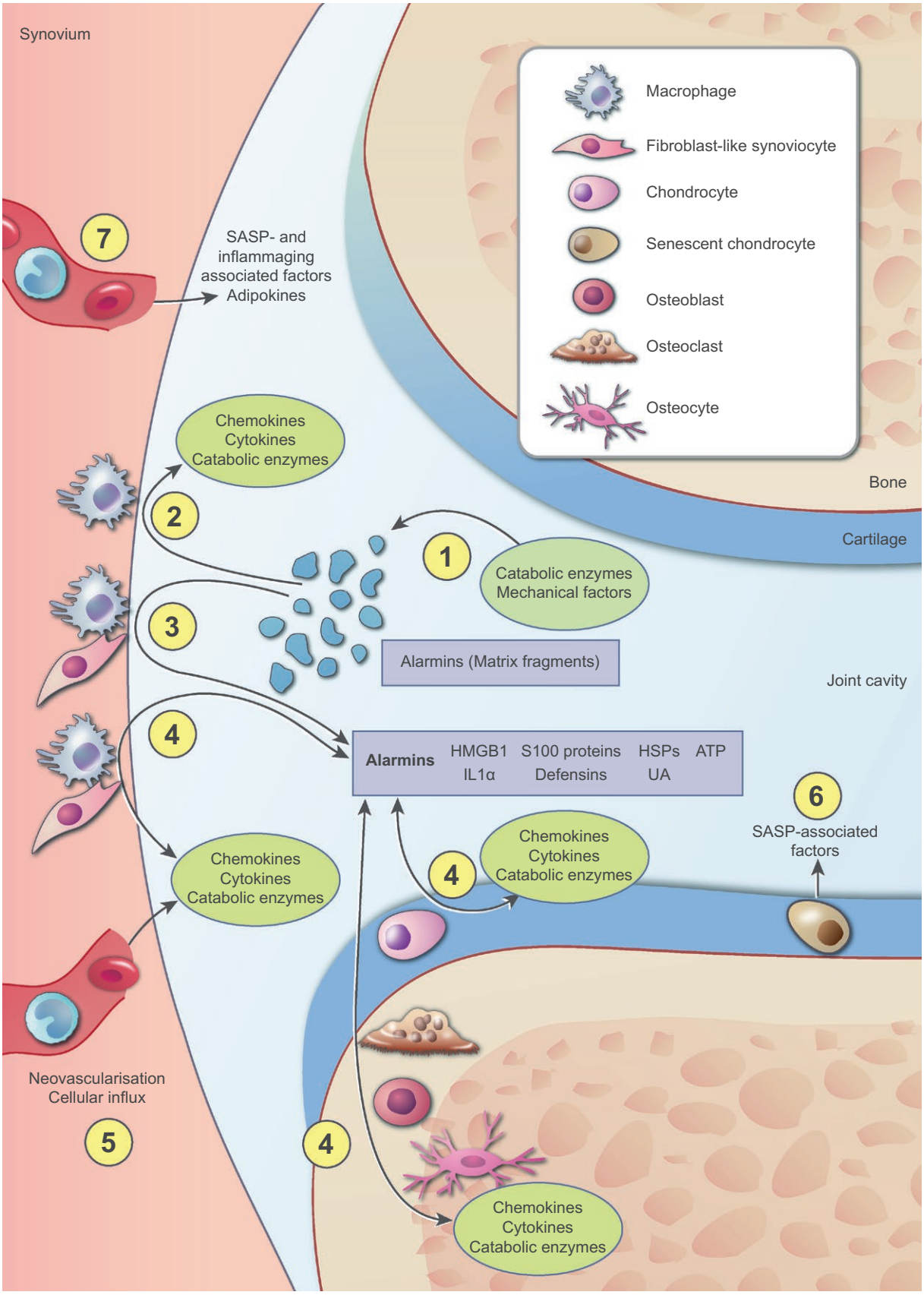
Synovium
Macrophage
Fibroblast-like synoviocyte
Chondrocyte
Senescent chondrocyte
Osteoblast
Osteoclast
Osteocyte
Bone
Cartilage
Joint cavity
7
SASP- and inflammaging associated factors
Adipokines
Chemokines
Cytokines
Catabolic enzymes
2
1
Catabolic enzymes
Mechanical factors
3
Alarmins (Matrix fragments)
4
Alarmins
HMGB1
S100 proteins
HSPs
ATP
IL1α
Defensins
UA
6
SASP-associated factors
Chemokines
Cytokines
Catabolic enzymes
4
Chemokines
Cytokines
Catabolic enzymes
Neovascularisation
Cellular influx
5
4
Chemokines
Cytokines
Catabolic enzymes

