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 \(\geq 50\%\) at 10 \(\mu\)g/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 pneumonitis, 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), chlamydial inclusions with respect to untreated controls, individual peptides were diluted twofold with SPG, from 80 to 2.5 \(\mu\)g/ml, in a volume of 0.15 ml in polypropylene tubes. An equal volume of *Chlamydia* 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 \(\times\) 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).

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 \(\geq 50\%\) the inclusion numbers of all 10 strains tested at a concentration of 10 \(\mu\)g/ml. BMAP-27, BMAP-28, and Bac7(1-35) had a similar effect at 80 \(\mu\)g/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 ≥50% the numbers of IFUs of all five *C. pneumoniae* strains tested at 10 μg/mL. The other peptides did not exert any inhibitory effect, even at 80 μg/mL. Animal chlamydiae were not sensitive to the concentration of cathelicidins tested, with the exception of the four *C. felis* isolates, which were affected by SMAP-29 and Bac7(1–35) at 80 μg/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, cationic peptides) have been produced (4, 22, 23, 24). In all these studies, the effects of mammalian peptides (protegrins, cationic peptides) have been produced (4, 22, 23, 24).

### TABLE 1. Activity of cathelicidin peptides against *Chlamydia* spp. \(^a\)

<table>
<thead>
<tr>
<th>Peptide</th>
<th><em>C. trachomatis</em> (strains (n = 10))</th>
<th><em>C. pneumoniae</em> (strains (n = 5))</th>
<th>Animal Chlamydia spp. (strains (n = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bac7(1–35)</td>
<td>80 (19) ± 1.4</td>
<td>&gt;80 (19)</td>
<td>&gt;80 (19) (^b)</td>
</tr>
<tr>
<td>SMAP-29</td>
<td>80 (3) ± 0.0</td>
<td>80 (3) ± 0.0</td>
<td>&gt;80 (19) (^b)</td>
</tr>
<tr>
<td>BMAP-27</td>
<td>80 (25) ± 2.6</td>
<td>80 (25) ± 2.6</td>
<td>&gt;80 (25)</td>
</tr>
<tr>
<td>BMAP-28</td>
<td>80 (26) ≥ 1.9</td>
<td>&gt;80 (26)</td>
<td>&gt;80 (26)</td>
</tr>
<tr>
<td>LL-37</td>
<td>&gt;80 (18)</td>
<td>&gt;80 (18)</td>
<td>&gt;80 (18)</td>
</tr>
<tr>
<td>PG-1</td>
<td>&gt;80 (18)</td>
<td>&gt;80 (18)</td>
<td>&gt;80 (18)</td>
</tr>
</tbody>
</table>

\(^a\) Results are shown as means ± standard errors of the means (three replications).

\(^b\) Four *C. felis* strains were susceptible to Bac7(1–35) and to SMAP-29 at a concentration of 80 ± 2.5 μg/mL.

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**REFERENCES**


