Allergology International 70 (2021) 30–38

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Invited Review Article

Mechanisms of toxicity mediated by neutrophil and eosinophil granule proteins

Lea Gigon [a], Shida Yousefi [a], Alexander Karaulov [b], Hans-Uwe Simon [a, b, *]

[a] Institute of Pharmacology, University of Bern, Bern, Switzerland
[b] Department of Clinical Immunology and Allergology, Sechenov University, Moscow, Russia

ARTICLE INFO

Article history:
Received 13 November 2020
Available online 1 December 2020

Keywords:
Cytotoxicity
Eosinophil
Granule protein
Innate immunity
Neutrophil

Abbreviations:
α1-PI, α1-proteinase inhibitor; α1-ACT, α1-antichymotrypsin; ALP, anti-leukoprotease; AMP, antimicrobial protein; BPI, bactericidal/permeability-increasing protein; CLC, Charcot-Leyden crystal protein; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EET, eosinophil extracellular trap; EPO, eosinophil peroxidase; ET, extracellular trap; fMLP, N-formylmethionyl-leucyl-phenylalanine; GPI, glycosylphosphatidylinositol; hCAP, human cationic antimicrobial protein; HNP, human neutrophil peptide; LBP, LPS-binding protein; MBP, major basic protein; MMP, metalloproteinase; MPO, myeloperoxidase; mtDNA, mitochondrial DNA; NET, neutrophil extracellular trap; NGAL, neutrophil gelatinase-associated lipocalin; PAF, platelet-activating factor; PMN, polymorphonuclear leukocytes; OS, reactive oxygen species; SLPI, secretory leukoproteinase inhibitor; TIMP-1, tissue inhibitor of metalloproteinase-1; TULIP, tubular-lipid binding protein

ABSTRACT

Neutrophils and eosinophils are granulocytes which are characterized by the presence of granules in the cytoplasm. Granules provide a safe storage site for granule proteins that play important roles in the immune function of granulocytes. Upon granulocytes activation, diverse proteins are released from the granules into the extracellular space and contribute to the fight against infections. In this article, we describe granule proteins of both neutrophils and eosinophils able to kill pathogens and review their anticipated mechanism of antimicrobial toxicity. It should be noted that an excess of granules protein release can lead to tissue damage of the host resulting in chronic inflammation and organ dysfunction.

Introduction

Granulocytes are polymorphonuclear (PMN) leukocytes that comprise neutrophils, eosinophils, and basophils, of which neutrophils are the most frequent type found in blood. These white blood cells are an essential part of the innate immune system and

* Corresponding author. Institute of Pharmacology, University of Bern, Inselspital, INO-F, CH-3010, Bern, Switzerland.
E-mail address: hus@pki.unibe.ch (H.-U. Simon).
Peer review under responsibility of Japanese Society of Allergology.

https://doi.org/10.1016/j.alit.2020.11.003

play important roles in fighting against infections, but, under pathological conditions, might also cause tissue injury.[1,2] Granules are formed during the differentiation and described as the hallmark of granulocytes that storage "pre-packed" proteins which can be released into the extracellular space upon stimulation. These diverse mediators, once released to the extracellular space, are able to encounter and destroy all types of microorganism, and, in the case of eosinophils and basophils, also known to orchestrate hypersensitivity reactions.[3] Granule proteins form together with a fibrous scaffold build of mitochondrial DNA (mtDNA) extracellular structures called extracellular traps (ETs) that can be formed by all three granulocyte types. ETs comprise an efficient mechanism of host defense through facilitating the binding and killing of invading bacteria and fungi in the extracellular space. Granule proteins with a high affinity for DNA are delivered into ETs and cause a high local concentration of antimicrobial proteins and simultaneously reduce the spread of harmful proteins such as proteinases to adjacent tissues.[4–10]

The formation of granules (granulopoiesis) in neutrophils occurs sequentially during myeloid cell differentiation and is regulated by several myeloid transcription factors that are active at specific stages of neutrophil development. Four morphologically distinct granule populations can be distinguished: azurophilic (primary), specific (secondary), gelatinase (tertiary) and secretory granules.[11,12] Azurophilic granules are formed during the promyelocytic stage and represent the largest granules in neutrophils. They contain the most toxic mediators including elastase, myeloperoxidase (MPO), cathepsins as well as defensins, and contribute mainly to the antimicrobial effects of neutrophils.[13–16] In the metamyelocyte stage, specific granules emerge that are characterized by a high content of the glycoprotein lactoferrin. In addition, they also contain antimicrobial compounds, including neutrophil gelatinase-associated lipocalin (NGAL), human cathelicidin (hCAP-18) and lysozyme.[16] The smallest granules, the gelatinase granules, are the last population formed during neutrophil maturation. They contain metalloproteases, including gelatinase and leukolysin, as well as of few antimicrobials.[16] These three classical granules are formed by budding from the Golgi.[16] In contrast, the secretory vesicles are formed through endocytosis at the end of neutrophil maturation, accordingly, they incorporate mainly plasma-derived proteins such as albumin.[16]

Eosinophil granules are subdivided in primary and secondary granules. Primary granules contain Charcot-Leyden crystal protein and eosinophil peroxidase (EPO). Secondary granules are composed of matrix with a crystalline core. Eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and EPO are found in the matrix, meanwhile major basic protein (MBP) is restrained in the crystalline core.[17]

Antimicrobial proteins (AMP) are ancient molecules also present in granules of both neutrophils and eosinophils evolved as part of host defense to eliminate pathogenic bacteria, fungi, parasites and viruses.[18] Their main modes of action consist of disruptions of bacterial membrane. Furthermore AMPs are able to vanish the electrochemical gradient of the membrane by pore formation or to interfere with DNA, protein or cell wall synthesis and inhibit protein folding after entering into the cytoplasm.[19] AMPs share some structural features: 1) smaller length than 60 amino acids, 2) often a net positive charge, 3) broad-spectrum antimicrobial activity at physiological conditions and 4) hydrophobic and hydrophilic amino acids clusters adopted in an amphipathic shape.[20]

Granules of both neutrophils and eosinophils contain proteases which are important for physiological processes. However, their excessive, prolonged or inappropriate proteolytic activity is involved in the offset of diseases.[21] Proteases are subdivided in four classes based on their biochemistry of the active site: 1) serine proteases, 2) metalloproteases, 3) thiol-proteases and 4) aspartate proteases. Serine proteases and metalloproteases are most active at neutral pH thus are involved in extracellular protein digestion.[21] On the other hand, thiol-proteases and aspartate proteases are most active at acidic pH and participate in the degradation of intracellular proteins.[21] Peroxidases in granulocytes are members of the heme peroxidase superfamily.[22] Oxidants and reactive oxygen species (ROS) are required for many physiological processes, nonetheless their excessive production is involved in pathophysiological processes. Peroxidases are able to interact with H_2O_2 and are required for its cytotoxicity.[23]

In this article, we summarize our current understanding on the mechanisms of toxicity of neutrophil and eosinophil granule proteins (Fig. 1, Table 1) as well as their regulation to inhibit their cytotoxic potential.

Eosinophil granule proteins

Primary granule proteins

Charcot-Leyden crystal (CLC) protein, also known as galectin 10, belongs to the galectin superfamily[24] and is only found in humans,

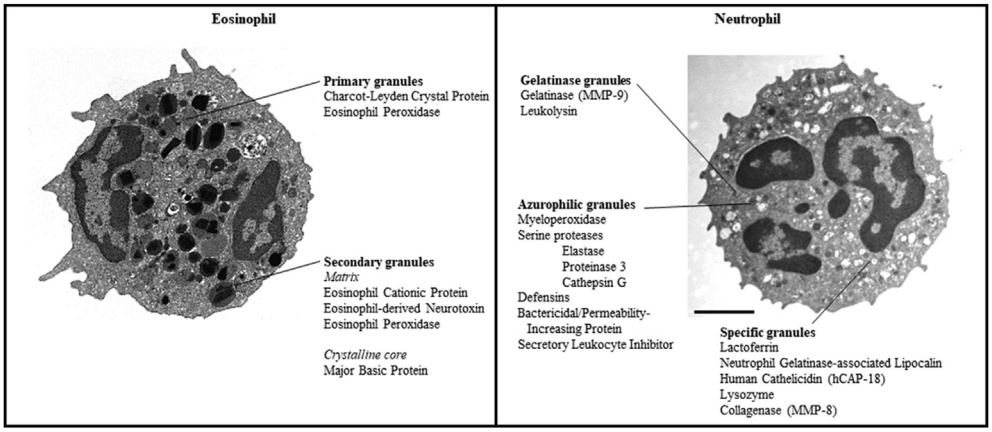

Fig. 1. Overview of granule proteins in eosinophils and neutrophils. The figure illustrates the different granule subsets of human eosinophil and neutrophil granulocytes. Eosinophil granules are divided in primary granules and secondary granules. The secondary granules are composed of two compartments: a matrix and a crystalline core. Neutrophil granules are developed during different stages of neutrophil development. The three subsets are defined by their morphology, their time of biosynthesis and their protein content.

