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James K. Chan, … , Nicole Horwood, Jagdeep Nanchahal

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Alarmins: awaiting a clinical response

James K. Chan,[1] Johannes Roth,[2] Joost J. Oppenheim,[3] Kevin J. Tracey,[4] Thomas Vogl,[2] Marc Feldmann,[1] Nicole Horwood,[1] and Jagdeep Nanchahal[1]

[1]Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom. [2]Institute of Immunology, University of Münster, Münster, Germany. [3]National Cancer Institute, Frederick, Maryland, USA. [4]Feinstein Institute of Medical Research, New York, New York, USA.

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Alarmins in health and disease

The alarmin family comprises structurally diverse and evolutionarily unrelated multifunctional endogenous molecules that are passively released from necrotic cells upon infection or tissue injury or are rapidly secreted by stimulated leukocytes and epithelia. In the absence of injury or infection, alarmins play important intracellular roles (Table 1). However, once released extracellularly, alarmins promote activation of innate immune cells and recruitment and activation of antigen-presenting cells engaged in host defense and tissue repair through pattern recognition receptors such as the TLRs, many of which have a key role in the detection of pathogens (refs. 1–3 and Table 1). In health, inflammation is self-limiting and a vital part of the innate host defense. It occurs in response to sterile injury or infection and involves the recruitment of phagocytes to remove cell debris and microbes. This is followed by resolution, with the recruitment of other cell types, including stem and endothelial cells, to restore tissue homeostasis. As potent mediators of inflammation, alarmins play a fundamental role in the pathogenesis of a wide range of sterile or infection-induced immune and inflammatory disorders (4–6). Crucially, their ability to enhance the adaptive immune response through their effects on antigen-presenting cells, including DCs, makes them a critical link between the innate and adaptive arms of the immune response (7). Hence, the alarmin family represents an intriguing therapeutic target, not only to dampen inflammation but also to uncouple the innate and adaptive immune responses in chronic pathologies, including autoimmune disorders. Furthermore, alarmins may serve as useful diagnostic and prognostic biomarkers in inflammatory disorders. While there is now a rapidly growing list of alarmins in the literature, the best characterized in health and disease are high-mobility group protein B1 (HMGB1), S100 proteins, and heat shock proteins (HSPs). In this Review, we will focus on these alarmins, as they have the clearest and most tangible clinical translational potential to date.

Modulation of the alarmin response to suppress inflammation

Dysregulation of inflammation underlies the pathophysiological process in many immune and inflammatory disorders. The identification of proinflammatory cytokines, in particular TNF-α, as a therapeutic target in the 1990s heralded a paradigm shift that led to impressive clinical benefits, most notably in patients suffering from RA (8). Unfortunately, cytokine blockade is not effective in a significant proportion of patients (9) and has been disappointing in the treatment of patients with acute inflammatory disorders, such as trauma-induced systemic inflammatory response syndrome (SIRS) or sepsis (10). The recent identification of alarmins as crucial mediators of the inflammatory processes in these disorders and the observation that their release is accompanied by an upregulation of their receptor suggests the alarmin signaling pathway as an alternative target (Figure 1).

Acute disorders

SIRS and sepsis. Severe trauma remains the most common cause of death under the age of 45 years. There is a biphasic distribution of mortality, with the initial peak corresponding to the "first hit," such as severe injury or hemorrhagic shock. This triggers the host innate immune system to mount an immediate inflammatory response, the magnitude of which depends on the severity of the injuries. If the patient survives, this systemic inflammation is further augmented by a "second hit," such as ischemia/reperfusion, surgical interventions, and opportunistic infections, accounting for the subsequent peak in mortality (11). The resultant "cytokine storm," which describes the overwhelming and sustained release of pro/anti-inflammatory mediators, including TNF-α, IL-1β, IL-6, and IL-10, is responsible for organ dysfunction as well as increased susceptibility to sepsis (12–14). Indeed, prognosis correlates with circulating levels of biomarkers of systemic inflammation, such as IL-6 and C-reactive protein (CRP).

