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Alarmins Link Neutrophils and Dendritic Cells

De Yang^{1,2}, Gonzalo de la Rosa², Poonam Tewary², and Joost J. Oppenheim²

¹Basic Science Program, SAIC-Frederick, Inc., Frederick, Maryland 21702–1201

²Laboratory of Molecular Immunoregulation, Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702–1201

Abstract

Neutrophils are the first major population of leukocyte to infiltrate infected or injured tissues and are crucial for initiating host innate defense and adaptive immunity. Although the contribution of neutrophils to innate immune defense is mediated predominantly by phagocytosis and killing of microorganisms, neutrophils also participate in the induction of adaptive immune responses. At sites of infection and/or injury, neutrophils release numerous mediators upon degranulation or death, among these are alarmins which have a characteristic dual capacity to mobilize and activate antigen-presenting cells. We describe here how alarmins released by neutrophil degranulation and/or death can link neutrophils to dendritic cells by promoting their recruitment and activation, resulting in the augmentation of innate and adaptive immune responses.

Introduction

Neutrophils are rapidly induced to degranulate in the inflammatory microenvironment by a wide variety of stimulants such as formyl-methionyl-leucyl-phenylalanine (fMLF), C5a, platelet-activating factor (PAF), lipopolysaccharide (LPS) and tumor necrosis factor (TNF) ¹. The granules of neutrophils sequentially release several hundred constitutively expressed proteins ², including proinflammatory mediators with maturational effects on dendritic cells (DCs) ³. Immature DCs present at sites of infection where activated neutrophils degranulate are thus potentially influenced by numerous granule-derived mediators. These include ‘alarmins’ that rapidly galvanize antigen-presenting cells (APCs) and activate innate and adaptive immune responses. We have termed them ‘alarmins’ because they represent the first host response to exogenous (infections) and endogenous (injuries) danger signals ^{4,5}. Alarmins have the dual capacities to recruit ⁶ and to activate inflammatory cells including DCs ^{4,5} using G α -protein-coupled receptor(s) (GiPCR) and activating receptor(s) respectively (Box 1). Alarmins rapidly marshal the host's innate inflammatory responses by activating recruited inflammatory cells to produce cytokines and to develop immature DCs into mature DCs capable of inducing antigen-specific adaptive immune responses ⁷. Neutrophil-derived alarmins include a number of human antimicrobial peptides such as α -defensins ⁸⁻¹⁰, cathelicidin ^{11,12} and lactoferrin ^{13,14}. In addition, cell injury/necrosis of neutrophils results in the release of nuclear binding proteins with alarmin activity, such as high-mobility group box-1 (HMGB1) protein ¹⁵⁻¹⁷. It must be pointed out that not all antimicrobial peptides or proteins released by degranulation and cell injury are alarmins. We have not been able to show that

Correspondence should be addressed to: Joost J. Oppenheim (Tel: 301–846–1551; Fax: 301–846–7042; email: oppenhei@ncifcrf.gov).

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neutrophil-derived azurocidin, transferrin, lysozyme, myeloperoxidase, bactericidal/permeability-increasing protein, elastase or cathepsin G are able to chemoattract and activate APCs (Table 1). Nevertheless, some of these molecules play important alternative roles in promoting innate immunity.

α -Defensins

α -Defensins are small (3–4 kDa) cationic host-derived antimicrobial peptides that have been identified in humans, monkeys and several rodent species. They were primarily isolated from the azurophilic granules of human neutrophils and thus are referred to as human neutrophil peptides (HNPs)^{8,18}. They are particularly abundant in neutrophils, certain macrophage populations and Paneth cells of the small intestine and are active at micromolar concentrations against many bacteria, fungi and enveloped viruses. Although mice lack neutrophil α -defensins, mouse Paneth cells of the intestinal tract do produce homologues of human α -defensins 5 and 6 (HD5 and HD6), called cryptdins. These peptides are characterized by six highly conserved cysteine residues and form three pairs of intrachain disulfide bonds that are paired C1 to C6, C2 to C4, and C3 to C5¹⁹.

