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Review

Host antimicrobial proteins as endogenous immunomodulators

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ABSTRACT

Host defense mechanisms are multilayered and involve physical as well as chemical barriers, antimicrobial factors as well as a broad set of immunocompetent cells. The mode of action of antimicrobial factors is variable, ranging from opsonisation and agglutination to direct killing of pathogens. In the last years it has become increasingly clear that some of these factors act as endogenous ligands that bind to distinct host receptors, as for example pathogen recognition receptors (PRRs), thereby influencing distinct immunological processes like chemotaxis, modulation of phagocytosis, dendritic cell maturation or the production of cytokines. By that way, these factors are implicated to protect the host by preventing and clearing of microbial infections.

1. Introduction

Effective host defense against pathogens requires a well-concerted interplay of many components of the organism. In order to avoid infection, higher organisms utilize epithelial barriers, specialized immunocompetent cells as well as host antimicrobial proteins (AMPs). For a long time it has been thought that the latter contribute to host protection solely by opsonisation, agglutination, neutralization or destruction of harmful invaders. However, recent observations have shown that some of these molecules have a broader impact in host defense, since they can both trigger and tune immunomodulatory processes as for example chemotaxis, phagocytosis, cytokine production, reactive oxygen species production and dendritic cell maturation. In having antimicrobial as well as immunomodulatory properties, these molecules thereby represent immunomodulatory AMPs (IAMPs).

Based on their mode of secretion, IAMPs can be subdivided into two groups: proteins of the first group are stored within specialized cells and are released after pathogen encounter, thereby representing inducible IAMPs. In contrast, proteins of the second group are secreted constitutively at endogenous surfaces, therefore representing constitutive IAMPs.

Additional duality of IAMPs is given, since some of them exert their antimicrobial action at luminal sites and show immunomodulatory activity when translocated to interstitial sites in case of infection.

Beside the characterization of novel endogenous immunomodulatory molecules, current research is focusing on the identification of their receptors, which mediate cellular signaling and initiate an immune response. To date several receptor families, as for example PRRs, have been shown to be involved in the recognition of endogenous structures. The main task of PRRs is the surveillance for microbes that contain pathogen associated molecular patterns (PAMPs). PAMPs are constituted by lipid, carbohydrate, peptide and nucleic structures that are expressed by different groups of microorganisms. A variety of PRRs have been identified up to date, like CD14 [1], scavenger receptors [2], the NOD-like receptor family, the RIG-I-like receptor family [3], C-type lectins [4] and the so far most extensively studied family, the toll-like receptors (TLRs) [5].

TLRs are responsible for a multiplicity of PRR–PAMP interactions and turned out to be additionally important sensors for endogenous structures. In humans the TLR family comprises at least 11 membrane proteins, located at the cell surface or on the membrane of endocytic vesicles and other intracellular organelles. Microbial components recognized by TLRs have been identified so far for TLR1 (e.g. mycobacterial araLAM), TLR2 (e.g. peptidoglycan), TLR3 (e.g. double stranded RNA), TLR4 (e.g. LPS), TLR5 (bacterial flagellin), TLR6 (e.g. mycoplasmal macrophage-activating lipopeptide 2 kDA),

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TLR7 and TLR8 (viral single-stranded RNA), TLR9 (e.g. unmethylated bacterial CpG DNA), and TLR11 (uropathogenic bacteria) [6–12].

Despite suggestions that engagement to TLRs is a privilege for exogenous structures [13], it is highly probable that TLRs can also bind to endogenous structures that appear in case of danger. It was shown that TLR4 signaling in dendritic cells can be triggered by extracellular matrix components, such as hyaluronic acid, heparan sulfate or fibronectin, which represent molecules that occur in case of tissue damage or infection [14–16]. The relevance of these interactions is not fully established at present but might represent an immunomodulatory mechanism in response to endogenous "danger" signals. Indeed, it has been reported that endogenous danger signals, which are released after tissue injury, play an important role in processes of tissue repair [17]. Furthermore, it has been indicated, that endogenous RNA and DNA play a role in autoimmune diseases by having the potency to induce the production of RNA and DNA specific autoantibodies, which afore involves TLR7 and TLR8 triggering [18].

In this review we will focus on the properties of host molecules that have both direct antimicrobial and immunomodulatory effects, and discuss their multifaceted impact on the establishment of site specific host defense mechanisms that are employed in the prevention and clearance of infections.

2. Immunomodulatory antimicrobial proteins (IAMPs)

2.1. Inducible IAMPs

In response to tissue injury or microbial infection, cells of the innate immune system, as for example neutrophils, eosinophils, basophils, NKs, monocytes or macrophages, secret AMPs. Some AMPs solely act as antimicrobial agents, others comprise both antimicrobial and immunomodulatory properties, therefore representing IAMPs. To underline the importance for host defense the term "alarmin" has been coined for these molecules [19]. Representatives of this group are defensins, cathelicidins or eosinophil derived neurotoxin (EDN) (Table 1).

