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**Chlamydia-secreted protease CPAF degrades host antimicrobial peptides**

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Abstract

*Chlamydia trachomatis* infection in the lower genital tract, if untreated, can ascend to the upper genital tract, potentially leading to complications such as tubal factor infertility. The ascension involves cell-to-cell spreading, which may require *C. trachomatis* organisms to overcome mucosal extracellular effectors such as antimicrobial peptides. We found that among the 8 antimicrobial peptides tested, the cathelicidin LL-37 that is produced by both urogenital epithelial cells and the recruited neutrophils possessed a most potent antichlamydial activity. Interestingly, this antichlamydial activity was completely inhibited by CPAF, a *C. trachomatis*-secreted serine protease. The inhibition was dependent on CPAF’s proteolytic activity. CPAF selectively degraded LL-37 and other antimicrobial peptides with an antichlamydial activity. CPAF is known to secrete into and accumulate in the infected host cell cytoplasm at the late stage of chlamydial intracellular growth and may be released to confront the extracellular antimicrobial peptides before the intra-inclusion organisms are exposed to extracellular environments during host cell lysis and chlamydial spreading. Thus, the finding that CPAF selectively targets host antimicrobial peptides that possess antichlamydial activities for proteolysis suggests that CPAF may contribute to *C. trachomatis* pathogenicity by aiding in ascending infection.

Key words: *Chlamydia trachomatis*, CPAF, proteolysis of antimicrobial peptides
1. Introduction

There are more than one million new *Chlamydia trachomatis* infections every year in the US [3], some of which, if untreated, can ascend to the upper genital tract and causes complications such as pelvic inflammatory diseases, ectopic pregnancy and infertility [29]. The *C. trachomatis* ability to undergo intracellular replication and intercellular spreading is thought to significantly contribute to *C. trachomatis* pathogenicity [41, 8, 9]. The *C. trachomatis* organisms initiate their intracellular infection by invading epithelial cells in the form of elementary bodies (EBs). Once internalized, the intracellular EBs rapidly differentiate into reticulate bodies (RBs) for replication. The progeny RBs have to differentiate back into EBs for exiting the infected cells and spreading to new cells. The intracellular life of *C. trachomatis* organisms occurs strictly inside cytoplasmic vacuoles termed inclusions, during which, the intra-inclusion chlamydial organisms secrete numerous proteins into host cell cytoplasm [2, 43], including a unique serine protease designated as chlamydial proteasome/protease-like activity factor or CPAF [44, 23]. However, the function and significance of these secreted proteins in chlamydial biology and pathogenesis remain largely unknown [14, 31, 36, 42, 30]. For example, CPAF was initially thought to degrade various intracellular targets for aiding in chlamydial evasion of host defense [46, 43, 45]. However, a recent study has shown that degradation of the intracellular targets may be an artifact produced during sample processing [4]. Then, what is the authentic target(s) of CPAF if not intracellular molecules?

It is known that *Chlamydia trachomatis* organisms can ascend from lower to upper genital tracts and cause upper genital tract pathologies. Although it is obvious that
ascending infection may require chlamydial organisms to spread from cells to cells, the
precise mechanisms of ascending infection remain unknown. It has been recently
reported that *C. trachomatis* organisms can spread via either cell lysis or inclusion
extrusion mechanisms [24]. In both cases, *C. trachomatis* organisms have to undergo
an extracellular phase and expose to mucosal extracellular environments where
abundant host immune effectors such as antimicrobial peptides can readily attack
chlamydial organisms. For example, it has been shown that various antimicrobial
peptides including the cathelicidin LL-37 can potently inhibit chlamydial infection and
antimicrobial peptides are produced in response to chlamydial infection [34, 22]. The
fact that *C. trachomatis* can ascend from lower to upper genital tracts suggests that *C.
trachomatis* has possessed the ability to overcome the extracellular immune effectors in
the urogenital tract mucosa. The question is whether *C. trachomatis* can use CPAF
molecules pre-stored in the cytoplasm of the infected cells to proteolytically target the
extracellular defense effector molecules.