Of the six human α -defensins identified, four (HNP 1–4) are produced and stored in human neutrophils^{8,19,20} and the other two, HD-5 and –6, are expressed by Paneth cells of the small intestine^{21,22}. Expression of HNPs is constitutive, and under physiological conditions the level of expression varies depending on the inflammatory status of the individual, with extremely high local concentrations of up to 2 mg/ml in regions of neutrophil infiltration^{3,23,24}. Initially characterized as antimicrobial agents, these neutrophil-derived peptides also have immunostimulatory properties that are mediated by direct interaction with receptors on cells of the host immune system³. These molecules are important mobilizers and activators of innate inflammatory responses at nanomolar concentrations and promote antigen-specific adaptive immune responses when co-administered with a given antigen *in vivo*^{3,10,25}.

We have demonstrated that HNP-1 and –2 are chemotactic for naïve T cells and immature DCs, but not for mature DCs⁹. Other groups have reported that these peptides are potent chemoattractants of monocytes, but fail to induce chemotaxis of neutrophils²⁶. HNP-1 also suppresses neutrophil migration in response to fMLF, but not to interleukin-8 (IL-8)²⁷. Others report that HNP-1 and HNP-3 chemoattract human monocyte-derived macrophages, naïve and memory T cells and human mast cell lines²⁸. It is now established that α -defensins chemoattract a variety of leukocytes to inflammatory sites depending on the study conditions and sources of peptides, and the chemotactic effect is mediated by an unidentified GiPCR^{9,28}. Apart from their direct chemotactic activity, HNPs, by activating as yet unidentified activating receptors, also induce the expression of cytokines and chemokines including IL-1, TNF, IL-8 and monocyte chemoattractant protein-1 (MCP-1)³.

In addition to their numerous roles in innate immunity, it is apparent that HNPs can also galvanize adaptive immune responses by inducing the maturation of DCs and acting as an immune adjuvant. Intranasal co-administration of defensins in mice HNP-1–3 with ovalbumin (OVA) enhanced both humoral and cellular responses and enhanced *ex vivo* production of both T helper 1 (Th1) cytokines such as interferon- γ (IFN- γ), cytokines such as IL-5, IL-6 and IL-10¹⁰. Furthermore, in a B-cell lymphoma model, mice administered α -defensins in combination with tumor-associated antigen conjugated to keyhole limpet hemocyanin (KLH) had better survival rates and enhanced humoral responses²⁵. Therefore, these peptides directly or indirectly provide signals to various APCs, including DCs, induce chemotaxis and antigen uptake, and upregulate the production of cytokines, thereby increasing antigen presentation and lymphocyte activation. Hence α -defensins act as alarmins and promote the recruitment and activation of host leukocytes.

Cathelicidins

Cathelicidins are a family of mammalian antimicrobial proteins that consist of an N-terminal putative signal peptide, a conserved cathelin-like domain and a C-terminal antimicrobial domain that varies remarkably in size (ranging from 12 to 97 amino acids)²⁹. More than 40 members of the cathelicidin family have been identified in different species; however, humans and mice each produce only one cathelicidin, called human cationic antimicrobial protein 18 (hCAP18) and the ortholog cathelin-related antimicrobial peptide (CRAMP), respectively^{3, 24,29}. The C-terminal antimicrobial domain released from hCAP18 is called LL-37, because it consists of 37 amino acids, starting with two leucines at the N-terminus²⁹. Cathelicidins are predominantly stored constitutively at high concentrations in the secondary granules of neutrophils, which, in the course of degranulation, release the C-terminal mature antimicrobial peptides (e.g. LL-37) by proteolytic cleavage^{3,24,29-31}. The mature cathelicidin peptides can be further processed in the tissues into smaller peptides with distinct biological activities^{31, 32}. In addition to neutrophils, other leukocytes, including monocytes/macrophages, mast cells and epithelial cells, can also generate cathelicidin, particularly in response to proinflammatory stimuli including cytokines, pathogen-associated molecular patterns (PAMPs) or tissue injury^{3,24,29}.

