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The dual role of cathelicidins in systemic inflammation

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ABSTRACT

Antimicrobial peptides are key components of the innate immune system. They act as broad-spectrum antimicrobial agents against Gram-positive and −negative bacteria, viruses, and fungi. More recently, antimicrobial peptides have been ascribed immunomodulatory functions, including roles in wound healing, induction of cytokines, and altering host gene expression. Cathelicidins are a class of antimicrobial peptide found in humans, mice, and rats, among others. Known as LL-37 in humans and cathelin-related antimicrobial peptide (CRAMP) in rodents, cathelicidins are produced by many different cells, including macrophages, neutrophils, and epithelial cells. The role of cathelicidins is somewhat confounding, as they exhibit both pro-and anti-inflammatory activity. A major obstacle in the study of cathelicidins is the inability of exogenous LL-37 or CRAMP to mimic the activity of their endogenous counterparts. Nevertheless, studies have shown that LL-37 is recognized by multiple receptors, and may stabilize or modulate Toll-like receptor signaling. In addition, cathelicidins play a role in apoptosis, inflammasome activation, and phagocytosis. However, many studies are revealing the dual effects of cathelicidins. For example, CRAMP appears to be protective in models of group A *Streptococcus* skin infection, pneumonia, and meningitis, but detrimental in cases of severe bacterial infection, such as septic shock. It is becoming increasingly clear that the activity of cathelicidins is modulated by complex interactions with the microenvironment, as well as the disease background. This article reviews what is currently known about the activity of cathelicidins in an attempt to understand their complex roles in systemic diseases.

1. Introduction

Antimicrobial peptides (AMPs), also known in higher eukaryotic organisms as host defense peptides, are ancient components of the innate immune system that can kill pathogens both directly (antimicrobial properties) and indirectly (immunomodulatory effects). Cathelicidins are a class of AMP comprising a single member each in rats, mice, and humans. In rodents, cathelicidin is known as CRAMP (cathelin-related antimicrobial peptide), while its human ortholog is LL-37 [1].

Since the first descriptions of their unexpected immunoregulatory functions, researchers have hypothesized that AMPs might have activities that go beyond defense against microbes, including roles in sterile inflammation [2], wound healing [3], angiogenesis [4], cell death [5], autoimmune disorders [6], and even in malignant transformation [7].

Here, we will review the current literature, evaluating the activity of cathelicidins under several conditions, in an attempt to understand their multiple functions in complex systemic diseases.

2. Technical challenges and molecular gaps

Both CRAMP and LL-37 are produced by many cell types, including macrophages, neutrophils, natural killer cells, and epithelial cells of the skin, intestine, airways, and ocular surface [8]. Cathelicidins exert both pro- and anti-inflammatory functions, which may be modulated by complex interactions with the microenvironment, cellular context, and disease background, a fact that we are just beginning to appreciate.

An important point that should always be kept in mind is the fact that AMPs are cytotoxic, and the effects of exogenous cathelicidins do not reproduce correctly the effects following endogenous secretion in murine macrophages. Experiments performed by our group showed that exogenous CRAMP, even when used at concentrations as low as 1–10 μM, induced significant cell damage, and aborted lipopolysaccharide-induced Toll-like receptor signaling [9], even though this kind of cytotoxicity may depend on the cell type, so it is difficult to say that LL37 is always toxic. It is not clear why the kinetics of endogenous cathelicidins are so hard to mimic *in vitro*. However, we believe that secretion *in vivo* may occur exponentially, or in association with other factors, which may somehow prevent disruption of the cell membrane and the ensuing leakage of intracellular contents and disturbance of certain signaling pathways. For this reason, results obtained from experiments performed using exogenous LL-37 or CRAMP must be considered

Abbreviations: CRAMP, cathelin related antimicrobial peptide; AMPs, antimicrobial peptides.

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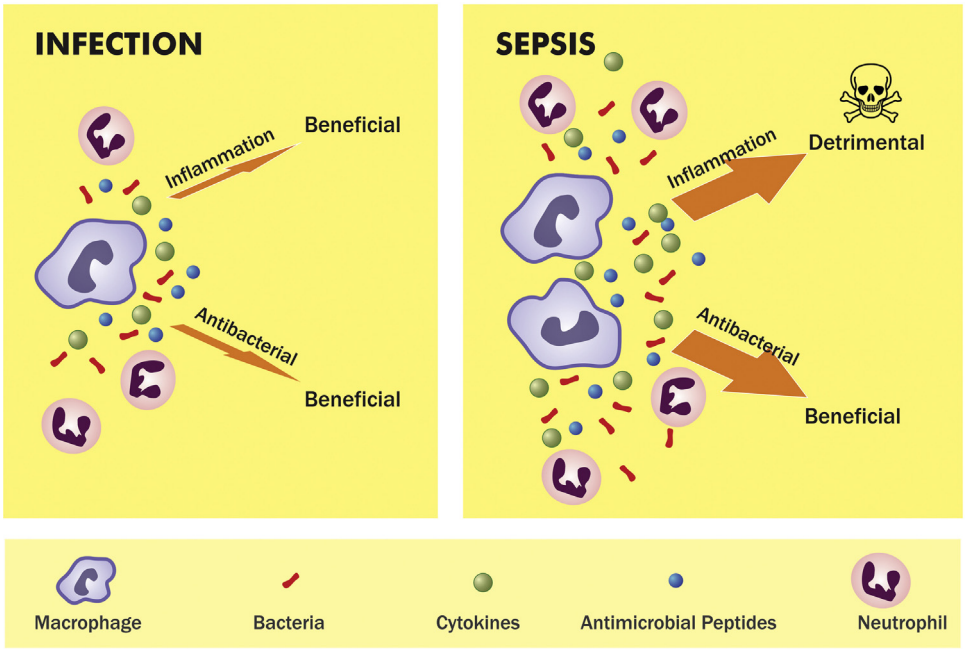

