**Tribolium castaneum defensin 1 kills Moraxella catarrhalis in an in vitro infection model but does not harm commensal bacteria**

Wilhelm Bertrams, Nora S. Lindhauer, Marie Christin Rieke, Anne Paas, Kerstin Hoffmann, Brandon Greene, Alexander Visekruna, Andreas Vilcinskas, Kerstin Seide, and Bernd Schmeck

**ABSTRACT**

*Moraxella catarrhalis* is a bacterial pathogen that causes respiratory tract infections in humans. The increasing prevalence of antibiotic-resistant *M. catarrhalis* strains has created a demand for alternative treatment options. We therefore tested 23 insect antimicrobial peptides (AMPs) for their activity against *M. catarrhalis* in a human in vitro infection model with primary macrophages, and against commensal bacteria. Effects on bacterial growth were determined by colony counting and growth curve analysis. The inflammatory macrophage response was characterized by qPCR and multiplex ELISA. Eleven of the AMPs were active against *M. catarrhalis*. Defensin 1 from the red flour beetle *Tribolium castaneum* significantly inhibited bacterial growth and reduced the number of colony forming units. This AMP also showed antibacterial activity in the in vitro infection model, reducing cytokine expression and release by macrophages. Defensin 1 had no effect on the commensal bacteria *Escherichia coli* and *Enterococcus faecalis*. However, sarcotoxin 1 C from the green bottle fly *Lucilia sericata* was active against *M. catarrhalis* and *E. coli*, but not against *E. faecalis*. The ability of *T. castaneum* defensin 1 to inhibit *M. catarrhalis* but not selected commensal bacteria, and the absence of cytotoxic or inflammatory effects against human blood-derived macrophages, suggests this AMP may be suitable for development as a new therapeutic lead against antibiotic-resistant *M. catarrhalis*.

**Introduction**

*Moraxella catarrhalis* is a Gram-negative, aerobic, diplococcus pathogen that colonizes the human respiratory tract. It produces non-hemolytic, round and opaque colonies on blood agar. The major virulence strategies of *M. catarrhalis* include complement resistance, the formation of protective biofilms, localization within lymphoid tissues to avoid immunosurveillance, and polyclonal non-specific B-cell activation to modulate adaptive immunity [1]. Respiratory tract colonization does not always lead to symptomatic disease, which is highly dependent on the patient’s age [2]. In the upper respiratory tract, *M. catarrhalis* can cause acute otitis media (OM). Approximately 80% of children have already experienced OM by the age of 3, and 15–20% of these cases are caused by *M. catarrhalis*. The prevalence of *M. catarrhalis* is ≤ 75% in children but only 1–3% in adults, a distribution that has been stable since the 1970s [3]. Infections with *M. catarrhalis* can be treated with antibiotics, but strains resistant to penicillin, ampicillin, and amoxicillin are now common [4]. Antibiotic-resistant Gram-negative bacteria are increasingly seen as threats to global healthcare systems, causing more than 670,000 infections and 33,000 deaths per year in Europe [5] with associated healthcare costs of more than €1 billion [6]. Alternative treatments for bacterial infections are therefore urgently needed.

Antimicrobial peptides (AMPs) offer one potential source of new drug leads against infectious diseases.

**CONTACT** Bernd Schmeck bernd.schmeck@uni-marburg.de

*Present address:* Vascular Biology Section, Evans Department of Medicine, Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, MA 02118, USA.

*Supplemental data for this article can be accessed here*

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
They are short peptides (typically 12–50 amino acids) that function as part of the innate immune system in all eukaryotic organisms. Their spectrum of activity includes viruses, bacteria, fungi and parasites. Insects provide a source of particularly diverse AMPs, which can be classified on the basis of their chemical attributes [7]. AMPs demonstrate a broad range of cellular mechanisms, from the promotion of angiogenesis to host cell chemotaxis, but electrostatic interaction with bacterial membranes and subsequent pore formation is the most important mechanism of direct bactericidal action [8]. In this regard, we previously described the effect of defensin 1 from the red flour beetle (Tribolium castaneum) against Streptococcus pneumoniae [7].