Cathelicidins such as LL-37 and CRAMP are α -helical peptides²⁹. Several of the cathelicidin peptides are chemotactic for various leukocytes including human and mouse neutrophils, monocytes, mast cells, T cells, and mouse DCs^{11,12,33-36}. The APC-chemoattracting activity of mouse cathelicidin (CRAMP) is verified by CRAMP-induced accumulation of mouse monocytes at the site of injection¹². Cathelicidins can also induce the activation of many types of cell, including monocytes, macrophages, mast cells, keratinocytes, and endothelial and epithelial cells^{3,11,24}. Furthermore, LL-37 promotes both the differentiation and activation of monocyte-derived DCs^{37,38}. In conjunction with DNA, cathelicidin promotes activation of plasmacytoid DCs, increasing their expression of co-stimulatory molecules and type-I IFN production, potentially exacerbating autoimmune diseases such as psoriasis^{37,39}. The APC-recruiting and activating activities of cathelicidins indicate that they function as alarmins⁴. Indeed, simultaneous administration of mouse cathelicidin, CRAMP, with the antigen ovalbumin enhanced ovalbumin-specific immune responses in mice¹².

Cathelicidins utilize the formyl peptide receptor-like 1 (FPRL1) to chemoattract human neutrophils, monocytes, T cells and multipotent mesenchymal stromal cells^{11,35,40}. CRAMP induces chemotaxis of mouse leukocytes using formyl peptide receptor 2 (FPR2), the mouse homolog of human FPRL1¹². Not only does FPRL1 or FPR2 mediate the chemotactic effect of cathelicidins, but it also accounts for the angiogenic activity of LL-37⁴¹. The capacity of LL-37 to promote IL-1 β production by monocytes is reportedly mediated by the ionotropic purinergic receptor P2X7⁴². However, the capacity of LL-37 to activate neutrophils and to inhibit their apoptosis has been attributed to both FPRL1 and P2X7⁴³. LL-37 interacts with self DNA to form a complex and to activate plasmacytoid DCs by triggering Toll-like receptor 9 (TLR9)³⁹. Therefore, in a similar way to other alarmins, cathelicidin uses a GiPCR to recruit cells and a pattern recognition receptor (e.g. P2X7 and/or TLR9) to activate leukocytes including APCs.

Although many laboratories, including ours, consider that cathelicidins have a clear proinflammatory profile, several groups have reported that they also exhibit anti-inflammatory capacities. LL-37 blocks the activation of human⁴⁴ or mouse DCs⁴⁵ induced by some TLR agonists including lipopolysaccharide (LPS). Cathelicidins inhibit the activation of mouse macrophages in response to small fragments of hyaluronan, an endogenous TLR4 ligand, which might be responsible for the exaggerated dinitrofluorobenzene-induced skin inflammation seen in cathelicidin knockout mice⁴⁶. However, transgenic mice overexpressing cathelicidin are

more resistant to cutaneous bacterial challenge⁴⁷. Furthermore, the fact that CRAMP knockout mice are more susceptible to bacterial challenge demonstrates the important role(s) that cathelicidin plays in promoting host immune defense^{3,24,29,48}.

Lactoferrin

Lactoferrin, a 703 amino acid (80 kDa) glycoprotein that belongs to the transferrin family of iron-binding proteins, was originally isolated from milk and shown to exhibit antimicrobial activity⁴⁹. Lactoferrin has anti-bacterial, anti-viral and anti-fungal activities based on iron deprivation⁴⁹. However, lactoferrin also has antimicrobial effects based on binding microbial LPS and glycosaminoglycans and other surface receptors⁴⁹⁻⁵¹. Furthermore, lactoferrin knockout mice exhibit reduced resistance to bacterial challenge⁵².