The prospect of treating polytrauma and sepsis as an inflammatory and immunological disease by immunomodulation remains unfulfilled (10, 15). Strategies involving blockade of cytokines and their receptors were promising in the preclinical setting, but disappointed in clinical trials (10, 16), perhaps in part due to the very early release of TNF-α precluding timely intervention. Currently, there is no FDA-approved agent for the treatment of SIRS or sepsis after activated droterecogin alfa, the recombinant form of human activated protein C, was recalled by the FDA in 2011.

In their role as proinflammatory mediators, alarmins provide valuable insights into the regulation of traumatic inflammation and the pathogenesis of SIRS and related conditions, including sepsis (17), which lead to multiorgan failure and death in up to

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Table 1
Alarmins: physiological and pathological functions

Alarmin	Origin	Intracellular physiological role	Extracellular actions	Release pathways	Receptors	Implicated diseases	Regenerative potential
HMGB1	All cell types including immune cells (non-histone nuclear protein)	Regulation of DNA transcription	Proinflammatory response (when bound to other DAMPs, e.g., LPS, IL-β1, DNA, RNA) (S1); chemotaxis, proliferation, and differentiation of immune and precursor cells, including neurites and myocardial precursors; angiogenesis; induction of adaptive immune response	(a) Passive release by necrotic cells; (b) During apoptosis, HMGB1 is oxidized on Cys106, rendering it tolerogenic rather than proinflammatory (S2); (c) Active secretion via alternative pathway (not via Golgi route): HMGB1 undergoes several forms of post-translational modifications, e.g., acetylation, phosphorylation, methylation	TLR2, TLR4, RAGE (subject to redox states; S3, S4)	Acute trauma and related conditions (S5–S9): sepsis (S5, S10–S13); ischemic brain injury (S14–S16); ischemia/reperfusion injury of the heart (S16, S17); organ transplantation (S18–S20) Chronic conditions: arthritides (S21–S23), SLE (S24), MS (S25), T1DM (S26), epilepsy (S27) Cancer (S28–S30)	Cardiac regeneration (S31–S34), revascularization (S35), skin wound healing (S36), bone repair (S37, S38), skeletal muscle (S39), nerve regeneration (S40)
S100A8/S100A9, S100A12 (humans only, absent in mouse)	Epithelial cells and phagocytes (40% of soluble cytosolic content in neutrophils)	Calcium regulation, cell motility	Pro-inflammatory response; neutrophil adhesion, migration, release from bone marrow; cytokine release by target cells, e.g., monocytes, endothelial cells; antibacterial and anti-parasite activity	(a) Active secretion by epithelial cells and innate immune cells via alternative pathway (i.e., bypass classical Golgi route); (b) Passive release by necrotic or damaged cells	S100A8/A9: TLR4, Carboxylated N-glycans/RAGE; S100A12: RAGE	Acute: sepsis (S41, S42), acute lung injury (S43, S44), asthma (S45) Chronic: arthritides (S46–S54), gout (S55), vascular inflammation (S56), giant cell arteritis (S57), atherosclerosis (S58), lung diseases (S59),inflammatory muscle diseases (S60), skin wound healing (S61, S62) Cancer (S63–S66)	Skin wound healing (S67–S69); liver regeneration (S70); musculoskeletal regeneration (S38, S47)
S100B (S71)	Astrocytes, oligodendrocytes, Schwann cells (cytosol)	Regulation of cell proliferation, differentiation, calcium homeostasis, transcription, motility, enzyme activity, transcription	Pro-proliferation, pro-differentiation	(a) Active release by astrocytes via activation of mGluR3; (b) Active release by Schwann cells via RAGE activation; (c) Passive release by damaged cells, e.g., in acute brain damage, melanoma	RAGE	Neuronal death (S40, S72), biomarker for traumatic and ischemic brain damage (S73, S74), neurodegeneration (S75), schizophrenia (S76)	Nerve regeneration (S40, S77)
HSP60 and HSP70	All cell types (HSP60: mitochondria, HSP70: cytosol and nucleus)	Molecular chaperones in folding and assembly of multimeric protein structures and nascent polypeptide chains, respectively	Autoantigens stimulating immunoregulatory pathways to suppress inflammation; can also bind other ligands, e.g., LPS, to regulate proinflammatory cytokine release (S78)	Passive release by necrotic cells	TLR2, TLR4, SRA1	Sepsis (S78)	Immunization with HSPs induces Tregs in autoimmunity (e.g., RA, T1DM) and tissue and tumor transplantation, suppressing disease and transplant rejection (S79)
β-Defensins	Keratinocytes and epithelial cells	N/A	Direct antimicrobial activity, enhance adaptive immunity	Active release	GPCRs, e.g., CCR6	Respiratory: cystic fibrosis, ARDS, infections Gastrointestinal: IBD	Direct antimicrobial activity by formation of pores in microbial cell
Cathelicidin hCAP18/LL-37	As above + neutrophils, mast cells, monocytes/macrophages	N/A	Direct antimicrobial activity, enhance adaptive immunity	Active release or degranulation	FPRL1	Respiratory: cystic fibrosis, ARDS, infections Gastrointestinal: IBD	Direct antimicrobial activity by formation of pores in microbial cell