Human antimicrobial peptides represent a large group of cationic peptides that
possess antimicrobial activities, including human alpha-defensins (HADs) or human
neutrophil peptides (HNPs), human beta-defensins (HBDs) and cathelicidin LL-37 [28, 1,
20, 10]. LL-37 is a C-terminal 37 amino acid peptide starting with double leucines (LL)
processed from human cationic antimicrobial protein 18 (hCAP, 18kDa) that contains a
highly conserved amino terminal cathelin-like domain and a variable carboxyl terminal
domain [28, 20]. HNPs are mainly produced by neutrophils while HBDs by epithelial
cells. Interestingly, both epithelial cells including those in the genital tract and the
recruited neutrophils can produce and release LL-37. These extracellular antimicrobial
peptides possess a broad spectrum of antibacterial activity by inducing pore formation in bacterial membrane and represent a powerful first line of defense [27, 20]. The strong antibacterial activity of antimicrobial peptides has selected many bacterial species to evolve countermeasures for evading the antimicrobial peptides [12, 35, 39]. For example, LL-37 is targeted for degradation by SufA, a serine protease of *Finegoldia magna* [25], streptopain of *S. pyogenes*, elastase of *P. aeruginosa*, gelatinase of *Enterococcus faecalis* and the 50 kDa metalloprotease (ZapA) of *Proteus mirabilis* [39].

The *C. trachomatis* organisms produce a unique serine protease CPAF that is further secreted into and accumulated in the cytoplasm of the infected cells at the late stage of chlamydial intracellular growth [44, 23]. Since *C. trachomatis* organisms have to overcome antichlamydial activity of extracellular antimicrobial peptides in the genital tract mucosal tissues during ascending infection, we hypothesize that CPAF stored in the cytoplasm of Chlamydia-infected cells may be released to degrade extracellular antimicrobial peptides before the intra-inclusion organisms are exposed to the extracellular environment during host cell lysis and chlamydial spreading. To test the hypothesis, we re-evaluated the antichlamydial activity of 8 human antimicrobial peptides and found that LL-37 displayed the strongest ability for inhibiting chlamydial infection. Then, we evaluated the effect of CPAF on LL-37 antichlamydial activity and found that CPAF can potently block the LL-37 antichlamydial activity and restore chlamydial infection. We further found that the blockade was due to CPAF’s ability to efficiently degrade LL-37. Thus, we have revealed a novel mechanism on how *C. trachomatis* organisms can use the serine protease CPAF pre-stored in the host cell
cytoplasm for evading extracellular immune effectors, which may benefit chlamydial spreading and promote chlamydial pathogenicity.

2. Materials and methods

2.1. Cell Culture and Chlamydial Infection

HeLa cells (human cervical carcinoma epithelial cells, ATCC cat# CCL2) and C. trachomatis L2/LGV-434/Bu and serovar D/UW3 organisms (both from ATCC) were used in the current study. The chlamydial organisms were propagated, purified, aliquoted and stored as described previously [44]. For infection, HeLa cells grown in either multiple well plates/tissue culture dishes/flasks were inoculated with chlamydial organisms as described previously [44]. The cultures were processed for immunofluorescence assays as described below.

2.2. Immunofluorescence assay

The immunofluorescence assay was carried out as described previously [19]. C. trachomatis-infected HeLa cells grown on coverslips in 24 well plates were fixed with 2% paraformaldehyde (Sigma, St. Luis, MO) dissolved in PBS for 1h at room temperature, followed by permeabilization with 2% saponin (Sigma) for an additional 1h. After washing and blocking, the cell samples were subjected to antibody and chemical staining. Hoechst (blue, Sigma) was used to visualize DNA. A rabbit anti-chlamydial organism antibody (raised with C. trachomatis D organisms, unpublished data), plus a goat anti-rabbit IgG secondary antibody conjugated with Cy2 (green; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was used to visualize chlamydial
inclusions under a fluorescence microscope. For quantitating chlamydial infection, five random views were counted from each coverslip and the results were expressed as average number of IFUs per view.