Table 1
Antimicrobial mechanisms mediated by granule proteins of eosinophils and neutrophils.

Granule Protein	Distribution	Mechanism of toxicity
Eosinophil		
CLC	Primary granules	vesicular transport of cationic RNases[26]; requirement for eosinophil granulogenesis[26]; involvement in inflammation[27]
EPO	Primary & secondary granules	oxidative inactivation of pathogens[29–35]; oxidative damage towards host endothelial cells[36]; inhibition of LPS and lipid A in gram-negative bacteria membranes[38]
EDN	Secondary granules	ribonuclease activity against viruses[43–45]; inactivation of extracellular virions[50]; ROS production & induction of apoptosis in keratinocytes[51]
ECP	Secondary granules	antibacterial & antiparasitic properties[39,46–48]; involvement in EETs[5,52,53]; bacterial cell membrane depolarization[54]; neutralization of LPS[55]; formation of amyloid-like fibrils[58]; ROS production & induction of apoptosis in keratinocytes[51]; upregulation of MMP9 expression[51]
MBP	Secondary granules	antibacterial & antiparasitic properties[46,48,59]; cytotoxicity against host tissue[59,60]; causes degranulation of human eosinophils[61]; enhancement of production of proinflammatory IL-8[61]; permabilization of cell membranes[48,59,62]; formation of amyloid-like fibrils[58]; involvement in EETs[5,52,64,65]; non-toxic extracellular deposits[59,63]
Granule Protein	Distribution	Mechanism of toxicity
Neutrophil		
MPO	Azurophilic granules	oxidative inactivation of pathogens[21,66,67]; chronic inflammation[67]; involvement in NETs[6]
Serine proteases (Elastase, Cathepsin G, Proteinase 3)	Azurophilic granules	antimicrobial activity against bacteria, yeast and fungi[21]; non-infections inflammatory diseases[69–74]; degradation of bacterial virulence factors[75,77]; involvement in NETs[4,79]; proteolytic processing of antimicrobial proteins[80–83]; induction of proapoptotic death pathway in neutrophils[84,85]; proteolytic modification of chemokines[86–89]; activation of NADPH oxidase[90]
Defensins (HNP1-4)	Azurophilic granules	antimicrobial activity against bacteria, viruses and fungi[91,92]; involvement in NETs[79]; permeabilization of bacterial membranes[21]; pore formation in lipid bilayers[96]; inhibition of virus fusion to host plasma membrane[92]; inhibition of serpins[98]
BPI	Azurophilic granules	neutralization of LPS[101]; bacterial cell membrane damage[102]; involvement in LPS uptake and antigen presentation by DCs[105]
SLPI	Azurophilic granules	inhibitor of serine proteases[108]; protection of local tissues against inflammation[109]; inhibition of bacterial translation[110]; fungicidal activity[113]
hCAP18/LL-37	Specific granules	modification of bacterial surface[117–120]; involvement in NETs[121]; antimicrobial activity against intracellular pathogens[124]
Lactoferrin	Specific granules	involvement in NETs[79]; bacteriostatic & bactericidal effect[127,128]; inhibition of proliferation of fungi and viruses[125]; modification of bacterial membrane[129]; interference with bacterial cellular mechanism[125]; increase of inflammatroy cytokines & chemokines[130]
NGAL	Specific granules	decrease of MMP-9 activity[132]; sequestration of neutrophil chemoattractants[134,135]; cofactor of microbial killing[136]; bacteriostatic effect[7,138]; involvement in NETs[139]
Lysozyme	Specific granules	bactericidal effect[141]; hydrolysis of bacterial cell wall[141]; involvement in NETs[79,142]
MMP-8	Specific granules	degradation of collagen[152]; modification of chemokines[153]
MMP-9	Gelatinase granules	degradation of collagen, elastin & gelatin[159]; inactivation of SLPI[161]; proteolytic processing of IL-1β & TNF precursor[162,163]
Leukolysin	Gelatinase granules	inactivation of α1-PI[167]

but not in mouse eosinophils.[25] Unlike other galectin members, CLC does not have the ability to bind β-galactosidase[24] or any known mammalian glycan structures.[26] CLC is found to interact with glycosylated EDN and ECP, two human eosinophil granule cationic RNases, but induces no inhibitory function upon binding. Upon IFN-γ activation of eosinophils, CLC associates rapidly with CD63 positive secondary granules and EDN, suggesting a role of CLC in vesicular transport of cationic RNases.[26] The presence of CLC is required for eosinophil granulogenesis during differentiation.[26] Furthermore, CLC has been shown to be involved in inflammation in allergic, parasitic and other eosinophil-associated diseases and inflammatory reactions.[27]

Eosinophil peroxidase (EPO, also known as EPX) catalyzes the two-electron redox reaction $H_2O_2 + X^- + H^+ \rightarrow HOX + H_2O$ similar to other peroxidases.[28] Unlike for MPO, the physiologic substrate for EPO is still mainly uncertain. Three rather unusual substrates including Br^-, NO_2^- and the pseudohalide thiocyanate (SCN^-) are involved in the oxidation by EPO and H_2O_2.[28] During degranulation, EPO can be released into large cytoplasmic vacuoles (phagosome) or directly onto the surface of a target.[29] Peroxidase–H_2O_2–iodide reaction leads to the iodination of protein and killing of bacteria.[30] EPO interacts with the superoxide generated by the NADPH oxidase to provide the bactericidal activity of eosinophils.[31] EPO exhibits cytotoxicity through the release of peroxidase-derived oxidants towards mammalian tumor cells,[32] HIV-1[33] and schistosomula of *Schistosoma mansoni*[34] when the peroxidase is combined with H_2O_2 and a halide. Eosinophils adhere to schistosomula coated with antibody and complement. Upon release EPO binds to the surface of schistosomula. The binding itself is non-toxic but the presence of surface-bound EPO can enhance the eosinophil-mediated toxicity against the parasite.[35] Similar effect mediated by EPO–H_2O_2–halide system occurs towards host endothelial cells and is observed in eosinophilic endocarditis.[36] *Mycobacterium tuberculosis* treated

with EPO exhibit alterations of the cell wall followed by cell wall fragmentation and cell lysis within 120 min, particularly in the presence of H_2O_2.[37] Binding of EPO to gram-negative bacteria membranes and subsequent inhibition of LPS and lipid A occurs in a haloperoxidase-independent manner.[38]

Secondary granule proteins

Eosinophil granule proteins EDN/RNase 2 and ECP/RNase 3 are reported to be closely related proteins and part of RNase A superfamily.[39] RNase A superfamily itself is an important part of the human AMP family.[40] Members of the RNase A superfamily share a similar primary sequence and structural similarities such as 6–8 conserved cysteine residues forming characteristics distinct disulfide bonds and conserved histidines, as well as a lysine in the active center to catalyze the ribonuclease activity.[41,42] The ribonucleases are reported in mucosal secretions, in several types of immune cells as well as in major organs.[42]

EDN exhibits ribonuclease activity against single stranded RNA viruses including respiratory syncytial virus,[43] hepatitis B virus[44] and HIV.[45] On the other hand, ECP has antibacterial and antiparasitic properties.[39,46–48] EDN has been shown to be less cationic and less cytotoxic than ECP and was originally described as a neurotoxin.[39] In contrast, ECP is uniquely expressed in eosinophils, more cationic and exhibits significant less ribonuclease activity compared to EDN or RNase A.[49] In fact, the cytotoxic activity of ECP is independent of its ribonuclease activity.[49] EDN displays the ability to penetrate the viral capsid thus gaining access to the viral RNA genome and inactivating extracellular virions by its ribonuclease activity.[50] Both EDN and ECP mediate cytotoxic effects on keratinocytes through the production of ROS and induction of apoptosis.[51] Additionally, ECP has been shown to promote keratinocyte cell–matrix detachment and to upregulate MMP9 expression.[51] ECP is released in association with mitochondrial DNA in the context of forming eosinophil extracellular traps (EETs) which are able to bind and to kill bacteria in the extracellular space.[5,52,53] High-affinity binding of ECP to lipopolysaccharide (LPS) and peptidoglycan causes destabilizing of the bacterial cell wall and subsequently cell membrane depolarization.[54] Moreover, ECP-LPS interactions trigger LPS aggregation and neutralization of LPS-stimulated TNF-α production.[55] ECP binds to the membrane phospholipid polar heads by electrostatic interactions. Following clustering of the liposome–protein complexes, liposome aggregation and destabilization of the lipid bilayer occurs that lead ultimately to membrane disruption and release of the liposome content.[56] ECP interacts preferably with anionic and zwitterionic phospholipids of cell membranes. The anionic lipids are exposed on the external side of microbial membranes but sequestered on cytoplasmic side of eukaryote host cell membrane explaining a potential mechanism of antibacterial properties.[57] Under in vitro condition, ECP was shown to form amyloid-like fibrils. The amyloid-type aggregates of ECP led to cell agglutination and bacteria clearance preceding the cell death.[58]