FPRL1, formyl peptide receptor-like 1; GPCR, G protein–coupled receptor; IBD, inflammatory bowel disease; mGluR3, metabotropic glutamate receptor 3; T1DM, type 1 diabetes.

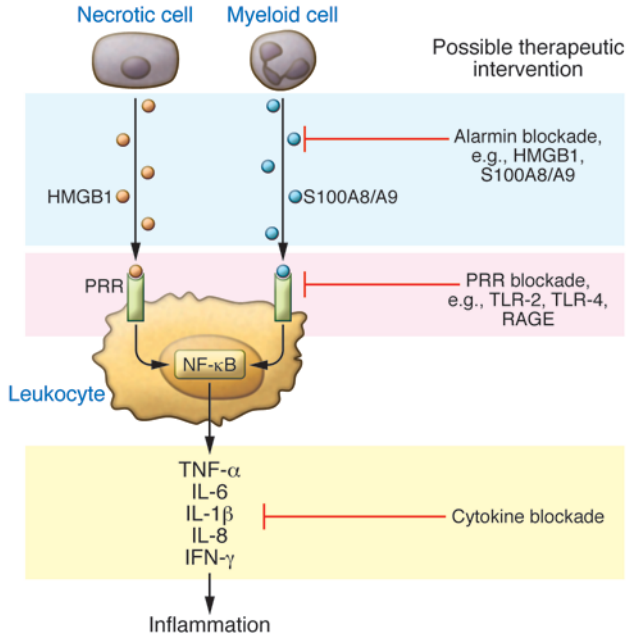

Figure 1
Alarmin pathway as potential therapeutic target in the innate inflammatory cascade. The upstream alarmin signaling pathways are potential therapeutic targets for immunomodulation in both acute and chronic inflammatory diseases. This has been achieved in animal models by directly targeting the alarmins using antibodies or competitive inhibitors, e.g., Box A (HMGB1), or by targeting the pattern recognition receptors (PRR) with antibodies or soluble decoy receptors. The convergence of S100 proteins and HMGB1 onto their receptors may be a rate-limiting step in this pathway and hence represents an attractive therapeutic target. Downstream cytokine blockade, including of TNF-α and IL-6, is used clinically in the treatment of chronic inflammatory diseases such as RA and inflammatory bowel disease, but remains ineffective in a significant proportion of patients. Cytokine blockade has been notably unsuccessful in patients with acute inflammatory disorders.

60% of patients (18). The best-characterized alarmin in this context is HMGB1. It is rapidly released into the circulation upon severe mechanical trauma (19) and in related conditions as well as sepsis (6). This is associated with a devastating and self-injurious innate immune response. HMGB1 levels are also elevated in other life-threatening conditions associated with acute inflammation, including stroke and acute myocardial infarction (19–21).