Fig. 1. Overview of the processes that contribute to inflammation in osteoarthritis. Nowadays it is well accepted that inflammation is present and actively involved in the pathogenesis of osteoarthritis (OA). The most broadly supported view is that cartilage extracellular matrix fragments, released as the result of an initial trauma or catabolic enzyme activity (a), increase synovial inflammation by stimulation of cells in the synovial lining. Activated synovial cells start to produce proinflammatory factors, such as chemokines, cytokines and catabolic enzymes (b). In addition, these cells secrete high levels of various alarmins, such as high-mobility group box-1 (HMGB1) and S100 proteins (c). These alarmins initiate a positive-feedback loop with reciprocal production of chemokines, cytokines and catabolic enzymes, on one hand, and alarmins on the other hand, not only in synovial cells, but also in cells in the cartilage and periarticular bone (d). Growth factors and chemokines add to the inflammatory process in the joint by stimulating neovascularization and influx of inflammatory cells that also start to produce chemokines, cytokines and catabolic enzymes (e). In addition, cellular senescence is associated with an increased release of factors, such as many cytokines and catabolic enzymes, referred to as senescence-associated secretory phenotype (SASP), which further stimulates the inflammatory state of the joint (f). Finally, systemic changes are associated with inflammation during OA. Increased systemic levels of inflammatory mediators as the result of SASP or ageing and adipokines as the result of increased fat mass contribute to the inflammatory milieu in the joint (g). Together, these factors are thought to be involved in the pathological processes that take place during OA, such as hypertrophic differentiation of chondrocytes and breakdown of the articular cartilage, fibrosis in the synovial tissue, sclerosis of the subchondral bone and ectopic bone formation (these processes are not depicted in this figure).

OA is mainly linked to activation of innate immunity by binding of damage-associated molecular patterns (DAMPs) to so-called pattern recognition receptors (PRRs) [32,33]. Of central importance in the PRR family are the Toll-like receptors (TLRs). TLR-2 and TLR-4 have been shown to bind a multitude of degraded cartilage extracellular matrix fragments. These fragments include low molecular weight hyaluronan, tenascin C, fibronectin, biglycan and aggrecan [34–37]. It is believed that these fragments might cause the initial trigger that starts the inflammatory response in the OA joint. Other local triggers of inflammation include activation of the complement system and stimulation of cells by crystals that are present in the majority of OA patients [38–40].

Next to local induction of inflammation by danger signals, low-grade systemic inflammation and ageing-induced inflammatory responses have been associated with OA development. Low-grade systemic inflammation is present in many OA patients. Serum levels of various cytokines are increased in OA patients [41]. Moreover, OA is strongly linked to obesity and the metabolic syndrome. Increased fat mass has been shown to result in higher systemic inflammatory factors, such as cytokines and various adipose tissue-produced factors called adipokines that have inflammatory functions [42–44].

Furthermore, ageing is associated with an altered inflammatory response, also referred to as inflammageing. Cellular senescence claims a central spot in this process. Senescent cells have an increased production of proinflammatory and catabolic mediators [45]. Indeed, proteins secreted as the result of this senescence-associated secretory phenotype (SASP), such as various cytokines, chemokines and MMPs, are abundant in OA tissues and fluids [46].