Major basic protein 1 (MBP-1) is a cationic protein stored in the crystalline core of the eosinophil secondary granule.[59] MBP-1 is produced as a pre-protein which lacks toxicity owing to the presence of the N-terminal domain. Interestingly, the C-terminal region crystallizes to prevent intracellular toxicity when the pro-MBP-1 is cleaved upon granule maturation. Activation of eosinophils decreases the pH within the granules leading to the breakdown of the crystalline core and release of MBP-1 as an active cytotoxic protein.[59] Damage induced by MBP-1 is not selective, hence is directed against pathogen and mammalian target cells.[46,59] MBP-1 exhibit toxicity against bacteria,[46,48,59] schistosomula of *S. mansoni*[46] as well as host tissue associated with eosinophil infiltration such as intestine, spleen, skin, mucosa and peripheral blood mononuclear cells.[59,60] MBP-1 and EPO are able to cause degranulation of human eosinophils as potent as sIgA beads, a strong agonist for eosinophils.[61] Furthermore, MBP-1 enhances production of the proinflammatory cytokine IL-8.[61] MBP-1 is able to bind and to permeabilize membranes similar to cytotoxicity mediated by ECP that occurs through pore formation on bacterial cell membranes.[48,62] Furthermore, local perturbations of bacterial cell membrane are observed following MBP-1 treatment.[59] Moreover, MBP-1 is able to form amyloids that facilitate antimicrobial activity,[59] comparable to reports of ECP amyloid.[58] Accelerating the fiber formation by heparin decreases toxicity and is in line with the observation of non-toxic large MBP-1 amyloid accumulation in tissues.[59] The extracellular deposits are proposed to function as sequestration of toxic oligomers[59] that can recruit inflammatory cells,[60] including macrophages for the process of phagocytosis.[63] MBP and ECP were found attached to the DNA scaffold of EETs in vitro,[5] in colons of Crohn's disease patients,[5] allergic asthma,[64] acute atopic dermatitis,[52] bullous pemphigoid[52] and eosinophilic esophagitis.[65]

Neutrophil granule proteins

Azurophilic granules

MPO is a heme-containing peroxidase of the peroxidase superfamily. In combination with H_2O_2 and a halide, it catalyzes the formation of reactive oxygen intermediates.[66] MPO is released from azurophilic granules of activated neutrophils either into the phagolysosome or directly into the extracellular space.[21] Hypohalous acids formed by MPO exhibit antibacterial, antiviral as well as antifungal properties and are involved in chronic inflammation when produced in excess.[67] MPO is found in colocalization with mtDNA within NETs.[6]

Serine proteases contain a catalytically essential serine residue at their active side.[21] They comprise the largest class of mammalian proteases and are mainly synthesized as inactive precursors. The exception are the three human leukocyte serine proteases elastase, cathepsin G and proteinase 3 that are stored in an active form within the azurophilic granules.[68] Neutrophil serine proteases exhibit antimicrobial activity against bacteria, yeast and fungi.[21] Furthermore, they are involved in several non-infections inflammatory diseases, including arthritis,[69] bullous pemphigoid,[70,71] chronic inflammatory lung diseases[72,73] and tissue damage following ischemia/reperfusion injury.[74] Cathepsin G-knockout mice are more susceptible to Gram-positive bacteria whereas mice lacking neutrophil elastase are more susceptible to Gram-negative bacteria and enterobacteria species.[75,76] Neutrophil elastase binds the bacterial membrane and directly degrades the outer membrane protein A of *Escherichia coli* or virulence factors of enterobacteria, resulting in the loss of bacterial integrity and cell lysis.[76,77] The human neutrophil serine proteases are upregulated on the cell surface upon activation. They mostly remain bound to the plasma membrane during exocytosis of the granules allowing the neutrophils to modulate their inflammatory response through the preservation of their catalytic activity.[78] Additionally, neutrophil serine proteases display extracellular activities. Serine proteinases are released in NET-forming neutrophils.[4,79] After exocytosis, proteinase 3 is required for proteolytic processing of the human cathelicidin to its active form LL-37.[80] Similarly, mature active IL-18 is released of epithelial cells after stimulation with IFN-γ in combination of proteinase 3 and LPS without the activity of caspase-1.[81] In contrast, neutrophil elastase downregulates the biological activity of IL-18 through proteolysis.[82] Neutrophil elastase and cathepsin G abolish the pro-inflammatory effect of bacterial

flagellin on epithelial cells through cleavage.[83] Interestingly, by cleaving caspase-8, cathepsin D is able to induce a proapoptotic death pathway in neutrophils themselves.[84,85] Moreover, neutrophil serine proteases are able to regulate chemokine activities by proteolytic modifications. For example, the activity and stability of CXCL8 and CXCL5 are enhanced following their N-terminal proteolytic modification by proteinase 3 and cathepsin G.[86,87] On the other hand, cleavage of CCL3, CXCL12 and CXCR4 results in lower chemotactic activity.[88,89] Recently, it has been shown that fibroblast activation protein − 1 alpha, a serine protease, is expressed in neutrophils and involved in the activation of NADPH oxidase.[90]

In neutrophils, four small cationic peptides of α-defensins called human neutrophil peptides 1, 2, 3 and 4 (HNP1-4) are secreted upon activation and exhibit antibacterial, antiviral as well as antifungal activity.[91,92] Defensins are characterized by a β-sheet-rich fold, a high cathodal electrophoretic mobility and a framework of six disulphide-linked cysteines. Three subfamilies are identified: α- and β-defensins that are formed by a triple-stranded β-sheet with a "defensin" fold and θ-defensins.[93] HNP1 and HNP3 differ only at the first residue, while HNP2 is proteolytic processed of one of these two defensins.[94] HNP1 and HNP3 have been characterized as NET-associated proteins.[79] Defensins are produced as prepropeptides. First steps of maturation include removing of the signal peptide and packaging of the inactive propeptides in azurophilic granules. The full process occurs within the granules mediated by neutrophil elastase and proteinase 3.[95] HNPs are acting against Gram-positive and Gram-negative bacteria through membrane permeabilization.[21] Defensins decrease the membrane potential of target cells within minutes through the creation of small membrane channels followed by the permeabilization of the cells after a short lag period. Interestingly, cells can be rescued by removing the HNP-containing media after 30 min, hence a second phase of injury and continuing presence of HNP is necessary for cell lysis.[91] HNP2 has been shown to form multimeric pores in lipid bilayers.[96] Defensin-mediated bactericidal activity occurs by outer membrane permeabilization following inner membrane permeabilization.[97] Synchronous inhibition of RNA, DNA and protein production, as well as loss of cellular metabolites and altered ionic cellular environment lead to loss of the bacterial colony-forming potential.[97] Defensins interfere and also disrupt the virus fusion to the host plasma membrane through inhibition of the interactions between viral glycoproteins and cellular receptors.[92] In contrast to antibacterial and antifungal mechanism, direct disruption of the virus lipid bilayer does not occur. Viral envelopes are mainly derived from host cell membranes helping the enveloped virus to escape the defensins.[92] Moreover, at sites of inflammations where defensins are present at high concentrations, they can bind serine proteinase inhibitors (serpins), particularly α1-proteinase inhibitor (α1-PI) and α1-antichymotrypsin (α1-ACT), to prevent their activity against serine proteases.[98] Within the phagolysosome of neutrophils, increased proteolytic activity of serine proteases through defensin-serpin binding may lead to enhanced phagocytic digestion of microbes.[98]

Bactericidal/Permeability-Increasing Protein (BPI) belong to the lipid transfer/LPS binding protein family that is characterized by the binding of lipid substrates[99] as well as to the tubular-lipid binding protein (TULIP) family.[100] BPI exerts bactericidal activity against Gram-negative bacteria through the neutralization of LPS by binding to the lipid A moiety.[101] Penetration of the inner bacterial cell membrane induces membrane damage leading eventually to cell death.[102] BPI-mediated toxicity occurs in two stages. In an early, reversible sublethal phase, BPI acts on the outer bacterial membrane and induces growth arrest. Penetration of the cell membrane leads to a time- and pH-dependent lethal stage with the involvement of cytoplasmic membrane damage.[102] BPI is able to reduce LPS-mediated neutrophil stimulation, pyrogenicity and LPS-induced TNF-α production.[103,104] BPI enhances the interaction of bacterial outer membrane vesicles with dendritic cells (DCs) and is involved in the LPS uptake and antigen presentation by DCs.[105] In Gram-positive infections, binding of BPI to bacterial lipopeptides and lipoproteins, as well as lipoteichoic acid enhances their inflammatory effects.[106] The LPS-binding protein (LBP) moiety of the TULIP family binds to the same component of LPS on gram-negative bacterial membrane as BPI. However, LBP helps to initiate the host immune response by bringing small amounts of LPS to effector cells.[106,107]