Lactoferrin is present in many mammalian secretions, including saliva, tears and at high concentrations in milk; the highest concentration of lactoferrin is found in colostrum (0.5–6.0 mg/ml)^{49,53}. Neutrophils are an important source of lactoferrin; they store this protein in their secondary granules, along with cathelicidin, and release it upon activation³⁰. Although under normal conditions lactoferrin levels in blood are about 1 µg/ml, which can reach up to 200 µg/ml under septic conditions⁵⁴ and are likely to be higher at the inflammatory site itself⁵⁵.

In addition to its antimicrobial properties, lactoferrin exerts many other effects on the immune system. Although we have not observed effects on neutrophil migration, others have reported that lactoferrin might have a direct chemotactic effect⁵⁶ or a chemorepulsive effect on neutrophils⁵⁷. In contrast, we have shown that lactoferrin is a weak chemoattractant for monocytes *in vitro*, but a more potent recruiter of murine myeloid cells *in vivo* through a GiPCR that remains to be identified¹³. This finding, along with the ability to induce the production of chemokines such as macrophage inflammatory protein-1α (MIP-1α) and MIP-2⁵⁸, and proinflammatory cytokine production, indicates that lactoferrin is a 'proinflammatory' stimulant.

Lactoferrin not only activates innate immunity, but also, when used at high concentrations (10–100 µg/ml), stimulates human monocyte-derived DCs and peripheral blood DCs^{13,14}. Such high concentrations of lactoferrin are pathophysiologically relevant since it is elevated to high concentrations in infectious states and can potentially be delivered locally at high concentrations by neutrophil granule release. This results in the activation of the adaptive immune system and consequently accounts for the immunoenhancing antitumor effects of lactoferrin⁵⁹⁻⁶¹.

Lactoferrin has been reported to interact with multiple receptors (as reviewed in detail by Suzuki⁶²). Lactoferrin inhibits LPS-mediated activation because of its ability to sequester endotoxin and iron and because it binds with soluble lipopolysaccharide-binding protein (LBP) and CD14⁶². We and others have, however, observed that lactoferrin by itself can activate the TLR4 pathway^{63,64}. Lactoferrin-induced cellular activation is diminished in macrophages and DCs when TLR4 is absent (e.g. APCs from C3H/HeJ mice) or its signal is blocked with anti-TLR4 antibodies⁶³. This capacity of lactoferrin to activate the TLR4 pathway in DCs might be very important in host defense in the absence of endotoxin, such as in internal traumatic injuries or neutrophil necrosis.

Nevertheless, other receptors must play a role in the activation of the immune system by lactoferrin, because even in the absence of TLR4, lactoferrin still induces some macrophage responses^{64,65}. Lactoferrin binds to the receptor for advanced glycation end-products (RAGE), asialoglycoprotein receptors and CD91; therefore, these receptors are also candidates for lactoferrin-induced mononuclear cell activation^{62,66,67}. Furthermore, DNA can complex with lactoferrin⁶⁸, so that TLR9 is a potential candidate receptor, especially in situations in which

cellular content is exposed to the extracellular environment, as in cell injury and necrosis. Although lactoferrin can polarize lymphocyte responses towards a Th2 phenotype⁵⁵, we and others have shown that when lactoferrin is used as an adjuvant, it rather favors Th1 lymphocyte responses^{13,69,70}.

Whether lactoferrin is pro- or anti-inflammatory is also controversial. Oral administration of lactoferrin reduces colon inflammation in dextran sodium sulfate (DSS) induced colitis in mice by increasing levels of IL-10 and IL-4 and reducing levels of TNF, IL-6 and IL-1⁷¹. However, *in vitro* and *in vivo* experiments have shown that lactoferrin activates both macrophages and DCs, increasing their production of proinflammatory cytokines^{13,14,69}. Moreover, oral administration of lactoferrin activates the enterogastric epithelia, inducing the production of cytokines such as IL-18 and type I IFN⁷², thus stimulating the immune system located beneath the apical surface of the enterocytes. As detection of lactoferrin in the stools has been used as a marker for human gastrointestinal disorders⁷³, it is likely that an increase in the level of this protein plays an important role in enhancing immune responses. Furthermore, we found that lactoferricin, a cleavage product of lactoferrin present in the gastrointestinal tract, still has alarmin activity. Whether lactoferrin enhances or suppresses immune responses in the gastrointestinal tract remains to be clarified.