In sterile injury, HMGB1 is released as an early mediator that activates the later release of TNF-α and other cytokines, and in animals systemic administration of HMGB1 is lethal (22). Numerous animals studies have shown that inhibition of HMGB1 with either neutralizing antibody or the recombinant antagonists Box A or N-terminal domain of thrombomodulin (23) is beneficial in hemorrhagic shock (24, 25), ischemia/reperfusion (26, 27), acute lung (28, 29), myocardial (30), and cerebral ischemic injury (31, 32). By contrast, HMGB1 appears to be a late mediator in sepsis (6, 22, 33), providing a clinically relevant time frame for pharmacological intervention by HMGB1 antagonism, and it has been effective in preclinical models of sepsis (22, 25, 33, 34). Moreover, the presence of autoantibodies to HMGB1 in sepsis is associated with a favorable outcome in critically ill patients (35). Another member of the HMGB family, HMGN1, has also recently been identified as a novel alarmin that is critical for lipopolysaccharide-induced immune responses (36).

S100A8 and S100A9, the most abundant cytoplasmic proteins of neutrophils and monocytes, are also mediators of inflammation (4). S100A8/S100A9 complexes are released during activation of phagocytes and mediate their effects via TLR4, leading to the production of TNF-α and other cytokines (37). Deficiency in these proteins conferred a survival advantage in models of sepsis (37, 38), and blockade of S100A8 and S100A9 suppressed LPS-induced proinflammatory activities (39). Importantly, S100A8/S100A9 levels were elevated on exposure to minimal amounts of LPS. Levels were also increased in septic patients and inversely correlated with survival (40).

An alternative strategy to targeting the upstream regulators of acute inflammation would be to target the pathways that perpetuate the inflammatory process, thereby preventing the "second hit" (41). For instance, TLR4 and receptor for advanced glycation end products (RAGE) have been shown to perpetuate the innate inflammatory response in septic shock (42), and their inhibition offered protection in animal studies (34). However, total abolition of the response may be counterproductive, as many microbial products share pattern recognition receptors such as TLR4 with alarmins, and inhibition may predispose the patient to infection. Therefore, targeting the alarmins directly would avoid the susceptibility to sepsis normally associated with immunosuppressive therapies. In addition, it would be helpful to develop biomarkers to identify those patients who would benefit from immunomodulatory therapy (10). Thus far, the efficacy of targeting the alarmin/receptor axis remains to be validated in controlled clinical trials.

Chronic and autoimmune disorders

It is now widely accepted that alarmins play a key role in the pathogenesis of inflammatory diseases (refs. 43, 44, and Table 1). They not only initiate but also amplify and sustain the inflammatory processes. Alarmins recruit immature DCs, which take up antigens and home to secondary lymphoid organs, where they present antigenic epitopes to naive T cells, resulting in the induction of the adaptive immune response (45–47). Persistent release of alarmins may lead to upregulation of ectopic MHC type I and II expression and presentation of previously unencountered antigens, as well as proliferation of antigen-specific T lymphocytes, thus preventing their activation-dependent apoptosis and promoting their polarization toward a Th1 phenotype. The overall effect is to drive a local hyperinflammatory environment (Figure 2). More recent data suggest that alarmins may also be able to direct commitment toward a Th2, Th7, or regulatory T cell fate (44).

S100A8, S100A9, and S100A12 in humans are highly expressed by phagocytes within the affected joints in inflammatory arthritis (48–50). They activate the endothelium; recruit and stimulate immune cells such as macrophages to produce proinflammatory cytokines, including TNF-α and IL-1β; and demonstrate cytotoxic effects, leading to tissue destruction (51–53). They also appear to be essential in the development of autoreactive $CD8^+$ T cells and systemic immunity (54). Strategies that target the proinflammatory properties of S100 proteins may therefore provide a novel approach for immunotherapy upstream of TNF-α and NF-κB activation, as illustrated by animal studies of hypersensitivity and chronic bowel inflammation (41). However, unlike S100 proteins, HMGB1 involvement appears to be independent of TNF-α (55). Anti–TNF-α therapy had no effect on HMGB1 expression (56),