Together, this shows that inflammation plays a clear role in OA development, although the nature of the inflammatory reaction is different from RA. Whereas RA is characterized by a severe synovial inflammation, a chronic but relatively low-grade inflammation is found in OA. Activation of the innate immunity in OA results in the production of cytokines, such as interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor (TNF)-α, activation of the complement system and production of matrix-degrading enzymes such as MMPs and ADAMTS4/5. More in-depth reviews concerning their involvement in OA can be found elsewhere [47–49].

Cells of the innate immune system, such as monocytes and macrophages, are mainly associated with the inflammatory response in the OA joint. Whereas OA patients show an altered T cell profile, the clear involvement of these cells in the disease pathogenesis is debatable [50,51]. Depletion of macrophages with intra-articular injection of clodronate-laden liposomes strongly reduced the MMP-mediated breakdown of articular cartilage and the formation of osteophytes in a preclinical model of OA [52,53]. Moreover, depletion of macrophages from a cell suspension made from human OA synovium reduced the cytokine response and activity of matrix-degrading enzymes [54].

Therefore, whereas OA might not comply with the classical view of inflammation with the presence of rubor, calor, dolor and tumour, and whereas there is no strong evidence of the presence of robust adaptive (auto)immune reactions, there is a clear involvement of both local and systemic inflammation in OA development.

However, results from clinical trials in which inflammatory cytokines, such as IL-1β and TNF-α, were targeted have been repeatedly disappointing (elegantly reviewed in [55]). Targeting more upstream regulators of acute inflammation that lead to the production and perpetuation of cytokine production might serve as an interesting alternative.

Alarmins

An example of such a group of factors that are released during the first phase of inflammation are the alarmins, a term proposed by Oppenheim *et al.* Initially defined as molecules that attract and activate antigen-presenting cells such as dendritic cells, alarmins are nowadays more broadly defined as structurally diverse and evolutionarily unrelated, endogenous molecules that are released upon cell stress,

which cause inflammation *in vivo* [56,57]. However, while osteoarthritis is characterized by an influx and activation of innate immune cells, little is known about the role of dendritic cells in this disease, although no profound adaptive (auto)immune responses are observed.

While defensins and high-mobility group box-1 (HMGB1) belong to the first identified, the list of alarmins has grown rapidly since then and now includes, but is not limited to, heat shock proteins (HSPs), uric acid (UA), adenosine triphosphate (ATP), IL-1α and s100 proteins [57,58]. An in-depth structural and functional characterization of the various alarmins is beyond the scope of this review and can be found elsewhere [57,59].

Many alarmins are intracellular proteins that are passively or actively released as the result of stress due to, for example, inflammation and tissue damage. Passive release can be the result of cell injury or death, such as necrosis or netosis. Active release of alarmins is regulated by mechanisms that are independent of the endoplasmatic reticulum and Golgi route. They include degranulation, secretion via the inflammasome and pyroptosis [56,60–62].

In the extracellular milieu alarmins bind to a range of receptors, among which the TLRs and receptor for advanced glycosylation end products (RAGE) are the most well-studied. TLR-4, in particular, is used by many alarmins [63–66].

Because of their quick release as the result of cell stress or non-programmed cell death, alarmins are among the first factors to be secreted and, as such, act as first responders to stimuli. Together with their ability to attract and activate immune cells, this puts them at the base of inflammatory responses.

Alarmins in inflammatory diseases

Alarmins play an important role in a wide range of disorders involving infection-induced and sterile inflammation, where they are associated with sustaining inflammation and the induction of tissue injury. HMGB1 and S100A8/A9 have been well characterized in the context of sepsis [67–69]. In conditions of sterile inflammation, such as haemorrhagic shock and ischaemic injury in multiple organs, HMGB1 appears to be a crucial factor in promoting inflammation [70–73].