In this study, we tested 23 insect AMPs for their activity against M. catarrhalis and selected two with the most promising activity for further analysis in an in vitro infection model based on primary human macrophages. We selected T. castaneum defensin 1, a β-sheet globular AMP stabilized by intramolecular disulfide bridges, and sarcotoxin 1 C from the green bottle fly Lucilia sericata [9], a linear α-helical AMP without cysteine residues. We investigated the ability of these AMPs to trigger cytokine release, host cell cytotoxicity, hemolysis, inflammation and immunosuppression. Our results may facilitate the development of AMP-based drug leads against antibiotic-resistant Gram-negative bacteria including M. catarrhalis.

Materials and methods

Antimicrobial peptides

The 23 insect-derived AMPs tested in this study were produced by solid-phase synthesis and purified by Coring System Diagnostix (Gernsheim, Germany), GenScript (Piscataway, NJ, USA) and Pepmic (Suzhou, China). The integrity of the AMPs was confirmed by liquid chromatography-mass spectrometry. The properties of the AMPs are summarized in Table S1, chromatograms for each peptide are provided in Table S2.

Culture and growth kinetics of M. catarrhalis

Colonies of M. catarrhalis were grown on sheep blood agar plates for 12 h at 37°C and 5% CO₂ before transfer to brain heart infusion (BHI) medium (Carl Roth, Karlsruhe, Germany) at an initial concentration of 1.6 × 10⁷ cells/ml (OD₆₀₀ = 0.08) as determined using an Ultraspec 10 cell densitometer (Amersham BioSciences, Little Chalfont, UK). Bacteria were cultivated in a shaking incubator at 37°C until the concentration reached 1.2 × 10⁸ cells/ml (OD₆₀₀ = 0.6) and then diluted to 2 × 10⁶ cells/ml (OD₆₀₀ = 0.011). To establish the optimal concentration ranges for AMP activity, defensin 1 was prepared as a two-fold dilution series from 12.5 µM to 1.56 µM and was added to the bacterial cultures, which were incubated as above for a further 16 h. Untreated cultures were used as controls. The OD₆₀₀ was measured automatically at 30-min intervals using an Infinite M200 Pro plate reader (Tecan Life Sciences, Männedorf, Switzerland). For AMP inhibitory testing without macrophages, M. catarrhalis was grown to the mid-exponential phase (~5 × 10⁸ cells/ml) in BHI medium in the presence of defensin 1 (12.5 µM) or sarcotoxin 1 C (0.39 µM) before dilution in PBS containing 0.15% gelatin, and directly plated on sheep blood agar. They were cultivated for a further 15 h at 37°C and 5% CO₂ in serial dilution before manual counting of the colonies on each plate.

Culture and growth kinetics of E. faecalis and E. coli

For the analysis of commensal bacteria, E. faecalis was grown on Columbia agar plates or in liquid BHI medium, and E. coli was grown on McConkey agar or in liquid LB medium. After overnight culture on agar plates at 37°C and 5% CO₂, both species were transferred to liquid medium at an initial OD₆₀₀ of 0.005. The cultures were maintained in a shaking incubator at 37°C, and the OD₆₀₀ was measured automatically at 30-min intervals as above.

Colony forming unit assay

The absolute number of bacterial cells after treatment with defensin 1 or sarcotoxin 1 C was determined by counting the colony forming units (CFUs). M. catarrhalis overnight cultures prepared as described above were grown to the mid-exponential phase (~5 × 10⁸ cells/ml) in BHI medium before dilution to 2 × 10⁷ cells/ml in PBS containing 0.15% gelatin, and blood-derived macrophages (BDMs) were infected with a multiplicity of infection (MOI) of 0.5 or 1. After incubation with bacteria for 1 or 5 h, we added 12.5 µM defensin 1 or 0.39 µM sarcotoxin 1 C to the cell cultures representing each incubation time point, based on the concentrations previously shown to inhibit bacterial replication. Cells were then incubated for an additional 16 h at 37°C and 5% CO₂ and then lysed with 1% saponin. Lysates were plated on sheep blood agar and cultivated for a further 15 h at 37°C and 5% CO₂ before manual counting of the colonies on each plate.
BDM cultivation and differentiation