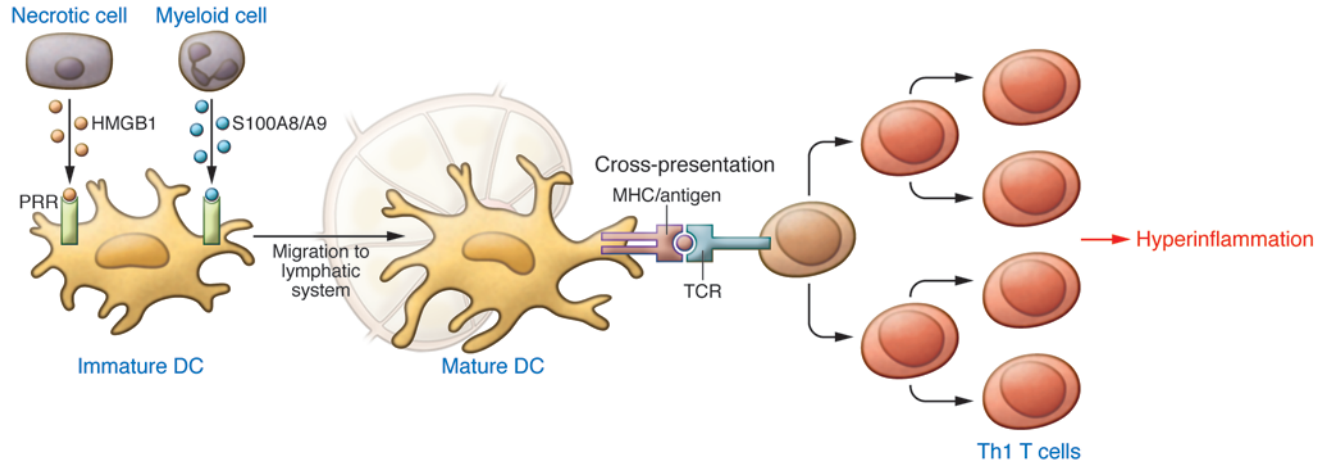

Figure 2
Induction of chronic and autoimmune inflammation by alarmins by upregulating the adaptive immune response. Alarmins recruit immature DCs and induce their functional maturation, leading them to take up antigens and home to secondary lymphoid organs. Here, they present antigenic epitopes to naive T cells, driving Th1 T cell polarization and inducing an adaptive immune response. Persistent release of alarmins upregulates ectopic MHC type I and II expression and presentation of previously unencountered antigens as well as uncontrolled proliferation of T cells, driving a hyper-inflammatory environment. Adapted with permission from *Immunological Reviews* (7).

but anti-HMGB1 antibodies and the antagonist Box A successfully inhibited the development of synovial inflammation and joint swelling in animal models of arthritis (55, 57, 58). Furthermore, binding of HMGB1 to other endogenous partners such as nucleosomes appears to break immunological tolerance and contribute to the pathogenesis of autoimmunity (59). Therefore, alarmins are attractive targets in RA and other chronic inflammatory disorders, especially for patients who do not respond to anti–TNF-α therapy.

In contrast, members of the HSP family, in particular HSP60 and HSP70, have been identified as alarmins whose upregulation may actually be beneficial to patients with inflammatory arthritis (60). HSP60 induces a subtype of regulatory T cells that suppress proinflammatory T cells (61–64). Transfer of these HSP-specific regulatory T cells inhibited inflammation in animal models of arthritis (60, 62). A recent phase II clinical trial yielded promising results (65, 66). This strategy illustrates how targeting the upstream components of the inflammatory cascade that leads to pathological cytokine production may offer a more effective strategy.

Cancer

Chronic inflammation and necrotic cell death are important features of tumorigenesis (67), and alarmins are upregulated in a number of cancers (68–73). The S100A8/S100A9 proteins promote proliferation and survival of tumor cells in vitro (74, 75), and upregulation of S100A9 in myeloid precursors results in the accumulation of cells that suppress T cell proliferation and enable immune evasion (76). S100A8/S100A9 have also been found to promote tumor spread (76–78). Anti–carboxylated glycan antibodies used to inhibit S100 protein-*N*-glycan binding reduced chronic inflammation and tumorigenesis in an in vivo model of colitis-associated carcinogenesis (75).