Secretory Leukoproteinase Inhibitor (SLPI), also known as anti-leukoprotease (ALP), is a endogenous serine protease inhibitor (serpin) produced by epithelial cells and some myeloid cells including neutrophils.[108] SLPI is mainly involved in the protection of local tissues against inflammatory consequences that are caused by proteases including cathepsin G, elastase and trypsin from neutrophils.[109] Furthermore, some studies propose antibacterial and antifungal capacity for SLPI.[110–112] While the proteinase inhibitory activities are shown to be located at the COOH-terminal domain, the antibacterial capacity against Gram-negative and Gram-positive bacteria depend on the NH_2-terminal domain.[111] SLPI induces toxicity against *E. coli* by binding to DNA and mRNA leading to inhibition of translation.[110] Fungicidal activity of SLPI emerge against metabolically active *Aspergillus fumigatus* and *Candida albicans*. On the other hand, metabolically quiescent *A. fumigatus* has been reported to be resistant against SLPI.[112]

Specific granule proteins

LL-37 is expressed by immune cells including neutrophils, monocytes, mast cells, NK cells, B and T cells, as well as stem cells.[113] LL-37 is the mature peptide of 37 amino acids released from the C-terminus of the propeptide hCAP18.[114] hCAP18 is processed by extracellular proteolysis of the C-terminal end of the human cationic antimicrobial protein (hCAP).[115] hCAP is the only cathelicidin family member, one of the major AMP families, known in humans. They are comprised of a highly conserved N-terminal signal peptide − "cathelin domain" − and a structurally variable cationic AMP at the C-terminus.[116] LL-37 is active against the anionic membrane of Gram-positive and Gram-negative bacteria, similar to other cationic AMPs. The positive charge of the peptide enhances the binding to negatively charged bacterial membranes rather than mammalian cell membranes.[117] The importance of the electrostatic attraction for the antibacterial capacity is underlined by the fact that modification of the bacterial surface influences the susceptibility of the cells to LL-37.[118–120] LL-37 and its propeptide hCAP18 are released within NETs. LL-37 binds to the DNA scaffold of the extracellular traps,[121] reportedly leading to the loss of its antibacterial capacity.[122,123] Stephan *et al.* reported the internalization of LL-37:DNA complexes derived from NETs by macrophages enabling antimicrobial activity of LL-37 against intracellular pathogens.[124]

Lactoferrin, also known as lactotransferrin, is a basic iron-binding glycoprotein of the secondary granules in neutrophils.[125,126] Leukocytes, macrophages, platelets and bacteria, as well as the gastrointestinal tract exhibit lactoferrin receptors.[125] Lactoferrin is found to be associated to NETs.[79] Lactoferrin exerts a bacteriostatic effect through iron sequestering inhibition of the bacterial proliferation.[127] Additionally, lactoferrin is reported to hold a bactericidal effect and inhibits the proliferation of fungi and viruses.[125] The protein modifies outer membrane of Gram-negative bacteria by binding and releasing LPS. This way, lactoferrin also enhances the entry of lysozyme into Gram-negative bacteria.[128] The modulation of bacterial cell membrane can be blocked by the

addition of Ca^{2+} and Mg^{2+}.[129] Through its iron-binding ability, lactoferrin may interfere with cellular mechanisms including DNA and RNA synthesis, protein synthesis, expression of lymphocyte surface markers, immunoglobulin secretion and interleukin-2 expression.[125] Lactoferrin serves also as ligand for TLR4 of rheumatoid arthritis synovial fibroblasts stimulating increased expressions of inflammatory cytokines and chemokines.[130] Furthermore, lactoferrin has been reported to inhibit spontaneous apoptosis in human neutrophils if the iron saturation status is low.[126]

Neutrophil gelatinase-associated lipocalin (NGAL, also known as lipocalin-2) is a member of the lipocalin family that is characterized by the ability of transporting small lipophilic substances, binding to specific cell-surface receptors and forming complexes with soluble macromolecules.[131] NGAL was first identified to be covalently associated with MMP-9.[132] Furthermore, NGAL-MMP-9 complex can be observed in a ternary complex with tissue inhibitor of metalloproteinase-1 (TIMP-1) that results in a 10-fold lower activity of MMP-9.[133] NGAL is able to bind to fMLP,[134] PAF[135] and leukotriene B4.[135] NGAL may also serve as a cofactor of microbial killing by other proteins through the binding to microbicidal, lipophilic ligands or modulating inflammatory responses induced by neutrophils.[136] Furthermore, NGAL has been found to bind to bacterial catecholate-type ferric siderophores preventing growth and spread of *Myobacterium tuberculosis*[137] and *E. coli*.[138] NGAL has also been identified in NETs and to be involved in their bactericidal effect in vitro.[139]

Lysozyme is a conserved hydrolytic antimicrobial protein that is well known for its importance to host defense. Lysozyme is expressed in phagocytes, hepatocytes, and epithelial cells of mucosal surfaces as well as in body fluids such as blood, tears, urine, saliva and milk.[140] Almost 100 years ago, Fleming *et al.* observed the bactericidal effect mediated by lysozyme.[141] Lysozyme provokes bacterial cell lysis through targeted hydrolysis of the bacterial cell wall.[141] Lysozyme is identified as a NET-associated protein using a proteomic approach.[79] Lysozyme is supposed to contribute to the antimicrobial activity of NETs due to its action on the bacterial wall.[142]

Matrix metalloproteases (MMPs) are characterized by their dependence on intrinsic Zn^{2+} ions and extrinsic Ca^{2+}. Their many function consist of degradation of all constitutes of the extracellular matrix.[68] Several MMPs are involved in the cleavage and inactivation of α_1-antitrypsin, an inhibitor of neutrophil elastase, cathepsin G and proteinase 3.[143] Likewise, serine proteases are able to inactivate protease inhibitor like TIMP-1[144] and cystatin C[145] that serve as inhibitors of MMPs. Metalloproteinase-8 (MMP-8; also known as neutrophil collagenase or collagenase 2) is a potent collagenase highly expressed by neutrophils[146] as well as rheumatoid synovial fibroblasts,[147] endothelial cells,[147] activated macrophages,[148] smooth muscle cells,[149] bronchial epithelial cells,[148] mast cells[150] and chondrocytes.[151] MMP-8 are secreted in tuberculosis-associated inflammation driving the tissue destruction through degradation of the collagen.[152] MMP-8 mediates responsiveness of neutrophils to LPS through chemokine modification.[153] Cleavage of collagen by MMP-8 facilitates neutrophil migration to sites of inflammation and injury behind collagen-rich barriers.[146]

Gelatinase granule proteins

Metalloproteinase-9 (MMP-9; also known as gelatinase B) is a member of the MMP family and is secreted by endothelial cells,[154] monocytes/macrophages,[155] mast cells,[156] eosinophils[157] and neutrophils.[158] MMP-9 degrades collagen, elastin and gelatin of the extracellular matrix upon exocytosis.[159] In allergic asthmatic patients MMP-9 is released by neutrophils after exposure to allergens.[160] MMP-9 cleaves and inactivate SLPI at sites of inflammation preventing its binding to LPS and inhibition of neutrophil elastase activity.[161] Several MMPs including MMP-9 process human IL-1β and TNF precursor into its biologically active form regulating their appearance at inflammatory sites.[162,163]

Leukolysin, also known as membrane-type 6 metalloproteinase (MT6-MMP, MMP25), is a GPI-anchored proteinase of the MMP family.[164] Leukolysin is expressed in leukocytes,[165] in SW480 colon carcinoma cells and several brain tumors including anaplastic astrocytomas and glioblastomas.[166] Leukolysin is the most efficient proteinase in the inactivation of α1-PI. α1-PI is found in high concentrations in interstital spaces and circulating plasma to protect tissue from neutrophil serine proteases. Action of leukolysin against α1-PI releases neutrophil elastase, proteinase 3 and cathepsin G from its inhibitor leading ultimately to tissue damage.[167]