Fig. 1. Illustration showing the role of cytokines and antimicrobial peptides in localized Infection and in Sepsis. The inflammatory role of antimicrobial peptides in Sepsis, however, remains under debate.

with caution. Gene knockout or knockdown are the gold standard techniques for investigation of cathelicidins.

There are many "molecular gaps" in our understanding of the effects of cathelicidins in the immune system. Although multiple receptors, including FPRL-1 [10], P2 × 7R [11], and several others [12,13], recognize LL-37, this is not enough to explain so many complex cellular functions, such as the ability of cathelicidins to both activate and inhibit cell responses. Several reports claim that cathelicidins "stabilize" receptors or "modulate" Toll-like receptor signaling, but a comprehensive molecular characterization of their mode of action remains to be done [8], even though convincing data has recently explained why LL37 and other cationic peptides can stimulate effectively TLR9 [14]. Oligomerization of LL-37, for example, affects its mode of interaction with biological membranes, and consequently, its cytotoxicity and receptor-mediated activities [15]. Indeed, formation of homodimers, heterodimers, or other oligomers could partially explain the opposing effects of LL-37 in different biological processes, a phenomenon that should be investigated further.

Cathelicidins can enhance or abrogate cell signaling in several health and disease states. They participate in the secretion of cytokines [9], chemotactic responses [16], apoptosis [17], inflammasome activation [18], and phagocytosis [19], among several other inflammatory and immune effects.

Interestingly, LL-37 has a high affinity for nucleic acids, binding both RNA and DNA. Because of this, LL-37 acts as a transmembrane shuttle for intracellular nucleic acid delivery [20]. Indeed, neutrophil AMPs are present in circulating DNA-containing immune complexes of systemic lupus erythematosus patients [21], who develop autoantibodies against both DNA and neutrophil antimicrobial peptides. Further, neutrophil extracellular traps released by activated neutrophils contain DNA-antimicrobial peptide complexes that trigger TLR9 in plasmacytoid dendritic cells [21]. Unexpectedly, however, extensive experiments performed by the Hoffmann group found no evidence of pathogenic involvement of cathelicidins in patient cohorts and mouse models of lupus and arthritis [22], suggesting that autoreactivity against cathelicidins does not seem to be indispensable for lupus and arthritis pathogenesis. However, other studies are needed to definitely show whether LL-37 and anti-LL37 antibodies are important in disease pathogenesis in lupus.

Experiments by our group showed that as well as interacting with extracellular DNA, LL-37 also migrates to the nucleus and binds genomic DNA, where it may participate in transcriptional processes [23].

3. Cathelicidins in the apparent chaos of systemic inflammation

Several groups have been investigating the role of LL-37 in pulmonary tuberculosis, a leading cause of death among infectious diseases. A recent study found significantly higher levels of LL-37 in bronchoalveolar lavage fluid from pediatric patients with pulmonary tuberculosis when compared with that from healthy children [24]. Interestingly, Afsal et al. showed that *in vitro* stimulation of macrophages with 1,25-dihydroxy vitamin D3 upregulated the expression of LL-37 in both pulmonary tuberculosis patients and healthy controls, but the increase was more prominent in patients without pulmonary cavities compared with patients suffering from cavitary pulmonary disease. This finding suggested that LL-37 production may be beneficial, particularly in less severe forms of tuberculosis [25]. Similarly, our group observed decreased expression of LL-37 in neutrophils from patients in septic shock [26], suggesting that cathelicidins are detrimental in more severe bacterial infections, but protective in less severe forms. Indeed, experiments using CRAMP knockout mice showed that cathelicidin is protective in experimental models of group A *Streptococcus* skin infection [27], pneumonia [28], and meningitis caused by both *Neisseria meningitidis* [29] and *Streptococcus pneumoniae* [30]. However, in these models the source infection remained predominantly localized. For the moment, the role of antimicrobial peptides in sepsis remains unclear, but data from our laboratory is putting in evidence that cathelicidins can be both pro or anti-inflammatory in sepsis, depending on the experimental model and bacterial agent (Pinheiro da Silva et al., *submitted*) (Fig. 1). Plasma levels of LL-37 are also reduced in untreated cases of human immunodeficiency virus-