All donors gave informed written consent (Ethics approval number: 161/17). Human BDMs were cultured as previously described [7]. Briefly, primary human monocytes were isolated from donor buffy coats by selection for CD14+ cells using CD14 Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes were grown in RPMI1640 medium with 1% human AB serum at 37°C and 5% CO2 on ultra-low attachment plates (Sigma-Aldrich, Munich, Germany). After 6 days, differentiated macrophages were detached, seeded at the desired density and incubated for 24h before further analysis.

Isolation of RNA from infected BDMs and real-time PCR analysis

BDMs were infected with *M. catarrhalis* as described above and the cells and supernatant were collected 16h post-infection. Total RNA was isolated by phenol-chloroform extraction followed by reverse transcription using the High-Capacity RNA-to-cDNA kit (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative real-time PCR (qRT-PCR) was performed with the following specific primer pairs to measure the expression of IL-1β (forward primer 5'-AGC TCG CCA GTG AAA TGA TGG-3' and reverse primer 5'-CAG GTC CTG GAA GGA GCA CTT C-3'), IL-8 (forward primer 5'-ACT GAG AGT TGA GAG TGG AC-3' and reverse primer 5'-AAC CCT CTG CAC CCA GTT TTC-3'), and RPS18 (forward primer 5'-GGG GCG GAA AAT AGC CTT TG-3' and reverse primer 5'-GAT CAC ACG TCC ACC TCA TC-3').

Multiplex ELISA

The presence of cytokines in the BDM supernatant after infection and treatment with 12.5 µM defensin 1 was assessed using the MAGPIX Multiplex ELISA (Luminex, Austin, TX, USA). BDM supernatants were prepared as recommended for the MAGPIX system. The cytokine panel comprised MPI1-α, MCP1, IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-23, LAP and TNF-α.

Statistical analysis

Statistical interpretation of the multiplex ELISA data required the incorporation of data points outside the detection range and adjustments for the effect of parallel technical measurements from the same biological samples. We therefore used the Hodges and Lehmann nonparametric aligned ranks test [10] to compare cytokine secretion by treatment time within treatment groups and between the following control and treatment groups:

| MOI          | Description                                      |
|--------------|--------------------------------------------------|
| 0.5          | Without defensin 1 (control)                     |
| 0.5          | With defensin 1 given 1 h post-infection         |
| 0.5          | With defensin 1 given 5 h post-infection         |
| 1            | Without defensin 1 (control)                     |
| 1            | With defensin 1 given 1 h post-infection         |
| 1            | With defensin 1 given 5 h post-infection         |
| LPS          | Without defensin 1                               |
| LPS          | With defensin 1 (added 1 h after LPS)            |

Statistical analysis was carried out using the R suite. All other statistical tests were carried out as indicated in the figure legends, with a significance threshold of *p* < 0.05.

Results

Defensin 1 and sarcotoxin 1 C show efficacy against *M. catarrhalis*

We screened a panel of 23 AMPs, 11 of which were effective against *M. catarrhalis* (Table S1). Among those, we selected defensin 1 and sarcotoxin 1 C for further analysis because they differ in structure and hence in their potential mechanism of action. Defensin 1 completely abolished the growth of *M. catarrhalis* when present at a concentration of 12.5 µM, but only delayed growth at a concentration of 6.25 µM (Figure 1a). Defensin 1 also reduced the bacterial burden in an infection assay of primary BDMs, as reflected by the lower *M. catarrhalis* CFU count compared to assays without the peptide (Figure 1b). Sarcotoxin 1 C completely inhibited the growth of *M. catarrhalis* at a concentration of 0.39 µM and caused a growth delay at 0.195 µM (Figure 1c). Sarcotoxin 1 C also reduced the bacterial load in an infection assay of primary BDMs when presented at a concentration of 0.39 µM (Figure 1d). While defensin 1 efficiently killed the bacteria, the effect of sarcotoxin 1 C on bacterial growth was partly reversible upon dilution of the AMP by plating (Figure S1).

