Activity of Cathelicidin Peptides against *Chlamydia* spp.

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The in vitro activity of six cathelicidin peptides against 25 strains of *Chlamydia* was investigated. SMAP-29 proved to be the most active peptide, reducing the inclusion numbers of all 10 strains of *Chlamydia trachomatis* tested by ≥50% at 10 μg/ml. This peptide was also active against *C. pneumoniae* and *C. felis.*

Members of the genus *Chlamydia* are obligate intracellular bacteria that can cause both human and animal diseases (15). There are three commonly recognized species infecting humans: *Chlamydia trachomatis,* *C. pneumoniae,* and *C. psittaci.* The first causes genital tract infections and is associated with neonatal conjunctivitis and pneumonia, the second causes acute respiratory infections and has been associated with cardiovascular diseases (11), and the third is a pathogen of birds and lower animals that infects humans only occasionally. Other chlamydial species are almost exclusively animal pathogens.

Several studies have suggested that polymorphonuclear leukocytes play an essential role in the response to chlamydial infections, and Register et al. (16) previously reported that granular protein extracts from these cells inactivated *C. trachomatis* and *C. psittaci.*

The antimicrobial peptides stored by mammalian leukocytes include defensins and cathelicidins (8). Peptides of the latter group are heterogeneous in size and sequence and exhibit marked structural diversity (10). They include linear peptides and disulfide-bridged cyclic peptides (1). In general, cathelicidin peptides display a potent and broad-spectrum activity and exert a protective effect in animal models of infection (2, 3, 10, 20).

Although their antimicrobial activity against bacteria, fungi, and protozoa has been extensively tested (2, 10, 17, 21), chlamydialiae have not been systematically tested. In this study, we therefore investigated the in vitro activity of six cathelicidin peptides against 25 strains of chlamydialiae of human and animal origin.

The strains we investigated included 10 isolates of *C. trachomatis,* 5 of *C. pneumoniae,* and 10 of chlamydial species infecting animals. *C. trachomatis* included strains of the serovars A, D, E, H, I, and LGV2 and four untyped isolates from urethral swabs of male patients with nongonococcal urethritis (5). *C. pneumoniae* comprised three recent isolates (13) and two reference strains, IOL-207 and CM-1. The 10 animal isolates included the reference strain 6BC of *C. psittaci* (parakeet) and 9 isolates (4 *C. felis,* 1 *C. abortus,* 3 *C. psittaci,* and 1 *C. pecorum*) from infected animals. All bacterial strains were grown in LLC-MK2 cells (6) on 24-well plates containing glass coverslips. Chlamydial elementary bodies (EBs) were purified by use of sucrose gradients (14), resuspended in 0.2 M sucrose-phosphate-glutamic acid (SPG), and frozen in 0.5-ml aliquots at −70°C.

The six cathelicidin peptides—SMAP-29 from sheep (18); LL-37 from humans (1); BMAP-27, BMAP-28, and Bac7(1-35) from cattle (7, 19); and PG-1 from pigs (12)—were chemically synthesized, purified by reversed-phase high-performance liquid chromatography, and characterized by electrospray ionization mass spectrometry as previously reported (18, 19). The purified peptides were lyophilized in 10 mM HCl and stored at 4°C. Stock solutions of each peptide at 1 g/liter were prepared in phosphate-buffered saline, pH 7.4, and stored frozen in 20-μl aliquots at −80°C until used. To determine the lowest peptide concentration required to achieve ≥50% reduction of chlamydial inclusions with respect to untreated controls, individual peptides were diluted twofold with SPG, from 80 to 2.5 μg/ml, in a volume of 0.15 ml in polypropylene tubes. An equal volume of 10° inclusion-forming units (IFU) per ml of purified chlamydial EBs in SPG medium was then added. After incubation at 23°C for 2 h, an aliquot of 0.1 ml from each sample was inoculated in triplicate onto LLC-MK2 cells grown on 24-well plates. The plates were centrifuged at 800 x g for 1.5 h at 33°C and then incubated for 72 h at 35°C, after replacement of the cell medium with 1 ml of chlamydial growth medium (6). After incubation, the cell cultures were fixed and stained for detection of inclusions by immunofluorescence (5). The number of IFU per coverslip was counted in 40 microscopic fields using a Zeiss UV microscope at a magnification of ×400. The data reported are the means of results from three independent experiments.

The activities of cathelicidin peptides against *Chlamydia* spp. are reported in Table 1. *C. trachomatis* was the most sensitive to peptides among all the species tested, and SMAP-29 was the most active peptide. In comparison with untreated controls, this compound reduced by ≥50% the inclusion numbers of all 10 strains tested at a concentration of 10 μg/ml. BMAP-27, BMAP-28, and Bac7(1-35) had a similar effect at 80 μg/ml. At this concentration LL-37 was ineffective, while PG-1 was active against *C. trachomatis* serotypes D, H, and LGV2.
microscopy of SMAP-29-treated *C. trachomatis* EBs indicates that the peptide causes loss of integrity of most of the particles (data not shown).

*C. pneumoniae* strains were less susceptible to peptides than *C. trachomatis*. SMAP-29 reduced by \( \geq 50\% \) the numbers of IFUs of all five *C. pneumoniae* strains tested at 10 \( \mu \)g/ml. The other peptides did not exert any inhibitory effect, even at 80 \( \mu \)g/ml. Animal chlamydial were not sensitive to the concentration of 80 \( \pm 2 \) \( \mu \)g/ml.

Only a few studies on the antichlamydial activity of cathelicidin peptides have been produced (4, 22, 23, 24). In all these studies, the effects of mammalian peptides (protegrins, \( \alpha \)-defensins, indolicidin, and a rhesus \( \theta \)-defensin) and of a model peptide (Novispirin G-10) were tested against *C. trachomatis*. In the present study we comparatively analyzed the action of six cathelicidin peptides against 25 strains of several *Chlamydia* spp. Chlamydiala showed a different susceptibility to the peptides, with *C. trachomatis* resulting the most susceptible. PG-1, the only cathelicidin peptide in the present study that was previously tested with chlamydiae, showed activity against *C. trachomatis* serovars D, H, and LGV2, thus confirming previous results (22, 24).

SMAP-29 proved to be the most active cathelicidin peptide against several bacterial species (10, 18). The present study confirms those observations also for chlamydiae. It also adds *Chlamydia* spp. among those susceptible to Bac7, reinforcing the notion that this peptide is mainly active against gram-negative bacteria (9).

Previous studies and the present one suggest that cathelicidin peptides have substantial in vitro activity against *C. trachomatis* and *C. pneumoniae*. Studies in animal models of chlamydial infection are needed to prove whether they will parallel in vitro results and indicate that these peptides are useful lead compounds for developing antichlamydial drugs.

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**REFERENCES**