Like the S100 proteins, HMGB1 has both intra- and extracellular roles in carcinogenesis (79) and tumor spread (80–83), probably through its ability to promote cell migration (51) and angiogenesis (84). Members of the S100 family interact with cytoskeletal elements, including microtubules and actin, leading to increased cellular motility and invasion (85, 86). Upregulation of HMGB1 expression has been found in melanoma and cancers of the prostate, pancreas, and breast (80, 87, 88) and is associated with invasion and metastasis. Blockade of HMGB1 and RAGE suppressed tumor growth and metastasis in murine models of lung and colon cancer (79, 89).

Other alarmins implicated in the promotion of cancer include defensins and cathelicidins. Defensins are a family of cysteine-rich, cationic peptides produced by cells involved in host defense against microbial infections. Expression of some defensins is constitutive, whereas for others it is regulated by damage-associated molecular patterns (DAMPs), cytokines, and growth factors in a tissue-specific manner. Human β-defensin–4 (DEFB4), the homolog of murine Defb29 that has been shown to have proangiogenic and protumorigenic functions, is upregulated seven-fold in stage III compared with stage I tumors (90). Human cationic antimicrobial protein 18 (hCAP-18) is the only known member of the cathelicidin family in humans. The active peptide LL-37 is overexpressed in breast, lung, and ovarian tumors, probably functioning as an autocrine survival factor (91–93). It may also promote tumor growth through angiogenesis and recruitment of $CD45^+$ cells (69).

Paradoxically, alarmins also display antitumor characteristics. Dying tumor cells following chemotherapy and radiotherapy release HMGB1, which can induce the maturation of DCs via TLR2 and TLR4 to promote a cytotoxic T lymphocyte response through cross-presentation of tumor antigens (94–96). Various defensins have been found to have tumor suppressor properties (97–99) and are significantly downregulated in various carcinomas, including DEFB1 in renal cell and prostate carcinomas and DEFB4A and α-defensin HNP-2 in cervical squamous cell carcinomas. Furthermore, although data on the role of α-defensins in cancer biology are currently lacking, they may be antiangiogenic, acting by disrupting fibronectin signaling via the α5β1 integrin (100). The apparently contradictory role of alarmins in cancers requires further investigation.

Table 2
Alarmins as biomarkers

Alarmin	Condition type	Refs.
S100A8, A9, A12	Sepsis, autoimmune (RA, JIA, psoriasis, etc.), respiratory (ARDS, cystic fibrosis), vascular (Kawasaki disease), gastrointestinal (IBD), neurological (MS)	S44, S54, S59, S73, S83–S94
S100B	Traumatic brain injury	S73
HMGB1	Sepsis, trauma, acute coronary syndrome, solid organ transplantation	S10, S18, S95–S99

ARDS, acute respiratory distress syndrome; JIA, juvenile idiopathic arthritis.

Alarmins as biomarkers

Similar to inflammatory biomarkers such as CRP, erythrocyte sedimentation rate (ESR), and IL-6, the levels of the S100 proteins correlate with disease activity in a number of inflammatory conditions (Table 2 and refs. 51, 101–103). However, S100 proteins show important advantages over traditional clinical or laboratory markers for specific indications, probably due to their local expression and release in direct response to tissue damage. Serum levels of S100A8/S100A9 were found to correlate better with disease activity and joint destruction in various inflammatory arthritides than classical markers of inflammation (104–106). In addition, serum S100A8/S100A9 and S100A12 can precisely stage severity and response to therapy (107) as well as predicting relapse (108, 109) and clinical progression, such as the development of erosive disease (110) and radiographic progression of joint damage (111). The suitability of both S100 proteins and HMGB1 as biomarkers for acute systemic inflammatory conditions such as sepsis and following major surgery is also currently under investigation (e.g., ref. 112). Last but not least, S100A8/S100A9 is the only parameter so far that allows early and reliable diagnosis of systemic onset juvenile idiopathic arthritis (SOJIA) and differentiates from infection, which is critical in instigating the appropriate treatment (50).