High-mobility group box-1 protein (HMGB1)

HMGB1, a member of the HMG superfamily, is a 215 amino acid non-histone chromosomal binding protein that is normally located in the nucleus where it regulates chromosome stability and the transcription of certain genes⁷⁴. This vital role of HMGB1 probably accounts for the fact that gene disruption has lethal consequences⁷⁵. HMGB1 is released extracellularly as a result of loss of membrane integrity upon necrosis of nucleated cells (including neutrophils). Mononuclear leukocytes can also be induced to express and secrete HMGB1 in response to treatment with various activators, such as pathogen associated molecular patterns (PAMPs) or proinflammatory cytokines^{15,76-79}. However, the release of HMGB1 by activated leukocytes occurs via a process distinct from the classical Golgi- and endoplasmic reticulum-dependent secretion pathway. The process involves a crucial initial acetylation on many of the 43 lysine residues of HMGB1 in the nucleus⁷⁷, followed by redistribution of HMGB1 from the nucleus to endolysosomes and finally exocytosis⁸⁰.

Extracellular HMGB1 has antibacterial activity⁸¹. HMGB1 acts as an alarmin because it induces both the migration¹⁷ and activation of DCs¹⁵⁻¹⁷, and it enhances antigen-specific immune responses that favor Th1 polarization^{15,82,83}. The first receptor identified for HMGB1 was RAGE⁸⁴. HMGB1-induced cell migration is, at least in part, mediated by RAGE because this effect can be partially inhibited by anti-RAGE neutralizing antibody^{17,85}. However, the capacity of HMGB1 to induce the migration of DCs and other cell types is also dependent on a GiPCR, because treatment of target cells with pertussis toxin, a specific GiPCR inhibitor, blocks HMGB1-induced cell migration^{17,85,86}. The activation of mouse macrophages and plasmacytoid DCs by HMGB1 is mediated in part by RAGE^{87,88}. In addition, HMGB1-induced NF- κ B activation and cytokine production in phagocytes relies on both TLR2 and TLR4⁸⁹. Recently, HMGB1 was detected in DNA-containing immune complexes often seen in the serum of patients with systemic autoimmune diseases (e.g. systemic lupus erythematosus)^{83,90}. The activation of B cells and/or plasmacytoid DCs by such HMGB1- and DNA-containing immune complexes or by complexes of HMGB1 and CpG oligodeoxynucleotides is crucially dependent on both RAGE and TLR9^{78,90}. Therefore, the activation of leukocytes, including DCs, by HMGB1 preparations appears to be mediated by multiple receptors including RAGE and TLR2, 4 and 9.

Highly purified eukaryotic HMGB1 induces little or no proinflammatory cytokines, suggesting that the potent leukocyte-activating effects of recombinant HMGB1 is really due to HMGB1 forming a complex with TLR ligands^{91,92}. This finding is supported by observations that induction of inflammatory responses by HMGB1 in the peritoneal cavity depends largely on the presence of both RAGE and TLR4⁹³. In addition, HMGB1 released by dying tumor cells *in vivo* has been shown to enhance anti-tumor immune responses by triggering TLR4-expressing DCs⁸². Furthermore, HMGB1 forms a complex with DNA in nucleosomes, which was released from 'late apoptotic' cells⁸³. Such HMGB1-containing nucleosomes not only induced the activation of macrophages and DCs leading to the production of proinflammatory cytokines (e.g. IL-1 β , IL-6, IL-10, and TNF- α) and DC maturation, but also stimulated the production of anti-dsDNA and anti-histone IgG responses in a TLR2-dependent manner⁸³. Therefore, it is likely that, *in vivo*, HMGB1 also acts as an activator for DCs and other cells in the form of complexes with other components (e.g. nucleosomes), which interact with multiple activating receptors including RAGE and TLR2, 4 or 9.