Moreover, alarmins are key players in tumour immunology, although they have been attributed paradoxical roles. On one hand, factors such as HMGB1 and S100A8/A9 are involved in tumour promotion via their capacities to promote cell proliferation, migration and production of matrix-degrading enzymes, which together result in tumour growth and metastases [74–77]. Furthermore, S100A8/A9 promotes the production and recruitment of myeloid-derived suppressor cells that diminish immunity [78]. On the other hand, alarmins can promote anti-tumour immunity by, for example, recruitment and activation of dendritic cells and stimulating T helper type 1 (Th1) immune responses [79–81].

Finally, it is broadly acknowledged that alarmins play central roles in many chronic inflammatory diseases, including many arthritides, inflammatory bowel disease and atherosclerosis [82–86]. A more detailed overview of the contribution of alarmins to inflammatory diseases is given elsewhere [57,59].

Alarmins in osteoarthritis

A growing body of evidence shows a central role for alarmins in the initiation and maintenance of the low-grade inflammatory response that is present during OA. Interestingly, high levels of many alarmins have been described in the synovial fluid of OA patients, including HMGB1, UA, ATP, thymosin β4 and various S100 proteins [40,87–92]. Other alarmins are described to have increased production and secretion, although the exact extracellular localization remains unknown, such as is the case for HSPs. Release of alarmins has been shown for multiple joint tissues, including the periarticular bone, cartilage and synovium. Their release is stimulated by proinflammatory and catabolic factors, but also by mechanical stress on the tissues via both active release and as the result of cell death in the inflammatory environment. In this section, a selective overview is provided of the alarmins that are present during OA and how they affect the disease pathology.

As described earlier in this narrative, cartilage extracellular matrix fragments such as biglycan, fibronectin, aggrecan and low molecular weight hyaluronan stimulate cell types in the joint via PRRs, leading to the production of inflammatory mediators and the attraction of inflammatory cells, and as such act as alarmins [34–37,93]. Moreover, they are taken up by synovial macrophages, resulting in the activation of proinflammatory responses in these cells.

IL-1α is predominantly released by macrophages as the result of stress-induced activation of the inflammasome [94]. Stimulation of chondrocytes with IL-1α increases nitric oxide production and MMP activity [95]. The role of IL-1 in the development of OA has been well investigated, but debate remains about its importance. Many studies describe the catabolic function of IL-1 on cartilage, although a preclinical model of OA induced in IL-1α/$\beta^{-/-}$ mice showed that both IL-1α and IL-1β are not involved in the disease pathology [48,96].

Other studies have shown increased UA levels in synovial fluid, which correlated with levels of IL-1β and IL-18 and with OA severity [40]. Furthermore, it was tentatively postulated that monosodium UA crystals,

which are key stimulators of inflammation in gout, might promote OA pathology via activation of the inflammasome which leads to the release of IL-1β and other alarmins [97].

Defensins comprise another group of alarmins that has been linked to a catabolic response in OA. Human β-defensin 3 expression in chondrocytes is induced by IL-1β and TNF-α. Stimulation of chondrocytes with recombinant β-defensin 3 protein increases the production of the collagenases MMP1 and MMP13, that are among the most potent matrix-degrading enzymes in OA, whereas it additionally decreases the production of tissue inhibitors of metalloproteinases (TIMP)1 and TIMP2 [98]. Other studies have shown an increased production of β-defensins 2 and 4 in OA cartilage and menisci, but further studies are required to elucidate the role played by these alarmins [99,100].

ATP is released from cells upon cell stress. In turn, ATP has been shown to stimulate both chondrocyte death and the calcification of cartilage [101]. Furthermore, a recent study shows the activation of pyroptosis in OA fibroblast-like synoviocytes by ATP together with TLR-4 ligands [102,103]. Finally, ATP increases pain sensitivity (hyperalgesia) via binding to the purinergic P2X3 and P2X2/3 receptors [89,104].

Although these studies suggest the involvement of these alarmins in OA, many aspects about their involvement remain unknown and therefore more (pre-)clinical studies are needed to further elucidate their importance and mechanism of action in the OA pathogenesis.