2.3. IC50 Titration

To obtain IC50 (minimal concentrations required for inhibiting 50% chlamydial infection) for human antimicrobial peptides, each peptide was serially diluted and incubated with chlamydial organisms in 200µl of SPG at room temperature for 2h. The incubation mixtures were then inoculated onto HeLa cell monolayer. Forty-eight hours after infection, chlamydial inclusions were visualized using an immunofluorescence assay as described above. The chlamydial infection was quantitated by counting 5 random views for each coverslip and the results were expressed as number of inclusion forming units (IFUs) per view. Based on the IFUs per view and concentrations of antimicrobial peptides used, a 50% of inhibition concentration was calculated for each antimicrobial peptide using an online IC50 calculation software (http://www.dxy.cn/bbs/topic/2987601). The titration was repeated 3 to 4 times with duplicates in each experiment. The final IC50 for each antimicrobial peptide was expressed as mean plus/minus standard deviation. For titrating IC50 of antimicrobial peptides against *E. coli*, serially diluted antimicrobial peptides were mixed with *E. coli* XL1-Blue (Stratagene, La Jolla, CA) in 100µl of PBS with shaking at 37°C for 2h. The incubation mixtures were plated on LB plates. The *E. coli* was pre-titrated so that the amount of *E. coli* used in the IC50 titration can form about 500 to 1000 colonies per plate in the absence of any antimicrobial peptides. After overnight culture at 37°C, the
number of colonies per plate was counted and the concentration of each antimicrobial peptide required for achieving 50% inhibition was calculated. As with the titration of antichlamydial activity, the anti-*E. coli* activity was repeated 3 to 4 times with duplicates in each experiment and the final results were expressed as mean plus/minus standard deviation. The following antimicrobial peptides were used in the current study: HNP1 (human neutrophil peptide 1 or human alpha-defensin 1, cat# 60743), HNP2 (cat# 60744, both from AnaSpec, Fremont, CA), HNP3 (cat# PDF-4416-s), HBD1 (human beta-defensin 1, cat# PDF-4337-s), HBD2 (cat# PDF-4338-s), HBD3 (cat# PDF-4382-s) & HBD4 (cat# PDF-4406-s, all 5 are from Peptides International, Louisville, Kentucky) and LL-37 (cat# 61302 with a sequence of LLGDFFRKSKKEKIGKEFKRIVQRIKDFLRNLVPRTES, AnaSpec or Peptide 2.0, Chantilly, VA).

### 2.4. Cell-free pre-incubation assay

A cell-free pre-incubation assay was used to measure both CPAF blockade of antichlamydial activity and CPAF degradation of antimicrobial peptides. Recombinant wild type (WT) CPAF or mutant CPAF carrying substitution of the catalytic residue glutamic acid (E) at the position of 558 with alanine (E558A, ref: [5, 23]) at varying concentrations were pre-incubated with each antimicrobial peptide in SPG {sucrose-phosphate-glutamic acid buffer, containing (per liter) 75g of sucrose, 0.52g of KH2PO4, 1.22g of Na2HPO4 & 0.72g of glutamic acid} or PBS (phosphate-buffered saline solution, pH 7.4) at 37°C for various periods of time as indicated in individual experiments. For blocking antichlamydial activity of antimicrobial peptides, the
pre-incubation was in 200µl SPG and each pre-incubation mixture was used to treat chlamydial organisms prior to infecting HeLa cells. For monitoring the degradation of antimicrobial peptides, the pre-incubation was in 10µl PBS and the pre-incubation mixtures were loaded onto a 17% SDS polyacrylamide gel. After electrophoresis separation, the gel was stained with a Coomassie blue dye (Sigma, MO) for visualizing protein bands.

3. Results

3.1. Antichlamydial activity of human antimicrobial peptides

We compared 8 human antimicrobial peptides including alpha-defensin-1 to 3, beta-defensin-1 to 4 and the cathelicidin LL-37 for their ability to inhibit chlamydial infection. When *C. trachomatis* serovar L2 organisms were pre-incubated with increasing amounts of LL-37, the infectivity was significantly decreased (Fig. 1). Using this infection assay, we titrated the 50% inhibition concentrations (IC50) for each of the 8 human antimicrobial peptides against both *C. trachomatis* serovar D an L2 organisms (Table 1). As a control, the IC50 of each antimicrobial peptide against *E. coli* was also measured. We found that the 3 alpha-defensins that are mainly produced by neutrophils but not urogenital epithelial cells displayed the weakest antichlamydial activity with an IC50 of from ~60 (alpha-defensin 2 against L2) to >100µg/ml (alpha-defensin 1 against both serovars D & L2). Among the 4 beta-defensins, we found that beta-defensin 3 had the strongest antichlamydial activity with an IC50 from ~7 (against L2) to ~16µg/ml (against D) followed by beta-defensin 2 with an IC50 of ~28 (against L2) to ~70µg/ml (against D) while the IC50s of beta-defensins 1 & 4 were >100µg/ml against either L2 or
D organisms. These observations are consistent with previous findings that human beta-defensin 3 but not beta-defensin 4 was inducible in genital tract epithelial cells in response to infection [26] and human beta-defensin 3 in cervico-vaginal secretion exhibited its antimicrobial activity via multiple mechanisms. Interestingly, the strongest antichlamydial activity was found with the cathelicidin family only member LL-37 peptide with an IC50 of ~5 (against L2) and ~10µg/ml (against D) respectively. This observation is supported by the concept that LL-37 is both produced by mucosal epithelial cells and neutrophils recruited into the mucosal tissues and plays a critical role in mucosal immunity [11, 17]. As a control, all 8 antimicrobial peptides exhibited an anti-\textit{E. coli} activity with an IC50 of ~18µg/ml for LL-37 and ~0.4 to ~3µg/ml for beta-defensins. Together, we have demonstrated that extracellular antimicrobial peptides especially LL-37 can potently inhibit chlamydial infection, suggesting that the antimicrobial peptides may play an important role in controlling chlamydial spreading by attacking the organisms exposed to extracellular environment.