Defensin 1 reduces the survival of *M. catarrhalis* and the inflammatory activation of macrophages

Defensin 1 showed efficacy in the CFU and infection assays, but for therapeutic development it must also demonstrate minimal toxicity toward human cells. We therefore tested the ability of defensin 1 to induce inflammation in an *in vitro* infection assay with BDMs. Previous work has shown that defensin 1 has minimal hemolytic activity up to a concentration of
100 µM and is nontoxic toward macrophages [7]. We exposed BDMs to defensin 1 at a concentration of 12.5 µM 1 or 5 h after infection with *M. catarrhalis* at MOI = 0.5 or MOI = 1 to mimic a clinical setting. Defensin 1 limited the expression of IL-1β and IL-8 at both post-infection time points and both MOI values, as determined by qRT-PCR (Figure 2). Defensin 1 also inhibited the bacteria-induced secretion of key cytokines IL-1β, IL-10, IL-12p70 and IL-23 at both post-infection time points and both MOI values, as determined by multiplex ELISA (Figure 3). The sterile activation of BDMs by LPS was not significantly affected by

---

**Figure 1. Defensin 1 and sarcotoxin 1 C inhibit the growth of *M. catarrhalis*.** Bacteria were grown to OD_{600} = 0.6 in BHI medium, diluted to OD_{600} = 0.011 and incubated with two-fold serial dilutions of defensin 1 (between 25 µM and 0 µM) or sarcotoxin 1 (between 1.56 µM and 0 µM) at 37°C. The OD_{600} nm was measured at 30-min intervals (a and c). Bacterial growth was also monitored following the infection of BDMs at the indicated MOIs, with AMP treatment beginning 1 or 5 h post-infection (defensin 1 = 12.5 µM, sarcotoxin 1 C = 0.39 µM, with uninfected cells as controls) (b and d). Statistical significance was assessed by two-way ANOVA (****p < 0.0001 vs. control).

---

**Figure 2. Defensin 1 reduces the abundance of cytokine mRNAs in BDMs infected with *M. catarrhalis*.** Treatment with 12.5 µM defensin 1 (minimal inhibitory concentration) significantly reduced the amount of *IL-1β* mRNA (a) and *IL-8* mRNA (b) expression for all MOIs and time points. LPS was used as a sterile positive control. Log_{2} transformed data are shown. Statistical significance was assessed by two-way ANOVA (**p < 0.01, *p < 0.05).
the presence of defensin 1, as previously also reported for TNF-α [7].

**Commensal E. faecalis and E. coli are unaffected by defensin 1 and only partly affected by sarcotoxin 1 C**

Antibiotics often cause severe clinical side effects by disrupting the commensal flora [11]. We therefore investigated the impact of defensin 1 on the commensal bacteria *E. faecalis* and *E. coli*. Whereas 12.5 µM defensin 1 was sufficient to completely inhibit the growth of *M. catarrhalis*, the same concentration only caused a minor growth delay in both commensal species (Figure 4a and b). Interestingly, 0.39 µM sarcotoxin 1 C was able to inhibit the growth of *E. coli* but not *E. faecalis* (Figure 4c and d).