Conclusions

Granule proteins are secreted from eosinophils and neutrophils into the extracellular space upon activation and consist of a variety of important mediators that are crucial for the function of granulocytes within the innate immunity. The released granule proteins display a great variety of weapons and diverse antimicrobial mechanism that are directed against different kind of pathogens (Table 1). On the other hand, excessive production of granule proteins of eosinophils and neutrophils plays a significant role in the pathogenesis of diverse human diseases. These findings arise the possibility to use granule proteins as diagnostic marker and drug targets for the development of new therapeutics against infectious and chronic inflammatory diseases. The understanding of the functional consequences of the molecular interactions between DNA and granule proteins within NETs and EETs requires additional experimentation.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Ramirez GA, Yacoub MR, Ripa M, Mannina D, Cariddi A, Saporiti N, et al. Eosinophils from physiology to disease: a comprehensive review. *Biomed Res Int* 2018;**2018**:9095275.
2. Lehman HK, Segal BH. The role of neutrophils in host defense and disease. *J Allergy Clin Immunol* 2020;**145**:1535–44.
3. Geering B, Stoeckle C, Conus S, Simon HU. Living and dying for inflammation: neutrophils, eosinophils, basophils. *Trends Immunol* 2013;**34**:398–409.
4. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004;**303**:1532–5.
5. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat Med* 2008;**14**:949–53.
6. Yousefi S, Mihalache C, Kozlowski E, Schmid I, Simon HU. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ* 2009;**16**:1438–44.
7. Morshed M, Hlushchuk R, Simon D, Walls AF, Obata-Ninomiya K, Karasuyama H, et al. NADPH oxidase-independent formation of extracellular DNA traps by basophils. *J Immunol* 2014;**192**:5314–23.
8. Yousefi S, Morshed M, Amini P, Stojkov D, Simon D, von Gunten S, et al. Basophils exhibit antibacterial activity through extracellular trap formation. *Allergy* 2015;**70**:1184–8.
9. Yousefi S, Stojkov D, Germic N, Simon D, Wang X, Benarafa C, et al. Untangling "NETosis" from NETs. *Eur J Immunol* 2019;**49**:221–7.
10. Yousefi S, Simon D, Stojkov D, Karsonova A, Karaulov A, Simon HU. In vivo evidence for extracellular DNA trap formation. *Cell Death Dis* 2020;**11**:300.
11. Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect* 2003;**5**:1317–27.
12. Borregaard N. Neutrophils, from marrow to microbes. *Immunity* 2010;**33**:657–70.
13. Pham CT. Neutrophil serine proteases: specific regulators of inflammation. *Nat Rev Immunol* 2006;**6**:541–50.
14. Lacy P. Mechanisms of degranulation in neutrophils. *Allergy Asthma Clin Immunol* 2006;**2**:98–108.