3.2. CPAF blocks the antichlamydial activity of host antimicrobial peptides

Since LL-37 is known to target by other bacterial proteases [25, 39] and CPAF is a serine protease secreted out of the chlamydial organisms and into host cell cytoplasm, we tested whether CPAF can block the antichlamydial activity of LL-37 (Fig. 2). We found that prior incubation of LL-37 with CPAF led to a complete blockade of the LL-37 antichlamydial activity. The blockade effect was observed when 1µg/ml of CPAF (equivalent to 0.014µM; The molecular weight of CPAF is ~70kDa) was mixed with 30µg/ml of LL-37 (equivalent to 7.5µM; The molecular weight of LL-37 is ~4kDa).
blockade peaked when CPAF was used at a final concentration of 10µg/ml (0.14µM).

Clearly, a CPAF molecule could block multiple molecules of LL-37, suggesting the involvement of proteolysis. Indeed, a mutant CPAF with an alanine substitution of the catalytic residue glutamic acid at the position of 558 (E558A) failed to block the antichlamydial activity of LL-37 even when CPAF(E558A) was used at a final concentration of 100µg/ml.

3.3. CPAF selectively degrades antimicrobial peptides with an antichlamydial activity.

We showed above that the powerful blockade of LL-37 antichlamydial activity by CPAF might depend on CPAF’s proteolytic activity. We next evaluated CPAF’s ability to degrade host antimicrobial peptides (Fig. 3A). We found that when CPAF was incubated with antimicrobial peptides for 30min, 0.1µg CPAF completely degraded 0.1µg LL-37, indicating that it only takes 30min for every one CPAF molecule to degrade 17.5 LL-37 molecules (since the molecular weight of CPAF is 17.5 times larger than that of LL-37). However, CPAF failed to significantly degrade any other antimicrobial peptides under the same experimental condition. Since LL-37 had the strongest antichlamydial activity among all antimicrobial peptides tested (table 1), the above observations support the concept that CPAF may selectively target host effectors with the strongest antichlamydial activity. When the incubation was extended to 2 hours, portions of beta-defensins 2 & 3 as well as alpha-defensin 2 or HNP2 (human neutrophil peptide 2) but not other defensin molecules were partially degraded by 100µg/ml of CPAF. Degradation of these three defensins by CPAF seemed to correlate with their
antichlamydial activity (Table 1), which further strengthens the concept that CPAF preferentially degrades antimicrobial peptides with antichlamydial activity. When the degradation time was shortened to 10 min, 0.16 µg of CPAF degraded >50% of 0.1 µg LL-37 and 0.32 µg of CPAF completely degraded all 0.1 µg LL-37 (Fig. 3B), demonstrating the extreme efficiency and capacity of CPAF for degrading LL-37. The CPAF mediated-degradation was specific since mutant CPAF(E558A) failed to degrade any significant amounts of antimicrobial peptides under all experimental conditions tested.

4. Discussion

In the current study, after confirming that human antimicrobial peptides especially the cathelicidin LL-37 possess a potent antichlamydial activity, we have provided the first experimental evidence that the *C. trachomatis* serine protease CPAF that is secreted into and accumulated in the cytoplasm of the infected cells at the late stage of chlamydial intracellular growth [44] can potently block the antichlamydial activity of the extracellular antimicrobial peptides by proteolytically targeting these peptides. The chlamydial ability to unleash active CPAF molecules pre-stored in the host cell cytoplasm to rapidly confront extracellular antimicrobial peptides upon cell lysis during chlamydial spreading may represent a novel mechanism for promoting *C. trachomatis* ascending infection.