HMGB1

HMGB1 levels are increased in synovial fluid of OA patients, which correlates with disease severity. HMGB1 is released from activated synovial cells, chondrocytes and necrotic bone cells under the influence of cytokines such as IL-1β and TNF-α [87,105–107]. Conversely, stimulation of human OA chondrocytes with HMGB1 results in higher secretion of IL-1β and TNF-α [106]. Interestingly, next to the direct stimulation of immune cells, HMGB1 can form complexes with pathogenic molecules including DNA and RNA but also with proinflammatory cytokines such as IL-1β, which synergistically activate the immune system [108,109]. In this way, HMGB1 stimulates osteoarthritic synoviocytes to produce the proinflammatory cytokines IL-6 and IL-8, and MMP1, MMP3 and MMP13 [110,111].

HSPs

Other alarmins that are involved in OA are HSPs, even though the various family members have been attributed divergent effects on joint homeostasis. Increased levels of HSP60, HSP70 and HSP90 are found within the OA joint [112,113]. HSP90 release results in the progression of cartilage degeneration and activation of the synovium, while increased extracellular levels of HSP60 and HSP70 have a chondroprotective effect and show immunomodulatory activities [114–116].

S100 proteins

Probably the most well-studied alarmins in the field of OA are the S100 family members. S100B is expressed by chondrocytes. Once released, S100B is thought to cause proinflammatory and procatabolic effects, mainly via RAGE-dependent signalling in chondrocytes [117]. This results in increased MMP13 expression in chondrocytes [118,119]. S100A4 is expressed in OA cartilage under the influence of, among others, IL-7, and stimulates chondrocytes to produce MMP13 via RAGE [120,121]. Furthermore, OA has been associated with increased S100A11 production, whose secretion from chondrocytes is stimulated by factors such as TNF-α and IL-8 [122,123]. In turn, stimulation of chondrocytes with S100A11 stimulates RAGE-dependent hypertrophic differentiation, a process that is closely associated with the progression of OA [123]. S100A12, which is closely related to S100A8 and S100A9, is markedly increased in synovial fluid of OA patients and correlates with disease severity [90,91]. Addition of S100A12 to human chondrocytes increases the expression of MMP13 and vascular endothelial growth factor (VEGF), again via RAGE-dependent signalling [124].

However, proof for the involvement of these S100 proteins in catabolic effects on chondrocytes that might lead to OA development was mainly obtained with *in-vitro* studies, whereas *in-vivo* studies using loss-of-function and gain-of-function experiments have rarely been performed.

S100A8/A9

S100A8 and A9 are by far the best-studied S100 proteins in the OA context. High levels of S100A8/A9 are present in the synovial fluid and serum of OA patients [92,125]. Among others, basic calcium phosphate (BCP) crystals that are present in the majority of OA patients stimulate the production of S100 proteins in macrophages [126]. Interestingly, strongly increased expression of S100A8/A9 has been described with ageing, the dominant risk factor for OA [127]. Induction of the experimental collagenase-induced OA (CiOA) mouse model that involves moderate synovitis in *S100a9*$^{-/-}$ mice, which additionally lack S100A8 protein in the periphery, results in a strongly decreased synovial inflammation and cartilage degradation, indicating the crucial involvement of S100A8/A9 in this model [125]. In contrast, the same study shows that S100A8/A9 is not of importance after induction of the destabilization of the medial meniscus (DMM), in which synovitis is scant. Higher S100A8/A9 serum levels were measured in early