15. Borregaard N, Sorensen OE, Theilgaard-Monch K. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol* 2007;**28**:340–5.
16. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. *Annu Rev Immunol* 2012;**30**:459–89.
17. Yousefi S, Simon D, Simon HU. Eosinophil extracellular DNA traps: molecular mechanisms and potential roles in disease. *Curr Opin Immunol* 2012;**24**: 736–9.
18. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;**415**:389–95.
19. Nguyen LT, Haney EF, Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol* 2011;**29**: 464–72.
20. Diamond G, Beckloff N, Weinberg A, Kisich KO. The roles of antimicrobial peptides in innate host defense. *Curr Pharm Des* 2009;**15**:2377–92.
21. Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest* 2000;**80**:617–53.
22. Nauseef WM. Biosynthesis of human myeloperoxidase. *Arch Biochem Biophys* 2018;**642**:1–9.
23. Kazura JW, Fanning MM, Blumer JL, Mahmoud AA. Role of cell-generated hydrogen peroxide in granulocyte-mediated killing of schistosomula of Schistosoma mansoni in vitro. *J Clin Invest* 1981;**67**:93–102.
24. Leonidas DD, Elbert BL, Zhou Z, Leffler H, Ackerman SJ, Acharya KR. Crystal structure of human Charcot–Leyden crystal protein, an eosinophil lysophospholipase, identifies it as a new member of the carbohydrate-binding family of galectins. *Structure* 1995;**3**:1379–93.
25. el-Hashimi W. Charcot-Leyden crystals. Formation from primate and lack of formation from nonprimate eosinophils. *Am J Pathol* 1971;**65**:311–24.
26. Grozdanovic MM, Doyle CB, Liu L, Maybruck BT, Kwatia MA, Thiyagarajan N, et al. Charcot-Leyden crystal protein/galectin-10 interacts with cationic ribonucleases and is required for eosinophil granulogenesis. *J Allergy Clin Immunol* 2020;**146**:377–89. e10.
27. Ackerman SJ, Zhou Z, Tenen DG, Clark MA, Tu Y-P, Irvin CG. Human eosinophil lysophospholipase (Charcot-Leyden crystal protein): molecular cloning, expression, and potential functions in asthma. In: Gleich GJ, editor. *Eosinophils in Allergy and Inflammation*. New York: Marcel Dekker; 1994. p. 21–54.
28. Wang J, Slungaard A. Role of eosinophil peroxidase in host defense and disease pathology. *Arch Biochem Biophys* 2006;**445**:256–60.
29. McLaren DJ, Mackenzie CD, Ramalho-Pinto FJ. Ultrastructural observations on the in vitro interaction between rat eosinophils and some parasitic helminths (Schistosoma mansoni, Trichinella spiralis and Nippostrongylus brasiliensis). *Clin Exp Immunol* 1977;**30**:105–18.
30. DeChatelet LR, Migler RA, Shirley PS, Muss HB, Szejda P, Bass DA. Comparison of intracellular bactericidal activities of human neutrophils and eosinophils. *Blood* 1978;**52**:609–17.
31. Persson T, Andersson P, Bodelsson M, Laurell M, Malm J, Egesten A. Bactericidal activity of human eosinophilic granulocytes against Escherichia coli. *Infect Immun* 2001;**69**:3591–6.
32. Jong EC, Klebanoff SJ. Eosinophil-mediated mammalian tumor cell cytotoxicity: role of the peroxidase system. *J Immunol* 1980;**124**:1949–53.
33. Klebanoff SJ, Coombs RW. Virucidal effect of stimulated eosinophils on human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* 1996;**12**:25–9.
34. Jong EC, Mahmoud AA, Klebanoff SJ. Peroxidase-mediated toxicity to schistosomula of Schistosoma mansoni. *J Immunol* 1981;**126**:468–71.
35. Jong EC, Chi EY, Klebanoff SJ. Human neutrophil-mediated killing of schistosomula of Schistosoma mansoni: augmentation by schistosomal binding of eosinophil peroxidase. *Am J Trop Med Hyg* 1984;**33**:104–15.
36. Slungaard A, Mahoney Jr JR. Bromide-dependent toxicity of eosinophil peroxidase for endothelium and isolated working rat hearts: a model for eosinophilic endocarditis. *J Exp Med* 1991;**173**:117–26.
37. Borelli V, Vita F, Shankar S, Soranzo MR, Banfi E, Scialino G, et al. Human eosinophil peroxidase induces surface alteration, killing, and lysis of Mycobacterium tuberculosis. *Infect Immun* 2003;**71**:605–13.
38. Allen RC, Henery ML, Allen JC, Hawks RJ, Stephens Jr JT. Myeloperoxidase and eosinophil peroxidase inhibit endotoxin activity and increase mouse survival in a lipopolysaccharide lethal dose 90% model. *J Immunol Res* 2019;**2019**: 4783018.
39. Gleich GJ, Loegering DA, Bell MP, Checkel JL, Ackerman SJ, McKean DJ. Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein: homology with ribonuclease. *Proc Natl Acad Sci U S A* 1986;**83**:3146–50.
40. Simanski M, Köten B, Schröder JM, Gläser R, Harder J. Antimicrobial RNases in cutaneous defense. *J Innate Immun* 2012;**4**:241–7.
41. Sorrentino S. The eight human "canonical" ribonucleases: molecular diversity, catalytic properties, and special biological actions of the enzyme proteins. *FEBS Lett* 2010;**584**:2194–200.
42. Gupta SK, Haigh BJ, Griffin FJ, Wheeler TT. The mammalian secreted RNases: mechanisms of action in host defence. *Innate Immun* 2013;**19**:86–97.
43. Domachowske JB, Bonville CA, Dyer KD, Rosenberg HF. Evolution of antiviral activity in the ribonuclease A gene superfamily: evidence for a specific interaction between eosinophil-derived neurotoxin (EDN/RNase 2) and respiratory syncytial virus. *Nucl Acids Res* 1998;**26**:5327–32.
44. Liu J, Li YH, Xue CF, Ding J, Gong WD, Zhao Y, et al. Targeted ribonuclease can inhibit replication of hepatitis B virus. *World J Gastroenterol* 2003;**9**:295–9.
45. Rugeles MT, Trubey CM, Bedoya VI, Pinto LA, Oppenheim JJ, Rybak SM, et al. Ribonuclease is partly responsible for the HIV-1 inhibitory effect activated by HLA alloantigen recognition. *AIDS* 2003;**17**:481–6.
46. Butterworth AE, Wassom DL, Gleich GJ, Loegering DA, David JR. Damage to schistosomula of Schistosoma mansoni induced directly by eosinophil major basic protein. *J Immunol* 1979;**122**:221–9.
47. Ackerman SJ, Gleich GJ, Loegering DA, Richardson BA, Butterworth AE. Comparative toxicity of purified human eosinophil granule cationic proteins for schistosomula of Schistosoma mansoni. *Am J Trop Med Hyg* 1985;**34**: 735–45.
48. Lehrer RI, Szklarek D, Barton A, Ganz T, Hamann KJ, Gleich GJ. Antibacterial properties of eosinophil major basic protein and eosinophil cationic protein. *J Immunol* 1989;**142**:4428–34.
49. Rosenberg HF. Recombinant human eosinophil cationic protein. Ribonuclease activity is not essential for cytotoxicity. *J Biol Chem* 1995;**270**:7876–81.
50. Domachowske JB, Dyer KD, Bonville CA, Rosenberg HF. Recombinant human eosinophil-derived neurotoxin/RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. *J Infect Dis* 1998;**177**:1458–64.
51. Amber KT, Chernyavsky A, Agnoletti AF, Cozzani E, Grando SA. Mechanisms of pathogenic effects of eosinophil cationic protein and eosinophil-derived neurotoxin on human keratinocytes. *Exp Dermatol* 2018;**27**:1322–7.
52. Simon D, Hoesli S, Roth N, Staedler S, Yousefi S, Simon HU. Eosinophil extracellular DNA traps in skin diseases. *J Allergy Clin Immunol* 2011;**127**: 194–9.
53. Kerstan A, Simon HU, Yousefi S, Leverkus M. Extensive accumulation of eosinophil extracellular traps in bullous delayed-pressure urticaria: a pathophysiological link? *Br J Dermatol* 2012;**166**:1151–2.
54. Torrent M, Navarro S, Moussaoui M, Nogues MV, Boix E. Eosinophil cationic protein high-affinity binding to bacteria-wall lipopolysaccharides and peptidoglycans. *Biochemistry* 2008;**47**:3544–55.
55. Pulido D, Garcia-Mayoral MF, Moussaoui M, Velázquez D, Torrent M, Bruix M, et al. Structural basis for endotoxin neutralization by the eosinophil cationic protein. *FEBS J* 2016;**283**:4176–91.
56. Torrent M, Sánchez D, Buzón V, Nogués MV, Cladera J, Boix E. Comparison of the membrane interaction mechanism of two antimicrobial RNases: RNase 3/ECP and RNase 7. *Biochim Biophys Acta* 2009;**1788**:1116–25.
57. Torrent M, Cuyás E, Carreras E, Navarro S, López O, de la Maza A, et al. Topography studies on the membrane interaction mechanism of the eosinophil cationic protein. *Biochemistry* 2007;**46**:720–33.
58. Torrent M, Odorizzi F, Nogués MV, Boix E. Eosinophil cationic protein aggregation: identification of an N-terminus amyloid prone region. *Biomacromolecules* 2010;**11**:1983–90.
59. Soragni A, Yousefi S, Stoeckle C, Soriaga AB, Sawaya MR, Kozlowski E, et al. Toxicity of eosinophil MBP is repressed by intracellular crystallization and promoted by extracellular aggregation. *Mol Cel* 2015;**57**:1011–21.
60. Gleich GJ, Frigas E, Loegering DA, Wassom DL, Steinmuller D. Cytotoxic properties of the eosinophil major basic protein. *J Immunol* 1979;**123**:2925–7.
61. Kita H, Abu-Ghazaleh RI, Sur S, Gleich GJ. Eosinophil major basic protein induces degranulation and IL-8 production by human eosinophils. *J Immunol* 1995;**154**:4749–58.
62. Young JD, Peterson CG, Venge P, Cohn ZA. Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* 1986;**321**:613–6.
63. Argilés A, García García M, Mourad G. Phagocytosis of dialysis-related amyloid deposits by macrophages. *Nephrol Dial Transpl* 2002;**17**:1136–8.
64. Dworski R, Simon HU, Hoskins A, Yousefi S. Eosinophil and neutrophil extracellular DNA traps in human allergic asthmatic airways. *J Allergy Clin Immunol* 2011;**127**:1260–6.
65. Simon D, Radonjic-Hösli S, Straumann A, Yousefi S, Simon HU. Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation. *Allergy* 2015;**70**:443–52.
66. Pattison DI, Davies MJ, Hawkins CL. Reactions and reactivity of myeloperoxidase-derived oxidants: differential biological effects of hypochlorous and hypothiocyanous acids. *Free Radic Res* 2012;**46**:975–95.
67. Rayner BS, Love DT, Hawkins CL. Comparative reactivity of myeloperoxidase-derived oxidants with mammalian cells. *Free Radic Biol Med* 2014;**71**:240–55.
68. Owen CA, Campbell EJ. The cell biology of leukocyte-mediated proteolysis. *J Leukoc Biol* 1999;**65**:137–50.
69. Adkison AM, Raptis SZ, Kelley DG, Pham CT. Dipeptidyl peptidase I activates neutrophil-derived serine proteases and regulates the development of acute experimental arthritis. *J Clin Invest* 2002;**109**:363–71.
70. Liu Z, Shapiro SD, Zhou X, Twining SS, Senior RM, Giudice GJ, et al. A critical role for neutrophil elastase in experimental bullous pemphigoid. *J Clin Invest* 2000;**105**:113–23.
71. Liu Z. Bullous pemphigoid: using animal models to study the immunopathology. *J Investig Dermatol Symp Proc* 2004;**9**:41–6.
72. Shapiro SD, Goldstein NM, Houghton AM, Kobayashi DK, Kelley D, Belaaouaj A. Neutrophil elastase contributes to cigarette smoke-induced emphysema in mice. *Am J Pathol* 2003;**163**:2329–35.
73. Chua F, Dunsmore SE, Clingen PH, Mutsaers SE, Shapiro SD, Segal AW, et al. Mice lacking neutrophil elastase are resistant to bleomycin-induced pulmonary fibrosis. *Am J Pathol* 2007;**170**:65–74.
74. Shimoda N, Fukazawa N, Nonomura K, Fairchild RL. Cathepsin g is required for sustained inflammation and tissue injury after reperfusion of ischemic kidneys. *Am J Pathol* 2007;**170**:930–40.