**Discussion**

Insects produce a broad repertoire of AMPs offering a rich source of potential new drug leads for the treatment of infections caused by antibiotic-resistant bacteria. We therefore screened a panel of 23 previously-described insect AMPs [7] against *M. catarrhalis*, a pathogen that causes respiratory infections in humans and is considered an emerging threat due to the increasing prevalence of antibiotic-resistant strains [4]. The most effective AMP in the panel was *T. castaneum* defensin 1, which was previously found to show low toxicity toward human BDMs [7]. Here we found that defensin 1 reduced the CFU counts of *M. catarrhalis* without harming BDMs in our in vitro infection model. It also limited the induction of the *IL-8* and *IL-1β* genes compared to untreated BDMs, and inhibited the secretion of cytokines such as IL-23, IL-12p70, IL-1β and IL-10. Despite its potent activity against *M. catarrhalis*, defensin 1 had a negligible impact on the growth of the commensal bacteria *E. faecalis* and *E. coli*. Another AMP in our panel with activity against *M. catarrhalis* was *L. sericata* sarcotoxin 1 C. This AMP was also effective against *E. coli*, but not against *E. faecalis*.

AMPs exert their function by targeting the bacterial cell wall, and are usually specialized for either Gram-negative or Gram-positive bacteria. Many AMPs are amphipathic with a positive net charge, so they bind to the negatively charged components of the bacterial cell wall and outer membrane. Some AMPs form pores...
in the bacterial cell membrane by direct binding and integration, leading to lysis and cell death [12]. Because AMPs target the structural integrity of bacteria, it is more difficult for them to evolve resistance mechanisms. The unique potency of defensin 1 against *M. catarrhalis* may reflect the presence of multiple disulfide bonds that help to stabilize the peptide and prevent degradation [13]. The β-sheet secondary structure may also increase stability by preventing the conformational changes that occur in α-helical AMPs. The enhanced stability of defensin 1 may explain its ability to inhibit the growth of *M. catarrhalis* in the BDM infection model, thus reducing the inflammatory host response triggered by bacterial cells. The timing of defensin 1 administration 1 or 5 h post-infection was chosen to mimic the clinical environment, where antimicrobial treatment tends to be initiated shortly after infection. We found that defensin 1 treatment 1 h post-infection was slightly more effective than the treatment after 5 h, which we attribute to a more comprehensive establishment of infection and more robust bacterial growth at the 5 h time point.

The approval of AMPs for the treatment of severe infections is pending [14], but it is important to note that AMPs not only act as potent direct antimicrobial agents but also as immunomodulators, thus helping to marshal the immune system against invading pathogens [15]. For example, AMPs can influence immune cell differentiation, the stimulation of chemotaxis, anti-endotoxin activity, initiation of adaptive immunity, and suppression of the TLR-mediated production of cytokines [16–18].

Among the 23 AMPs we tested, only defensin 1 was previously found to be effective against the Gram-positive pathogen *Streptococcus pneumoniae* [7]. It is unclear whether the efficacy of defensin 1 indeed lies in the structure of the peptide and whether other AMPs with different structures would also show activity against *M. catarrhalis*. We therefore also investigated *L. serica* sarcotoxin 1C, which has a linear α-helical structure without disulfide bridges. This AMP was efficient at a concentration of 0.39 µM, making it considerably more potent than defensin 1 in stopping bacterial growth in solution.

Given their different chemical structures and the different efficacies of defensin 1 and sarcotoxin 1 against *M. catarrhalis*, we tested whether their capacity to inhibit growth was bactericidal or bacteriostatic. While defensin 1 completely prevented bacterial growth also after its dilution, *M. catarrhalis* resumed growth after treatment with sarcotoxin 1C. This suggests that defensin 1 has bactericidal capacity, while the effect of sarcotoxin 1C is primarily bacteriostatic, as has been described before for the respective molecule class [19,20].