Alarmins link neutrophils to DCs

Neutrophil-derived alarmins contribute to the recruitment of DCs in several ways (Figure 1). First, certain α -defensins and HMGB1 are chemotactic for immature DCs and can contribute directly to local recruitment of DCs^{9,17}. Lactoferrin and cathelicidin, however, are chemotactic for monocytes^{11,13,26,28,94}, thus contributing to the recruitment of potential precursors of DCs^{94,95}. Furthermore, neutrophil-derived alarmins indirectly contribute to recruitment of DCs by inducing the production of chemokines, such as MCP-1 and/or RANTES^{3,13,16,17,96}. Therefore, alarmins help to increase local recruitment of DCs, enhancing both innate/inflammatory responses and presumably antigen uptake. Moreover, neutrophil-derived alarmins stimulate phenotypic and functional maturation of DCs either by themselves or in complexes with DNA^{39,90}. When neutrophils are activated, they release both granule proteins (including alarmins) and chromatin that form extracellular fibers called neutrophil extracellular traps (NETs, also see the article by Papayannopoulos and Zychlinsky in this special issue)⁹⁷. These NETs bind and concentrate the products of degranulation (e.g. LL-37 and defensins), which not only facilitates the destruction of microbial invaders⁹⁷, but also provides a potent co-stimulatory element consisting of complexes of DNA and alarmins. Thus, neutrophil-derived alarmins serve as a bridge between neutrophils and DCs, promoting inflammatory and immune responses by inducing the recruitment and maturation of antigen-presenting DCs.

Numerous studies have shown the participation of alarmins in the development of inflammation and immune responses. The levels of neutrophil-derived alarmins are high under many inflammatory conditions, whereas blockade of some of these mediators has been shown to ameliorate the manifestation of acute inflammatory reactions^{3,23,24,29,51,55,98}. In addition, administration of exogenous α -defensin(s), cathelicidin, lactoferrin or HMGB1 together with an antigen promotes antigen-specific immune responses^{10,12-15,25,70,83}. Furthermore, many levels of neutrophil-derived alarmins are increased markedly either locally or systemically in autoimmune disorders^{39,90,99,100}, in which case alarmins might promote autoimmune reactions^{39,83}.

Although we have focused on the immunostimulatory activities of alarmins derived from neutrophils in this review, alarmins produced by many different cells under distinct conditions have diverse effects that are more than just immunostimulatory. Cathelicidin inhibits LPS-induced DC maturation and inflammatory reaction in part by neutralizing LPS⁴⁴⁻⁴⁶. LL-37 produced in tumors promotes tumor progression by recruiting multipotent mesenchymal stromal cells and promoting angiogenesis⁴⁰. HMGB1 and LL-37 released by tumor tissues might contribute to tumor progression because of their proinflammatory and proangiogenic effects^{40,101}. Overall, the available evidence suggests that, when neutrophils infiltrate infected

or damaged tissues and become activated in acute inflammatory reactions, the release of alarmins results in proinflammatory responses that alert rather than suppress host immune defense.

Conclusion

One can ask whether there is a real need to propose a new term such as ‘alarmin’? Alarmins certainly act in concert with PAMPs in response to infections, but might play a more pivotal role in response to traumatic injuries. The fact that neutrophils as well as other leukocytes and epithelial cells can rapidly release stored proteins or peptides that mobilize and activate host immune responses distinguishes these molecules from cytokines. Cytokines certainly respond to danger signals in defense of the host, but they need to be induced and are usually not chemotactic. Alarmins can be considered a subset of damage-associated molecular patterns (DAMPs), but alarmins really mediate responses to danger and infection, and only become dangerous if released in excess. Alarmins have no structural similarities; however, they are all highly charged cationic molecules, which accounts for their antimicrobial activities. A number of them share an unknown tertiary structure that might enable them to interact with TLRs. Although their positive charge properties might account for their capacity to interact with host receptors, there are numerous charged antimicrobial proteins that are not alarmins. Perhaps the best testimony in favor of the alarmin concept is the fact that it has led to the identification of some unexpected molecules as initiators of host defense.