*C. trachomatis* organisms can only safely stay inside the initially infected epithelial cells until the infected cells are dislodged from the epithelial tissues and flushed out of the host. Thus, to maintain longevity in the infected host, *C. trachomatis*
organisms must be able to spread from cell to cell, which inevitably requires the organisms to expose to the extracellular environments where immune effectors such as antimicrobial peptides are readily available for attacking the chlamydial organisms. It is known that *C. trachomatis*-infected epithelial cells can release cytokines to both recruit inflammatory cells including neutrophils to the site of infection and activate adjacent cells to secrete more cytokines [9, 38]. Neutrophils were detected underneath the *C. trachomatis*-infected cells and thought to help push the infected cells to dislodge from the epithelial tissue [37]. Although neutrophils may fail to phagocytose the organisms that are still hidden inside epithelial cells, neutrophils can secrete cytokines and effector molecules such as antimicrobial peptides to create a hostile extracellular environment for *C. trachomatis* organisms. Clearly, *C. trachomatis* organisms have to overcome the antichlamydial activity of extracellular effectors in order to spread from cell to cell, which is likely required for ascending infection. The question is what molecular mechanisms *C. trachomatis* organisms have evolved for evading the extracellular immune effectors.

Many bacterial species are known to secrete various proteases to the surface of the bacteria or out of the bacteria for degrading antimicrobial peptides [12, 25, 35, 39]. The intra-inclusion *C. trachomatis* organisms are known to secrete a serine protease designated as CPAF into host cell cytoplasm [44]. In fact, CPAF is accumulated in large quantity in the cytoplasm of the infected cells prior to lysis of the infected cells [44]. We hypothesize that the pre-stored CPAF in the host cell cytoplasm may be rapidly released to proteolytically eliminate the extracellular antimicrobial peptides upon cell lysis before the intra-inclusion organisms are exposed to the extracellular environments. This hypothesis is supported by the followings: First, CPAF selectively targets
antichlamydial peptides with an antichlamydial activity. Among all 8 antimicrobial peptides tested, LL-37 displayed the strongest antichlamydial activity and was most efficiently degraded by CPAF. We found that one CPAF molecule could degrade multiple copies of LL-37 peptides within a short period of time, suggesting that CPAF molecules released from the infected cell cytoplasm have the capacity to rapidly clear a safe extracellular passage for chlamydial organisms to spread to the next target cells. The CPAF degradation of LL-37 was specific since the mutant CPAF(E558A) failed to degrade any significant amount of LL-37. Second, CPAF accumulation in the host cell cytoplasm is more obvious with productive infection [44, 21], suggesting that the increased storage of CPAF in host cell cytoplasm may be aimed for targeting host extracellular effectors when the productive infection results in cell lysis. Third, mature CPAF is stable and has a long half-life [15, 16, 23], suggesting that active CPAF can survive the mucosal extracellular environments once released from the infected cells. Fourth, although CPAF was initially thought to target various intracellular molecules, a recent report demonstrated that CPAF degradation of intracellular molecules might occur during sample processing [4], suggesting that the authentic substrates of CPAF may lie elsewhere, including immune effectors in the extracellular environment. This assumption is consistent with the fact that CPAF is a late expression gene and the protein is accumulated in the late stage of chlamydial intracellular infection. Proteins encoded by late genes are thought to benefit the next infection cycle. Thus, CPAF may play a role in promoting chlamydial cell-to-cell spreading. Finally, this hypothesis is also consistent with the previous findings that immunization of mice with CPAF can induce
partial protection against chlamydial challenge infection [33] and antibodies from women urogenitally infected with *C. trachomatis* neutralized CPAF activity [40].

We are aware that although we have presented experimental evidence for CPAF neutralization of the antichlamydial activity of the host AMP LL-37, these *in vitro* observations will have to be validated in animal models, which is underway.

**Conflict of interest:** The authors declare no conflict of interest.

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**References**


**Figure legends and Table note**

**Fig. 1. Antichlamydial activity of human antimicrobial peptide LL-37.** The *C. trachomatis* LGV2 organisms were treated without (panel a) or with LL-37 at the concentrations of 5 (b), 30 (c) or 90µg/ml (d) as listed on top of the figure. The organisms were then used to infect HeLa cell monolayer grown on coverslips in 24 well plates. Forty-eight hours after infection, the cultures were processed for immunofluorescence labeling with an antichlamydial organism antibody (green) and Hoechst DNA dye (blue). The number of inclusions was counted and the results were summarized in table 1. Note that LL-37 at 5µg/ml significantly inhibited chlamydial infection.