We furthermore tested the effect of defensin 1 and sarcotoxin 1C on the commensals *E. faecalis* and
E. coli. Selectivity of AMPs may provide an important therapeutic advantage over broad-spectrum antibiotics because AMPs targeting pathogens but not commensals would avoid the common side effects of antibiotic therapy. Intriguingly, whereas sarcotoxin 1 C efficiently inhibited the growth of Gram-negative E. coli as previously reported [21], it showed no activity against Gram-positive E. faecalis. Sarcotoxin 1 C was previously shown to inhibit 90% of clinical multidrug-resistant isolates of Enterobacter cloacae, Acinetobacter baumannii and Salmonella enterica, and pharmacological profiling revealed a good in vitro therapeutic index, no cytotoxicity or cardiotoxicity, an inconspicuous broad-panel off-target profile, and no acute toxicity in mice at a dose of 10 mg/kg [21]. Defensin 1 had had no effect against either commensal species in a previous study [20]. Cationic murine α-defensins, which have a predominantly β-sheet secondary structure and contain disulfide bonds, have been shown to kill E. coli [22]. Sarcotoxin 1 C and defensin 1 are cationic AMPs, allowing them to bind the outer membrane of Gram-negative bacteria and the cell wall of Gram-positive bacteria [23]. More mechanistic data are required to shed light on the selective action of the AMPs we describe and their association with different bacterial membrane and cell wall structures.

In summary, we have characterized the activity of defensin 1 against M. catarrhalis and confirmed its negligible activity against the commensals E. faecalis and E. coli. In contrast, we found that sarcotoxin 1 C was active against M. catarrhalis and E. coli, but ineffective against E. faecalis. We conclude that both sarcotoxin 1 C and defensin 1 are promising leads for the development of new antibiotics against M. catarrhalis infections, and that defensin 1 is particularly suitable due to its negligible effect against selected commensal flora. While the effects of defensin 1 and sarcotoxin 1 have partly been described before, the strength of our study lies in the direct comparison of their action against commensal and pathologic bacteria. With M. catarrhalis, we chose an important pathogen with potentially chronic disease manifestation, which we show for the first time to be sensitive to defensin 1 and sarcotoxin 1 C. The clinical implications of AMPs against pathogens of the airways necessitates development of topical application of AMPs to the lung epithelium, which requires the large-scale production of AMPs as aerosol formulations [24]. Recently, successful attempts have been made to neutralize Pseudomonas aeruginosa with AMPs coupled to nanoparticles in a mouse model [25], and also to render AMPs inhalable by spray-drying [26]. In the advent of spreading antibiotics resistance, AMPs hold great potential as successors or support of classical antibiotic treatment.

Acknowledgments
The authors thank Richard M. Twyman for manuscript editing.

Disclosure statement
The authors have nothing to disclose.

Funding
Parts of this work were funded by the Kempkes Foundation (04/2016) to WB, the Bundesministerium für Bildung und Forschung (e:Med CAPSYS - FKZ 01ZX1604E; JPI-AMR – FKZ 01KI1702; ERACoSysMed2 – SysMed-COPD – FKZ 031L0140; http://www.bmbf.de/) to BS, German Center for Infectious Disease Research (DZIF) to NSL, Deutsche Forschungsgemeinschaft (SFB-TR-84 TP C01; http://www.sfb-tr84.de/) to BS, Hessisches Ministerium für Wissenschaft und Kunst (LOEWE Medical RNomics - FKZ 519/03/00.001-(0003); http://www.proloewe.de/medicalrnomics) to BS.

ORCID
Wilhelm Bertrams @ http://orcid.org/0000-0002-0180-2529
Bernd Schmeck @ http://orcid.org/0000-0002-2767-3606