2.1.1. Defensins

Mammalian defensins are cysteine-rich endogenous antibiotic peptides of the innate immune system. Three different types of defensins have been described so far: α-defensins, β-defensins and θ-defensins. The family of defensins shares several structural properties, as for example three intramolecular disulfide bonds, cationic net charge and lack of glycosyl and acyl side-chain modification. Furthermore, all defensins are synthesized as prepropeptides and are differently processed depending on the site of expression [20]. While human α-defensins, also known as human neutrophil peptides (HNPs), are mainly produced in leukocytes, Paneth cells of the small intestine and in the female reproductive tract, human β-defensins (hBD-1–hBD-4) are widely expressed in the epithelium and in leukocytes [21–23].

Defensins are important effector molecules against enveloped viruses, bacteria, fungi and protozoa, and protein concentrations ranging from 0.5 to 5 μM were shown to kill a wide range of microbes *in vitro* [21]. Defensins have the ability to attack susceptible microorganisms and destroy the structure of target cell membranes [20,21]. Several members of each defensin family were shown to act as microbicides against distinct Gram-negative and Gram-positive bacteria, as well as fungi and viruses *in vitro*. When treated with HNP the outer membrane of *Escherichia coli* became permeable. This permeabilization furthermore coincided with the cessation of RNA, DNA and protein synthesis, and with a decreased bacterial viability [24].

Long known in plants, the θ-defensin retrocyclin was the first defensin that was described to have antiviral activity in vertebrates. Retrocyclin was shown to bind avidly to viral membrane glycoproteins, and it is hypothesized that this mechanism is responsible for its antiviral activity. As demonstrated in *in vitro* studies, retrocyclin protected human cells from infection by HIV-1 [25].

In a recent study, the human α-defensin HNP1 was shown to exert a dual antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). The authors of this study suggested that defense against VHSV is mediated by interfering of HPN1 with VHSV-G protein-dependent fusion on the one hand, and the inhibition of VHSV replication in target cells by up-regulating genes related to the type I interferon response on the other hand [26].

In addition to these direct antimicrobial activities, defensins also have other effects that influence immune responses. The human neutrophil defensins HNP1 and HNP2 were shown to exert significant chemotactic effects on monocytes. Subsequently to their release after pathogen encounter these molecules guide monocytes to the site of infection [27]. Chemoattraction mediated by defensins was also shown to influence the migratory behaviour of adaptive immune cells, as for example T-cells. In *in vivo* assays subcutanous injection of 1 μg of HNP1 and HNP2 resulted in the infiltration of modest numbers of $CD3^+$ T-cells [28]. Yang et al. showed that the human β-defensin hBD is also chemoattractive for memory T-cells and immature dendritic cells. In this study HEK293 cells were transfected with several chemokine receptors, like CCR1, CCR5, CXCR4 and CCR6, but only transfection with the latter induced migratory activity of HEK293 cells. Dendritic cells express CCR6 on their surface and it was demonstrated that migration in response to hBD is abrogated by blocking of CCR6 [29].

A further property of the defensins was revealed when it was shown that murine β-defensin (mDFβ) activates immature DCs. Stimulation of immature DCs with mDFβ2 led to the expression of proinflammatory chemokines and cytokines, as for example RANTES, macrophage-derived chemokine (MDC), interferon-γ inducible protein (IP-10), MIP1α and MIP/β, tumor necrosis factor-α (TNF-α) and the interleukins 1 (IL-1) and 12 (IL-12). Signaling induced by mDFβ2a was dependent on TLR4 since C3H/HeJ mice, which harbor a mutation in their *tlr* locus are not able to develop mature DCs in response to mDFβ2 [30].

Table 1
Inducibly expressed endogenous molecules, their activities and putative receptors

Endogenous molecule	Antimicrobial activity	Chemotaxis	Phagocytosis	ROS	Cytokine	Receptors	Reference
Defensins							
HNP1	Microbicidal	Yes	?	?	Yes	?	[26–28]
HNP2	Microbicidal	Yes	?	?	?	?	[27,28]
HBD2	Microbicidal	Yes	?	?	Yes	CCR6	[22,29]
θ-Defensin	Antiviral	?	?	?	?	?	[25]
mDFβ	Microbicidal	?	?	?	Yes	TLR4	[30]
EDN	Antiviral	Yes	?	?	Yes	TLR2	[37–40]
LL-37	Microbicidal	Yes	?	Yes	Yes	FPRL1	[43,48–50,52]