symptomatic OA patients that experience progression of joint space width narrowing between baseline measurements and the 2-year follow-up measurement compared to non-progressors [125]. S100A8/A9 has been shown to result in increased synovial activation, as determined by a number of inflammatory cell layers [125]. A later study confirmed that S100A8/A9 predominantly mobilizes pro-inflammatory Ly6C^{high} monocytes towards the inflamed synovium [128]. This phenomenon might be at least partially responsible for the cartilage breakdown via the production of cytokines and matrix-degrading enzymes. Indeed, stimulation of human OA synovial tissue and macrophages with S100A9 results in increased production of proinflammatory (e.g. IL-1β, IL-6, IL-8, and TNF-α) and catabolic factors such as MMP1, MMP3 and MMP9, which probably runs via TLR-4 signalling, as this has been shown to be the dominant S100A8/A9 receptor in myeloid cells [69,129,130]. However, in addition to possible indirect effects on cartilage degeneration via stimulation of cells in the synovium, direct stimulation of chondrocytes with S100A8 and S100A9 proteins also strongly promotes the production of various proinflammatory cytokines such as IL-6 and IL-8, the chemokine monocyte chemoattractant protein 1 and MMP1, MMP3, MMP9 and MMP13, which was shown to run via TLR-4 as dominant receptor [131]. Next to the production of these proinflammatory and catabolic mediators, S100A8/A9 is also involved in nociceptive pain sensation, independent of the degree of synovitis that is associated with different S100 levels ([132] and personal unpublished findings).

Interestingly, S100A8/A9 was shown not only to activate the catabolic aspects of OA, but additionally promotes the anabolic process of ectopic bone/osteophytes formation, both in the CiOA experimental model and in early human OA, possibly via MMP-mediated remodelling of the cartilage matrix that allows osteophytes to increase in size [133]. This is underlined by the finding that S100A8/A9 induces Wnt signalling, which has been shown to promote bone formation [134]. These findings are in agreement with other studies that address a more immunomodulatory rather than only a proinflammatory effect of S100A8/A9([135,136] and personal unpublished findings).

Together, these studies make a case for the crucial involvement of alarmins, and particularly S100A8/A9, in the development of disease pathology during OA.

Dampening the alarm as therapy

In this section, a short overview of how these alarmins might serve as therapeutic target for this crippling disease will be given. An overview of possible ways to target alarmins can be found in Fig. 2. A first route to inhibit the function of alarmins is to block their expression, although a clinical application might not be feasible because current techniques, such as siRNA or shRNA, do not allow widespread targeting of the very high expression of many alarmins. Furthermore, care should be taken not to 'overinhibit' their intracellular expression because of the pivotal physiological functions that alarmins carry out. This is highlighted by the findings that both *S100a8* and *Hmgb1*-deficient mice are not viable [137,138]. As a second option, blocking receptors of alarmins using antibodies, blocking peptides or small-molecule inhibitors might appear to be an attractive therapy, mainly because a multitude of alarmins bind to only a few receptors, such as RAGE, TLR-2 and TLR-4. Experimental evidence shows that blocking these receptors can lessen the impact of alarmins in inflammatory processes [131,139–141]. However, a major drawback of such an approach is that many infectious agents share the same PRRs with alarmins, of which the TLRs are particularly indispensible in host defence. Blocking PRRs will therefore most probably result in undesired adverse effects. Similarly, the use of soluble forms of PRRs, such as soluble TLR-4 and soluble RAGE, can scavenge alarmins and therefore decrease the cellular signalling, but comprises the same risk of undesired side effects.

Two more feasible ways of inhibiting the extracellular function of alarmins on the immune system are to block their secretion or target the alarmins themselves. Because alarmins lack a signal sequence for secretion they are secreted via alternative pathways not involving the endoplasmatic reticulum and Golgi complexes; blocking their release should target these pathways [142–144]. A possible advantage is that this would inhibit the simultaneous release of multiple alarmins without interfering with the secretion of classically released proteins. HMGB1 is released via lysosomes. The natural compound glycyrrhizin has been shown to block the secretion and additionally inhibits the function of HMGB1 [145]. Furthermore, ethyl pyruvate inhibits the nuclear-to-cytoplasmic translation of HMGB1, thereby decreasing its secretion [146,147]. In addition, this compound has been shown to block the NLRP3 inflammasome. The release of several alarmins is thought to follow mechanisms similar to IL-1, which implies a contribution of the inflammasome. For this reason, compounds such as ethyl pyruvate and specific caspase 1 inhibitors would block the release of these alarmins. S100A8/A9 is secreted by a tubulin-dependent mechanism [144]. Colchicine blocks tubulin polymerization and has been shown to decrease S100A8/A9 release [144,148]; however, the net outcome of these often relatively aspecific methods to block the release of alarmins on the development of OA needs further investigation, given the differential effects of, for example, S100A8/A9 *versus* HSPs on the cartilage. Moreover, the exact secretion mechanisms