Box 1: What are alarmins?

Alarmins are structurally distinct endogenous mediators that can recruit and activate antigen-presenting cells (particularly dendritic cells), and consequently possess the capacity to enhance innate and adaptive immune responses. Alarmins are usually constitutively present in cells, such as leukocytes and epithelial cells (including keratinocytes), as components of the granules, cytoplasm and nucleus. However, in addition, most alarmins like cytokines can be induced in response to proinflammatory cytokines and pathogen-associated molecular patterns (PAMPs). Unlike cytokines, alarmins are rapidly released by degranulation and/or cell necrosis in response to infection or tissue injury. Alarmins are endogenous peptides that are released in host defense against danger signals, and therefore, can be considered as a subset of damage associated molecular patterns (DAMPs).

Alarmins recruit leukocytes from the blood and activate many nearby cells. The recruitment and activation of neutrophils, monocytes/macrophages, dendritic cells and NK cells by alarmins enhances the inflammatory response. The capacity of an alarmin to recruit antigen-presenting cells directly is predominantly mediated by a G α -protein-coupled receptor (GiPCR), although alarmins can also indirectly recruit leukocytes by stimulating the production of chemotactic factors including chemokines. The activating effects of alarmins resulting in maturation of dendritic cells are often mediated by their capacities to interact with activating receptors such as Toll-like receptors (TLRs). Currently identified alarmins include defensins, cathelicidins (e.g. LL-37), eosinophil-associated ribonucleases (e.g. eosinophil-derived neurotoxin), high-mobility group proteins (e.g. high-mobility group box-1 protein), granulysin, and iron-binding proteins (e.g. lactoferrin). Molecules that may prove to be alarmins include some of the heat-shock proteins, the calcium chelating S100 family proteins and certain lipid metabolites.

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Figure 1.

Alarmins link neutrophils (N) and dendritic cells (DC). Neutrophils infiltrating the site of tissue injury and infection release alarmins, such as defensins, cathelicidin, lactoferrin and HMGB1. Other cell types, such as injured or otherwise activated epithelial cells (Ep), can also contribute to the local production of alarmins. Locally generated alarmins directly recruit various subsets of leukocytes (including dendritic cells) based on their chemotactic effect. However, alarmins also activate various leukocytes, such as monocytes/macrophages (Mφ) that are either resident or freshly recruited. Recruitment and activation of phagocytic cells by alarmins certainly contributes to the enhancement of the innate defense response through increasing the local

number of phagocytes, augmenting phagocytosis, and killing of pathogens, and increasing the production of cytokines. Recruitment and activation of DCs by alarmins results in not only an increase in the local number of DCs but also in the phenotypic and functional maturation of DCs i.e. immature DC (iDC) to mature DC (mDC) conversion, which contributes to the enhanced uptake, processing and presentation (upon arrival at the secondary lymphoid organs) of antigen(s) to naïve T cells, and consequently the enhancement of adaptive immune responses.

Table 1

Neutrophil proteins with alarmin activity

Neutrophil protein	Alarmin activity and receptor(s)			
	APC recruitment		APC activation	
	Activity	Receptor	Activity	Receptor
α -Defensins Cathelicidins	+	GiPCR	+	Unknown
	+	FPRL1 FPR2	+	P2X7 TLR9
Lactoferrin HMGB1	+	GiPCR	+	TLR4
	+	RAGE GiPCR	+	RAGE TLR2 TLR4 TLR9

Abbreviations: APC, antigen-presenting cell; FPR, formyl peptide receptor; FPRL1, FPR-like receptor 1; GiPCR, an unidentified Gai-protein-coupled receptor; HMGB1, high-mobility group box-1 protein; RAGE, receptor for advanced glycation end-products; TLR, Toll-like receptor.