75. Belaaouaj A, McCarthy R, Baumann M, Gao Z, Ley TJ, Abraham SN, et al. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. *Nat Med* 1998;**4**:615–8.
76. Weinrauch Y, Drujan D, Shapiro SD, Weiss J, Zychlinsky A. Neutrophil elastase targets virulence factors of enterobacteria. *Nature* 2002;**417**:91–4.
77. Belaaouaj A, Kim KS, Shapiro SD. Degradation of outer membrane protein A in Escherichia coli killing by neutrophil elastase. *Science* 2000;**289**:1185–8.
78. Owen CA, Campbell MA, Sannes PL, Boukedes SS, Campbell EJ. Cell surface-bound elastase and cathepsin G on human neutrophils: a novel, non-oxidative mechanism by which neutrophils focus and preserve catalytic activity of serine proteinases. *J Cel Biol* 1995;**131**:775–89.
79. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. *Plos Pathog* 2009;**5**:e1000639.
80. Sørensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 2001;**97**:3951–9.
81. Sugawara S, Uehara A, Nochi T, Yamaguchi T, Ueda H, Sugiyama A, et al. Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. *J Immunol* 2001;**167**:6568–75.
82. Robertson SE, Young JD, Kitson S, Pitt A, Evans J, Roes J, et al. Expression and alternative processing of IL-18 in human neutrophils. *Eur J Immunol* 2006;**36**:722–31.
83. López-Boado YS, Espinola M, Bahr S, Belaaouaj A. Neutrophil serine proteinases cleave bacterial flagellin, abrogating its host response-inducing activity. *J Immunol* 2004;**172**:509–15.
84. Conus S, Perozzo R, Reinheckel T, Peters C, Scapozza L, Yousefi S, et al. Caspase-8 is activated by cathepsin D initiating neutrophil apoptosis during the resolution of inflammation. *J Exp Med* 2008;**205**:685–98.
85. Conus S, Pop C, Snipas SJ, Salvesen GS, Simon HU. Cathepsin D primes caspase-8 activation by multiple intra-chain proteolysis. *J Biol Chem* 2012;**287**:21142–51.
86. Padrines M, Wolf M, Walz A, Baggiolini M. Interleukin-8 processing by neutrophil elastase, cathepsin G and proteinase-3. *FEBS Lett* 1994;**352**:231–5.
87. Nufer O, Corbett M, Walz A. Amino-terminal processing of chemokine ENA-78 regulates biological activity. *Biochemistry* 1999;**38**:636–42.
88. Delgado MB, Clark-Lewis I, Loetscher P, Langen H, Thelen M, Baggiolini M, et al. Rapid inactivation of stromal cell-derived factor-1 by cathepsin G associated with lymphocytes. *Eur J Immunol* 2001;**31**:699–707.
89. Ryu OH, Choi SJ, Firatli E, Choi SW, Hart PS, Shen RF, et al. Proteolysis of macrophage inflammatory protein-1alpha isoforms LD78beta and LD78alpha by neutrophil-derived serine proteases. *J Biol Chem* 2005;**280**:17415–21.
90. Wang X, Gessier F, Perozzo R, Stojkov D, Hosseini A, Amirshahrokhi K, et al. RIPK3–MLKL–mediated neutrophil death requires concurrent activation of fibroblast activation protein-α. *J Immunol* 2020;**205**:1653–63.
91. Lichtenstein A. Mechanism of mammalian cell lysis mediated by peptide defensins. Evidence for an initial alteration of the plasma membrane. *J Clin Invest* 1991;**88**:93–100.
92. Holly MK, Diaz K, Smith JG. Defensins in viral infection and pathogenesis. *Annu Rev Virol* 2017;**4**:369–91.
93. Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 2003;**3**:710–20.
94. Gabay JE, Scott RW, Campanelli D, Griffith J, Wilde C, Marra MN, et al. Antibiotic proteins of human polymorphonuclear leukocytes. *Proc Natl Acad Sci U S A* 1989;**86**:5610–4.
95. Tongaonkar P, Golji AE, Tran P, Ouellette AJ, Selsted ME. High fidelity processing and activation of the human α-Defensin HNP1 precursor by neutrophil elastase and proteinase 3. *PLoS One* 2012;**7**:e32469.
96. Wimley WC, Selsted ME, White SH. Interactions between human defensins and lipid bilayers: evidence for formation of multimeric pores. *Protein Sci* 1994;**3**:1362–73.
97. Lehrer RI, Barton A, Daher KA, Harwig SS, Ganz T, Selsted ME. Interaction of human defensins with Escherichia coli. Mechanism of bactericidal activity. *J Clin Invest* 1989;**84**:553–61.
98. Panyutich AV, Hiemstra PS, van Wetering S, Ganz T. Human neutrophil defensin and serpins form complexes and inactivate each other. *Am J Respir Cel Mol Biol* 1995;**12**:351–7.
99. Kirschning CJ, Au-Young J, Lamping N, Reuter D, Pfeil D, Seilhamer JJ, et al. Similar organization of the lipopolysaccharide-binding protein (LBP) and phospholipid transfer protein (PLTP) genes suggests a common gene family of lipid-binding proteins. *Genomics* 1997;**46**:416–25.
100. Alva V, Lupas AN. The TULIP superfamily of eukaryotic lipid-binding proteins as a mediator of lipid sensing and transport. *Biochim Biophys Acta* 2016;**1861**:913–23.
101. Wittmann I, Schönefeld M, Aichele D, Groer G, Gessner A, Schnare M. Murine bactericidal/permeability-increasing protein inhibits the endotoxic activity of lipopolysaccharide and gram-negative bacteria. *J Immunol* 2008;**180**:7546–52.
102. Mannion BA, Weiss J, Elsbach P. Separation of sublethal and lethal effects of the bactericidal/permeability increasing protein on Escherichia coli. *J Clin Invest* 1990;**85**:853–60.
103. Marra MN, Wilde C, Griffith J, Snable J, Scott R. Bactericidal/permeability-increasing protein has endotoxin-neutralizing activity. *J Immunol* 1990;**144**:662–6.
104. Marra MN, Wilde C, Collins M, Snable J, Thornton M, Scott R. The role of bactericidal/permeability-increasing protein as a natural inhibitor of bacterial endotoxin. *J Immunol* 1992;**148**:532–7.
105. Schultz H, Hume J, Zhang DS, Gioannini TL, Weiss JP. A novel role for the bactericidal/permeability increasing protein in interactions of Gram-negative bacterial outer membrane blebs with dendritic cells. *J Immunol* 2007;**179**:2477–84.
106. Bülow S, Zeller L, Werner M, Toelge M, Holzinger J, Entzian C, et al. Bactericidal/permeability-increasing protein as an enhancer of bacterial lipoprotein recognition. *Front Immunol* 2018;**9**:2768.
107. Schröder NW, Schumann RR. Non-LPS targets and actions of LPS binding protein (LBP). *J Endotoxin Res* 2005;**11**:237–42.
108. Jacobsen LC, Sørensen OE, Cowland JB, Borregaard N, Theilgaard-Mönch K. The secretory leukocyte protease inhibitor (SLPI) and the secondary granule protein lactoferrin are synthesized in myelocytes, colocalize in subcellular fractions of neutrophils, and are coreleased by activated neutrophils. *J Leukoc Biol* 2008;**83**:1155–64.
109. Gipson TS, Bless NM, Shanley TP, Crouch LD, Bleavins MR, Younkin EM, et al. Regulatory effects of endogenous protease inhibitors in acute lung inflammatory injury. *J Immunol* 1999;**162**:3653–62.
110. Miller KW, Evans RJ, Eisenberg SP, Thompson RC. Secretory leukocyte protease inhibitor binding to mRNA and DNA as a possible cause of toxicity to Escherichia coli. *J Bacteriol* 1989;**171**:2166–72.
111. Hiemstra PS, Maassen RJ, Stolk J, Heinzel-Wieland R, Steffens GJ, Dijkman JH. Antibacterial activity of antileukoprotease. *Infect Immun* 1996;**64**:4520–4.
112. Tomee JF, Hiemstra PS, Heinzel-Wieland R, Kauffman HF. Antileukoprotease: an endogenous protein in the innate mucosal defense against fungi. *J Infect Dis* 1997;**176**:740–7.
113. Wang G, Narayana JL, Mishra B, Zhang Y, Wang F, Wang C, et al. Design of antimicrobial peptides: progress made with human cathelicidin LL-37. *Adv Exp Med Biol* 2019;**1117**:215–40.
114. Gudmundsson GH, Agerberth B, Odeberg J, Bergman T, Olsson B, Salcedo R. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur J Biochem* 1996;**238**:325–32.
115. De Smet K, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol Lett* 2005;**27**:1337–47.
116. Méndez-Samperio P. The human cathelicidin hCAP18/LL-37: a multifunctional peptide involved in mycobacterial infections. *Peptides* 2010;**31**:1791–8.
117. Wang G, Mishra B, Epand RF, Epand RM. High-quality 3D structures shine light on antibacterial, anti-biofilm and antiviral activities of human cathelicidin LL-37 and its fragments. *Biochim Biophys Acta* 2014;**1838**:2160–72.
118. Saar-Dover R, Bitler A, Nezer R, Shmuel-Galia L, Firon A, Shimoni E, et al. D-alanylation of lipoteichoic acids confers resistance to cationic peptides in group B streptococcus by increasing the cell wall density. *Plos Pathog* 2012;**8**:e1002891.
119. Shah NR, Hancock RE, Fernandez RC. Bordetella pertussis lipid A glucosamine modification confers resistance to cationic antimicrobial peptides and increases resistance to outer membrane perturbation. *Antimicrob Agents Chemother* 2014;**58**:4931–4.
120. Koziel J, Bryzek D, Sroka A, Maresz K, Glowczyk I, Bielecka E, et al. Citrullination alters immunomodulatory function of LL-37 essential for prevention of endotoxin-induced sepsis. *J Immunol* 2014;**192**:5363–72.
121. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med* 2011;**3**:73ra19.
122. Weiner DJ, Bucki R, Janmey PA. The antimicrobial activity of the cathelicidin LL37 is inhibited by F-actin bundles and restored by gelsolin. *Am J Respir Cel Mol Biol* 2003;**28**:738–45.
123. Neumann A, Völlger L, Berends ET, Molhoek EM, Stapels DA, Midon M, et al. Novel role of the antimicrobial peptide LL-37 in the protection of neutrophil extracellular traps against degradation by bacterial nucleases. *J Innate Immun* 2014;**6**:860–8.
124. Stephan A, Batinica M, Steiger J, Hartmann P, Zaucke F, Bloch W, et al. LL37: DNA complexes provide antimicrobial activity against intracellular bacteria in human macrophages. *Immunology* 2016;**148**:420–32.
125. Levay PF, Viljoen M. Lactoferrin: a general review. *Haematologica* 1995;**80**:252–67.
126. Francis N, Wong SH, Hampson P, Wang K, Young SP, Deigner HP, et al. Lactoferrin inhibits neutrophil apoptosis via blockade of proximal apoptotic signaling events. *Biochim Biophys Acta* 2011;**1813**:1822–6.
127. Weinberg ED. Iron withholding: a defense against infection and neoplasia. *Physiol Rev* 1984;**64**:65–102.
128. Ellison 3rd RT, Giehl TJ. Killing of Gram-negative bacteria by lactoferrin and lysozyme. *J Clin Invest* 1991;**88**:1080–91.
129. Ellison 3rd RT, LaForce FM, Giehl TJ, Boose DS, Dunn BE. Lactoferrin and transferrin damage of the Gram-negative outer membrane is modulated by Ca2+ and Mg2+. *J Gen Microbiol* 1990;**136**:1437–46.
130. Umekita K, Miyauchi S, Nomura H, Umeki K, Okayama A. Neutrophil-derived lactoferrin induces the inflammatory responses of rheumatoid arthritis synovial fibroblasts via Toll-like receptor 4. *Clin Exp Rheumatol* 2019;**37**:834–41.