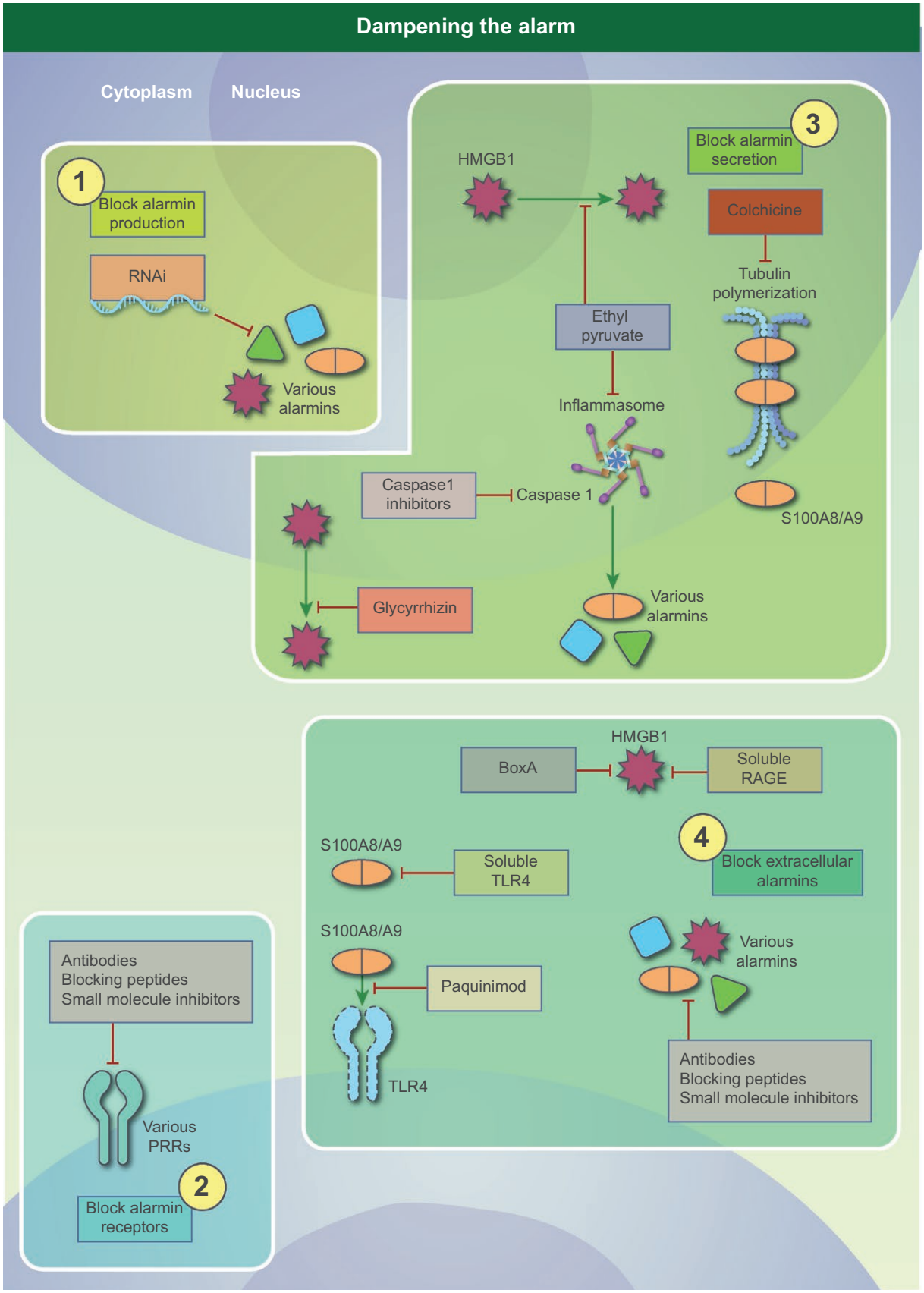

Fig. 2. Targeting alarmins as potential therapies to dampen osteoarthritis. Alarmins are thought to play important roles in the inflammatory process during osteoarthritis (OA). This provides a vast amount of opportunities to target these factors. First, the expression of alarmins can be inhibited using RNA interference techniques, such as short inhibitory (si)RNAs or short hairpin (sh)RNAs (a). However, care should be taken with this approach concerning the pivotal (often intracellular) roles that alarmins play under physiological conditions. Another possibility to inhibit the effects of alarmins is to block their pattern recognition receptors (PRRs) (b). This can be achieved using, among others, antibodies, blocking peptides or small molecule inhibitors against the desired receptor. A possible drawback of this approach is that these receptors, such as the family of Toll-like receptors (TLRs), are crucially involved in the body's defence against pathogens, meaning that blocking these receptors strongly increases the risk of infections. A third way to inhibit the activity of alarmins is to block their secretion (c). Alarmins are secreted via unconventional ways, involving among others lysosomal, inflammasome- or cytoskeleton-dependent pathways. Interference with these secretion machineries with, for example, colchicine to inhibit S100A8/A9 secretion, ethyl pyruvate or glycyrrhizin to inhibit high-mobility group box-1 (HMGB1) secretion or caspase 1 inhibitors to reduce inflammasome-dependent alarmins therefore prevent alarmins from conducting their proinflammatory functions in the extracellular space. Finally, specific inhibition of alarmins, once secreted into the extracellular environment, could be used to dampen inflammatory responses (d). To this end, specific antibodies, blocking peptides or small-molecule inhibitors can be used. BoxA has been shown to successfully inhibit HMGB1. Moreover, paquinimod effectively interferes with the binding of S100A9 to TLR-4. Soluble receptors, such as TLR-4 and receptor for advanced glycosylation end products (RAGE) can be used to scavenge alarmins and thereby decrease alarmin-induced cellular signalling.