Alarmins as regenerative therapy

The effects of alarmins, whether beneficial or detrimental, appear to depend on timing of release, dose, and context. Excessive and chronic presence of alarmins and unremitting alarmin-induced events exacerbate injury, but when expressed in a transient and self-limited manner upon injury and acute inflammation, they mediate repair (113). This dual role is exemplified by the proinflammatory cytokine TNF-α. While sustained upregulation has a destructive role in many inflammatory conditions, TNF-α also acts as a growth factor for myelin-producing cells (114), differentiation factor for mesenchymal stem cells (115), and potential therapeutic in the infarcted myocardium (116) or bone fractures (117).

The regenerative role of extracellular HMGB1 is largely mediated by its chemoattractant effects (84, 118–121) as well as its ability to promote cell proliferation (120, 122) and neo-angiogenesis (84, 120, 121). The β-defensin family and cathelicidins also exhibit proangiogenic (90), chemotactic, and proliferative properties (123, 124). Thus far, research on the use of alarmins in regenerative therapy is limited to preclinical studies, the greatest challenge being to understand how to enhance the regenerative processes in postnatal human tissues, where most cells are terminally differentiated and tissues heal with fibrosis following injury.

Exogenous application of alarmins has shown promise in cutaneous wounds. Skin wound repair is problematic in diabetes mellitus due to a dysregulated inflammatory response compounded by an increased microbial load, excessive protease activity, and vascular compromise (125). The antimicrobial alarmins are particularly attractive for cutaneous wound healing due to their additional antimicrobial activities. The pre-form hCAP18 is upregulated in human skin upon wounding, but its levels are low in chronic ulcers. Moreover, antibodies against LL-37 inhibited re-epithelialization (126). Human DEFB3 expression through viral transfection led to accelerated wound closure in *Staphylococcus aureus*–infected diabetic wounds in a pig model (127). HMGB1 expression is reduced in diabetic skin (125). Topical application of HMGB1 to wounds accelerated healing in diabetic mice but not normoglycemic mice, whereas topical Box A impaired wound healing in normoglycemic mice, suggesting that the latter may already have optimal levels of HMGB1 (125). S100A8 and S100A9 also appear to promote skin wound healing (128), and wound fluid from non-diabetic patients with non-healing venous leg ulcers showed that S100A8 and S100A9 were significantly reduced (129).

The use of exogenous alarmins to recruit and induce proliferation and differentiation of resident stem cells to enhance wound healing was demonstrated initially in a murine model of myocardial infarction (122, 130). Local administration of HMGB1 led to improved structural and functional outcomes after infarction (131). Furthermore, cardiac-specific overexpression of HMGB1 conferred significant protection against tissue damage and was associated with improved cardiac function (132), while anti-HMGB1 antibodies exacerbated injury (133).

Despite the evidence that alarmins promote tissue homeostasis, there are also data suggesting the contrary. For instance, although HMGB1 is a potent neurotrophic mediator, it also contributes to neuronal cell death in cerebral ischemia (32), and downregulation conferred significant protection (31, 32, 134). Activation of proinflammatory pathways by HMGB1 also exacerbated myocardial injury. Serum HMGB1 levels are elevated in patients with myocardial infarction and correlate with poor clinical outcomes (21). Treatment with HMGB1 inhibitor, Box A, significantly reduced infarct size and tissue damage in an ischemia/reperfusion injury model of the murine heart, and systemically administered rHMGB1 increased the severity of damage (30). While these findings appear to conflict with other studies that suggest that exogenous HMGB1 promoted cardiac regeneration (30, 122), this discrepancy may be explained by the low dose of HMGB1 being administered during a critical time window when its expression was low in the latter studies.

The harmful role of alarmins is particularly evident in chronic conditions. For example, activated macrophages promote destruction and impair regeneration via secretion of S100A8 and S100A9 in inflammatory muscle diseases (135), and blockade of RAGE restores effective cutaneous wound healing in diabetic mice (136).

Comparison of acute and chronic wounds in humans identified elevated levels of S100A8 and S100A9 from the exudate of nonhealing wounds (137). However, this may again be attributed to a dose-dependent effect: for example, low doses of S100B have been found to promote neurite outgrowth, whereas high doses led to apoptosis (138, 139).