References
[1] Augustyniak D, Seredynski R, McClean S, et al. Virulence factors of Moraxella catarrhalis outer membrane vesicles are major targets for cross-reactive antibodies and have adapted during evolution. Sci Rep. 2018;8(1):4955.
[2] Murphy TF, Parameswaran GI. Moraxella catarrhalis, a human respiratory tract pathogen. Clin Infect Dis. 2009;49(1):124–131.
[3] Verduin CM, Hol C, Fleer A, et al. Moraxella catarrhalis; from emerging to established pathogen. Clin Microbiol Rev. 2002;15(1):125–144.
[4] Ioannidis JP, Worthington M, Griffiths JK, et al. Spectrum and significance of bacteremia due to Moraxella catarrhalis. Clin Infect Dis. 1995;21(2):390–397.
[5] Cassini A, Hogberg LD, Plachouras D, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European economic area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19(1):56–66.
[6] O’Neill J (2016) Tackling drug-resistant infections globally: final report and recommendations.
[7] Lindhauer NS, Bertrams W, Pöppel A, et al. Antibacterial activity of a Tribolium castaneum
defensin in an in vitro infection model of Streptococcus pneumoniae. Virulence. 2019;10 (1):902–909.
[8] Nguyen LT, Haney EF, Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. Trends Biotechnol. 2011;29(9):464–472.
[9] Poppel AK, Vogel H, Wiesner J, et al. Antimicrobial peptides expressed in medicinal maggots of the blow fly Lucilia sericata show combinatorial activity against bacteria. Antimicrob Agents Chemother. 2015;59 (5):2508–2514.
[10] Hodges JL, Lehmann EC. Rank methods for combination of independent experiments in the analysis of variance. Ann Math Stat. 1962;33:482–497.
[11] Becattini S, Taur Y, Pamer EG. Antibiotic-induced changes in the intestinal microbiota and disease. Trends Mol Med. 2016;22(6):458–478.
[12] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415(6870):389–395.
[13] Rajamuthiah R, Jayamani E, Conery AL, et al. A defensin from the model beetle Tribolium castaneum acts synergistically with tefalovacan and daptomycin against multidrug resistant staphylococcus aureus. PLoS One. 2015;10(6):e0128576.
[14] Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006;24(12):1551–1557.
[15] Kosikowska P, Lesner A. Antimicrobial peptides (AMPs) as drug candidates: a patent review (2003–2015). Expert Opin Ther Pat. 2016;26(6):689–702.
[16] Davidson DJ, Currie AJ, Reid GSD, et al. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J Immunol. 2004;172(2):1146–1156.
[17] Van Der Does AM, Bogaards SJP, Ravensbergen B, et al. Antimicrobial peptide hLF1-11 directs granulocyte-macrophage colony-stimulating factor-driven monocyte differentiation toward macrophages with enhanced recognition and clearance of pathogens. Antimicrob Agents Chemother. 2010;54(2):811–816.
[18] Mahlapuu M, Hakansson J, Ringstad L, et al. Antimicrobial peptides: an emerging category of therapeutic agents. Front Cell Infect Microbiol. 2016;6:194.
[19] Johns R, Sonenshine DE, Hynes WL. Control of bacterial infections in the hard tick Dermacentor variabilis (Acari: ixodidae): evidence for the existence of antimicrobial proteins in tick hemolymph. J Med Entomol. 1998;35(4):458–464.
[20] Tonk M, Knorr E, Cabezas-Cruz A, et al. Tribolium castaneum defensins are primarily active against Gram-positive bacteria. J Invertebr Pathol. 2015;13 2:208–215.
[21] Hirsch R, Wiesner J, Marker A, et al. Profiling antimicrobial peptides from the medical maggot Lucilia sericata as potential antibiotics for MDR Gram-negative bacteria. J Antimicrob Chemother. 2019;74 (1):96–107.
[22] Wilson CL, Ouellette AJ, Satchell DP et al. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science. 1999;286(5437):113–117.
[23] Bechinger B, Gorr SU. Antimicrobial peptides: mechanisms of action and resistance. J Dent Res. 2017;96(3):254–260.
[24] Tonk M, Vilcinskas A. The medical potential of antimicrobial peptides from insects. Curr Top Med Chem. 2017;17(5):554–575.
[25] Falciani C, Zevolini F, Brunetti J, et al. Antimicrobial peptide-loaded nanoparticles as inhalation therapy for pseudomonas aeruginosa infections. Int J Nanomedicine. 2020;15:1117–1128.
[26] Kwok PC, Grabarek A, Chow MYT, et al. Inhalable spray-dried formulation of D-LAK antimicrobial peptides targeting tuberculosis. Int J Pharm. 2015;491 (1–2):367–374.