for many alarmins remain unknown and the above approaches will only block the active release of alarmins, whereas passive release as the result of cell death is not targeted.

A last approach would consist of selectively blocking the alarmins themselves. In this case it will be important to identify the dominant alarmin in a particular inflammatory disease. Successful attempts to inhibit inflammation have been described using neutralizing antibodies directed against HMGB1 and S100A8/A9 proteins in preclinical studies [149–153]. Of particular interest is the recent identification of the amino acid sequence in the active S100A8/A9 complex that activates TLR-4 signalling. Specific antibodies directed against this epitope might consequently block the activation of TLR-4 signalling and activation of the inflammatory response [154]. Also, small-molecule inhibitors, either natural or synthetic, can be used to target alarmins, for example quinoline compounds such as paquinimod and laquinimod for S100A8/A9 [155,156]. Interestingly, paquinimod reduced synovial inflammation, osteophyte formation and cartilage damage in a preclinical model for OA [133]. However, the efficacy of many of the above approaches to reduce OA pathology remains to be investigated.

Conclusion

Whereas OA might not comply with all the classical signs of inflammation, there is nowadays a wealth of evidence that inflammation is part of the OA pathology and is actively involved in the disease pathogenesis via promotion of catabolic responses, either directly in chondrocytes or by promoting synovial inflammation and its contribution to pain sensation. Of particular interest in this process are the alarmins, which lie at the basis of the inflammatory response. Further understanding of the functioning of alarmins in inflammation brings to light novel and promising targets for the development of innovative DMOADs, in which the S100A8/A9 proteins are expected to claim a central spot based on their broad involvement in the OA disease process.

Disclosure

No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript. The authors have no competing interests to declare.

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