131. Flower DR. The lipocalin protein family: structure and function. *Biochem J* 1996;**318**:1–14.
132. Triebel S, Bläser J, Reinke H, Tschesche H. A 25 kDa α2-microglobulin-related protein is a component of the 125 kDa form of human gelatinase. *FEBS Lett* 1992;**314**:386–8.
133. Kolkenbrock H, Hecker-Kia A, Orgel D, Kinawi A, Ulbrich N. Progelatinase B forms from human Nneutrophils. Complex formation of monomer/lipocalin with TIMP-1. *Biol Chem* 1996;**377**:529.
134. Sengeløv H, Boulay F, Kjeldsen L, Borregaard N. Subcellular localization and translocation of the receptor for N-formylmethionyl-leucyl-phenylalanine in human neutrophils. *Biochem J* 1994;**299**:473–9.
135. Bratt T, Ohlson S, Borregaard N. Interactions between neutrophil gelatinase-associated lipocalin and natural lipophilic ligands. *Biochim Biophys Acta* 1999;**1472**:262–9.
136. Kjeldsen L, Cowland JB, Borregaard N. Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse. *Biochim Biophys Acta* 2000;**1482**:272–83.
137. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cel* 2002;**10**:1033–43.
138. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, et al. Lipocalin 2-deficient mice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury. *Proc Natl Acad Sci U S A* 2006;**103**:1834–9.
139. Li H, Feng D, Cai Y, Liu Y, Xu M, Xiang X, et al. Hepatocytes and neutrophils cooperatively suppress bacterial infection by differentially regulating lipocalin-2 and neutrophil extracellular traps. *Hepatology* 2018;**68**:1604–20.
140. Ragland SA, Criss AK. From bacterial killing to immune modulation: recent insights into the functions of lysozyme. *Plos Pathog* 2017;**13**:e1006512.
141. Fleming A. On a remarkable bacteriolytic element found in tissues and secretions. *Proc R Soc Lond Ser B Biol Sci* 1922;**93**:306–17.
142. Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: is immunity the second function of chromatin? *J Cel Biol* 2012;**198**:773–83.
143. Michaelis J, Vissers M, Winterbourn C. Cleavage of alpha 1-antitrypsin by human neutrophil collagenase. *Matrix Suppl* 1992;**1**:80–1.
144. Itoh Y, Nagase H. Preferential inactivation of tissue inhibitor of metalloproteinases-1 that is bound to the precursor of matrix metalloproteinase 9 (Progelatinase B) by human neutrophil elastase. *J Biol Chem* 1995;**270**:16518–21.
145. Abrahamson M, Buttle D, Mason R, Hansson H, Grubb A, Lilja H, et al. Regulation of cystatin C activity by serine proteinases. *Biomed Biochim Acta* 1991;**50**:587.
146. Khatwa UA, Kleibrink BE, Shapiro SD, Subramaniam M. MMP-8 promotes polymorphonuclear cell migration through collagen barriers in obliterative bronchiolitis. *J Leukoc Biol* 2010;**87**:69–77.
147. Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, et al. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem* 1997;**272**:31504–9.
148. Prikk K, Maisi P, Pirilä E, Sepper R, Salo T, Wahlgren J, et al. In vivo collagenase-2 (MMP-8) expression by human bronchial epithelial cells and monocytes/macrophages in bronchiectasis. *J Pathol* 2001;**194**:232–8.
149. Herman MP, Sukhova GK, Libby P, Gerdes N, Tang N, Horton DB, et al. Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: a novel collagenolytic pathway suggested by transcriptional profiling. *Circulation* 2001;**104**:1899–904.
150. Naesse EP, Schreurs O, Helgeland K, Schenck K, Steinsvoll S. Matrix metalloproteinases and their inhibitors in gingival mast cells in persons with and without human immunodeficiency virus infection. *J Periodontal Res* 2003;**38**:575–82.
151. Cole AA, Chubinskaya S, Schumacher B, Huch K, Szabo G, Yao J, et al. Chondrocyte matrix metalloproteinase-8. Human articular chondrocytes express neutrophil collagenase. *J Biol Chem* 1996;**271**:11023–6.
152. Ong CW, Elkington PT, Brilha S, Ugarte-Gil C, Tome-Esteban MT, Tezera LB, et al. Neutrophil-derived MMP-8 drives AMPK-dependent matrix destruction in human pulmonary tuberculosis. *Plos Pathog* 2015;**11**:e1004917.
153. Tester AM, Cox JH, Connor AR, Starr AE, Dean RA, Puente XS, et al. LPS responsiveness and neutrophil chemotaxis in vivo require PMN MMP-8 activity. *PLoS One* 2007;**2**:e312.
154. Kelly EA, Busse WW, Jarjour NN. Increased matrix metalloproteinase-9 in the airway after allergen challenge. *Am J Respir Crit Care Med* 2000;**162**:1157–61.
155. Falkencrone S, Poulsen LK, Bindslev-Jensen C, Woetmann A, Odum N, Poulsen BC, et al. IgE-mediated basophil tumour necrosis factor alpha induces matrix metalloproteinase-9 from monocytes. *Allergy* 2013;**68**:614–20.
156. Kanbe N, Tanaka A, Kanbe M, Itakura A, Kurosawa M, Matsuda H. Human mast cells produce matrix metalloproteinase 9. *Eur J Immunol* 1999;**29**:2645–9.
157. Kelly EA, Liu LY, Esnault S, Quinchia Johnson BH, Jarjour NN. Potent synergistic effect of IL-3 and TNF on matrix metalloproteinase 9 generation by human eosinophils. *Cytokine* 2012;**58**:199–206.
158. Chakrabarti S, Zee JM, Patel KD. Regulation of matrix metalloproteinase-9 (MMP-9) in TNF-stimulated neutrophils: novel pathways for tertiary granule release. *J Leukoc Biol* 2006;**79**:214–22.
159. Beeh KM, Beier J, Kornmann O, Buhl R. Sputum matrix metalloproteinase-9, tissue inhibitor of metalloprotinease-1, and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. *Respir Med* 2003;**97**:634–9.
160. Ventura I, Vega A, Chacón P, Chamorro C, Aroca R, Gómez E, et al. Neutrophils from allergic asthmatic patients produce and release metalloproteinase-9 upon direct exposure to allergens. *Allergy* 2014;**69**:898–905.
161. Vandooren J, Goeminne P, Boon L, Ugarte-Berzal E, Rybakin V, Proost P, et al. Neutrophils and activated macrophages control mucosal immunity by proteolytic cleavage of antileukoproteinase. *Front Immunol* 2018;**9**:1154.
162. Gearing A, Beckett P, Christodoulou M, Churchill M, Clements J, Crimmin M, et al. Matrix metalloproteinases and processing of pro-TNF-α. *J Leukoc Biol* 1995;**57**:774–7.
163. Schönbeck U, Mach F, Libby P. Generation of biologically active IL-1β by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1β processing. *J Immunol* 1998;**161**:3340–6.
164. Kojima S-i, Itoh Y, Matsumoto S-i, Masuho Y, Seiki M. Membrane-type 6 matrix metalloproteinase (MT6-MMP, MMP-25) is the second glycosyl-phosphatidyl inositol (GPI)-anchored MMP. *FEBS Lett* 2000;**480**:142–6.
165. Pei D. Leukolysin/MMP25/MT6-MMP: a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. *Cell Res* 1999;**9**:291–303.
166. Velasco G, Cal S, Merlos-Suárez A, Ferrando AA, Alvarez S, Nakano A, et al. Human MT6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. *Cel Res* 2000;**60**:877–82.
167. Nie J, Pei D. Rapid inactivation of alpha-1-proteinase inhibitor by neutrophil specific leukolysin/membrane-type matrix metalloproteinase 6. *Exp Cel Res* 2004;**296**:145–50.