Challenges and future directions

The therapeutic potential for immunomodulation by targeting alarmins and their signaling pathways appears promising and needs to be tested in clinical trials. However, there are several key issues that remain to be addressed.

First, it remains unclear whether there exists a hierarchy of dominance in the inflammatory effects of alarmins. For example, both HMGB1 and S100 proteins have been shown to be critical inflammatory mediators in RA, but which is the master regulator, and how do we decide which to target?

Second is the practical issue of how to inhibit alarmins in inflammatory conditions. While neutralizing antibodies or antagonists have been successful in experimental models, effective blockade of their proinflammatory activities clinically may prove challenging given the high extracellular concentrations encountered at sites of inflammation, such as in the synovial joints. An approach that targets the steps that are rate limiting may prove more effective — for example, inhibition of the S100A8/S100AA9-dependent transendothelial leukocyte migration by blocking S100-*N*-glycan binding to endothelial cells (140, 141) or inhibition of the active release of S100A8/S100A9 by selectively targeting the "alternative pathway" in phagocytes. Furthermore, the concomitant release of multiple alarmins, all of which appear to drive and perpetuate inflammation, presents a challenge, as their inhibition may lead to differential effects, as exemplified by the beneficial effects of S100A8/S100A9 and HMGB1 inhibition versus that of HSP upregulation in inflammatory arthritis. Furthermore, the convergence of alarmins on pattern recognition receptors, for example S100A8/S100A9 and HMGB1 on TLR4 and RAGE, may mean that inhibitors of these receptors or their downstream intracellular signaling cascades may represent a more efficient approach (refs. 142–144 and Figure 2). However, a potential drawback of this approach is that many microbial products share patterns recognition receptors such as TLR4, and hence therapeutic approaches directed against the receptors may increase the risk of infection.

The third and greatest challenge concerns the issue of balance. Like many signaling molecules, alarmins exhibit both harmful and beneficial effects within the same disease context. While dampening of inflammation may be desirable in certain pathological contexts, total abolition of the host defense is detrimental, leaving the patient susceptible to opportunistic infections and tumorigenesis as well as impairing repair and remodeling pathways. Conversely, the alarmin signaling axis can be manipulated to activate transient and self-limited inflammation and pathways that orchestrate tissue homeostasis. This is likely to be achieved by a better understanding of how the microenvironment and dosage contribute to the net effects of alarmins. For example, it has been found that low doses of S100B induce trophic effects in neurites, whereas high doses induce apoptosis (138). Furthermore, we must understand how to clinically modulate the local environment so as to activate the innate protective pathways that initiate regenerative and repair processes while downregulating the self-injurious pathways that inhibit repair and drive excessive and deleterious cytokine release. An area that deserves particular attention is the mechanism by which alarmins mediate the interaction between alarmin-stimulated DCs and tissue-repair macrophages, as this would offer the prospect of a rational, mechanism-based approach to promote wound repair.

The translational potential of alarmins and their signaling pathways is not restricted to the clinical conditions mentioned in this Review. For example, the immunological adjuvant activity of all alarmins and antimicrobial properties of many also mean that they have therapeutic potential in the development of therapies and vaccines against viruses, fungi, and cancer (145–147). However, this is relatively new territory, and such advances are more distant on the translational horizon.

Over the past century, tremendous strides have been made in understanding the immune system, leading to significant translational successes, including the development of immunosuppressive drugs in the field of organ transplantation (148) and, more recently, biologic therapies, including anti–TNF-α therapy in the treatment of RA and other chronic autoimmune conditions (149). Further understanding of apparently conflicting roles of alarmins in inflammation and repair is beginning to yield novel approaches for translation to the clinical arena.

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Address correspondence to: James Chan, Kennedy Institute of Rheumatology, University of Oxford, 65 Aspenlea Road, London W6 8LH, United Kingdom. Phone: 44.773.878.1762; Fax: 02085630399; E-mail: james.chan@kennedy.ox.ac.uk.

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