**Fig. 2. Blocking antichlamydial activity of LL-37 by CPAF.** (A) The infection conditions were the same as described in Fig. 1 legend except that the LL-37 peptide was pre-incubated without (panel b) or with wild type (WT) CPAF at the concentrations of 1 (c), 10 (d) or 100µg/ml (e) or mutant CPAF(E558A) at 100µg/ml (f) prior to LL-37 treatment of chlamydia organisms. Note that the *C. trachomatis* LGV2 infection (a) was severely inhibited by 30µg/ml of LL-37 (b). However, this inhibition was reversed by pre-incubation of LL-37 with wild type CPAF (c to e) but not mutant CPAF (f). The images
were taken at 200X amplification. (B) Both *C. trachomatis* serovars D (solid bar) and LGV2 (open) were used for evaluating the CPAF-mediated blockade of LL-37 antichlamydial activity as described Figs. 1 & 2A legends above. The inclusions were counted under 600X amplification for 5 random views per coverslip and the results were expressed as number of inclusions per view. The data presented came from 3 to 4 independent experiments. Note that CPAF at 10µg/ml completely blocked the antichlamydial activity of LL-37.

**Fig. 3. CPAF degradation of antimicrobial peptides.** (A) Eight human antimicrobial peptides including LL-37 (panel a), α-defensin 1 or human neutrophil peptide 1 (HNP1, b), HNP2 (c), HNP3 (d), human β-defensin 1 (HBD1, e), HBD2 (f), HBD3 (g) or HBD4 (h) were each used as substrate (0.1µg) for degradation by varying amounts of CPAF for 30 or 120 min in a total volume of 10µl. Each mixture was loaded to the corresponding lane of a 17% SDS polyacrylamide gel for visualizing the remaining peptides via Coomassie blue staining. Note that 0.1µg of wild type CPAF completely degraded 0.1µg LL-37 within 30min while 1µg of mutant CPAF with substitution of the catalytic residue glutamic acid (E) at the position of 558 with alanine (E558A) failed to degrade any LL-37 even after 120 min incubation. The wild type CPAF also partially degraded HNP2, HBD2 & HBD3 but not other defensin molecules after 120 min incubation. (B) CPAF was 2 fold serially diluted for further titrating its ability to degrade LL-37. The wild type CPAF at 0.16µg degraded >50% while at 0.32µg degraded all of the 0.1µg LL-37 within 10min. The inactive mutant CPAF and active CPAF N-terminal (CPAFn) and C-terminal (CPAFc) fragments as well as residual antimicrobial peptides
visualized with the Coomassie blue dye were marked on the right of the figure. Partial or complete degradation of antimicrobial peptides was marked with single or double stars respectively.

Table 1. Anti-\textit{C. trachomatis} and anti-\textit{E. coli} activities of antimicrobial peptides

A total of 8 human antimicrobial peptides were titrated for their ability to inhibit growth of \textit{C. trachomatis} serovars D and L2 and \textit{E. coli}. The concentrations of antimicrobial peptides required for inhibiting 50\% of microbial growth (IC50) were determined for each antimicrobial peptide against each type of organisms. The data expressed as µg/ml in the format of mean plus/minus standard deviation came from 3 to 4 independent experiments.

<table>
<thead>
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<th>\textit{E. coli}</th>
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<td></td>
<td>\textit{D}</td>
<td>\textit{L2}</td>
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<tr>
<td>Cathelicidin</td>
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<tr>
<td>α-defensins</td>
<td>HNP1</td>
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<td></td>
<td>HNP2</td>
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<tr>
<td></td>
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<td>&gt;100</td>
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<td>&gt;100</td>
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<td>HBD2</td>
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Fig. 1, Tang et al
A

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<th>10</th>
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<td>30</td>
<td>30</td>
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<td>100 µg/ml</td>
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B

![Bar graph showing the number of inclusions per view](image)

Infection: D L D L D L D L D L

LL-37(µg/ml): - 30 - 30 - 30 - 30 - 30 - 30

CPAF(µg/ml): - - 1 10 100 100 100 100

CPAF (WT) CPAF (E558A)