Loss of ciliary activity in organ cultures of rat trachea treated with lipo-oligosaccharide from *Haemophilus influenzae*

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Summary. Organ culture of rat trachea was used as an experimental model to examine the ability of lipo-oligosaccharide from *Haemophilus influenzae* to damage respiratory tract mucosal tissue. Lipo-oligosaccharide from two strains of *H. influenzae* produced a significant decrease in the ciliary activity of tracheal rings observed over a 3–5 day period. No loss of ciliary activity was observed with the lipid-free moiety of the lipo-oligosaccharide.

Introduction

Haemophilus influenzae is the commonest cause of bacterial meningitis in infants and young children and may also cause other serious systemic infections in this age group. It is also associated with exacerbations of chronic bronchitis in adults (Turk, 1982). Despite the importance of *H. influenzae* as a human pathogen, its pathogenicity is incompletely understood. To increase our understanding of this, several experimental systems, including an animal model of meningitis (Moxon *et al.*, 1974) and the use of organ cultures of respiratory mucosa inoculated with *H. influenzae* (Denny, 1974; Johnson *et al.*, 1983) have been developed. With the latter system it was shown that ciliary activity in cultures of tracheal mucosa inoculated with *H. influenzae* was lost because of sloughing of epithelial cells (Denny, 1974; Johnson *et al.*, 1983). It was also observed that sterile supernates from infected organ cultures contained a soluble, heat-stable factor that caused loss of ciliary activity when transferred to fresh organ cultures (Denny, 1974). Although the heat-stable toxic factor was not identified, Denny speculated that it might be *H. influenzae* endotoxin. In the present study, the concept that *H. influenzae* endotoxin might be capable of damaging tracheal mucosa has been investigated by determining the effect that purified lipo-oligosaccharide from *H. influenzae* has on the ciliary activity of organ cultures of rat trachea.

Materials and methods

Lipo-oligosaccharide

Lipo-oligosaccharide (LOS) was extracted from *H. influenzae* strains 1060 and S-2. Strain 1060 was isolated from a child with meningitis at the Texas Children's Hospital, Houston, TX. Strain S-2 is a capsule-deficient mutant of strain Eagan (Moxon *et al.*, 1984). The LOS was isolated from each strain by enzyme digestion and phenol-water extraction (Johnson and Perry, 1976; Inzana, 1983). Lipid-free oligosaccharide was isolated from strain Eagan as described by Inzana *et al.* (1985). The samples of LOS and oligosaccharide were prepared in the laboratory of one of the authors (TJI) and after lyophilisation, were sent to APJ for assessment of their biological activity in rat trachea organ cultures.

Preparation of organ cultures of rat trachea

Young male and female specific-pathogen-free Sprague-Dawley or Lewis rats, bred at the Clinical Research Centre, were used. Tracheas were removed aseptically from animals by the method of Johnson *et al.* (1983) and placed in a petri-dish containing Eagle's minimal essential medium (MEM) buffered at pH 7.2 with 50 mM HEPES and supplemented with ampicillin 10 µg/ml. The MEM was prepared with sterile pyrogen-free water as described by Johnson *et al.* (1983). Each trachea was cut into a series of rings which were placed individually in tissue-culture tubes containing 1 ml of MEM and then incubated at 37°C on a roller drum (Cherry and Taylor-Robinson, 1970).

Treatment of organ cultures

In initial experiments, organ cultures were incubated

after inoculation, each of three tracheal rings treated with 25 µg/ml showed $\geq 50\%$ loss of ciliary activity, while each of three untreated organ cultures and two of three organ cultures treated with 2.5 µg/ml showed $\leq 10\%$ decrease in ciliary beating. Therefore in further studies with LOS from strain 1060 LOS was used at a concentration of 25 µg/ml. As shown in the figure (Aa), organ cultures of trachea from Sprague-Dawley rats treated with LOS showed a statistically significant decrease in ciliary activity when compared to untreated organ cultures after observation for 4 days ($p=0.016$). After these experiments, the unavailability of Sprague-Dawley rats meant that subsequent studies had to be performed with tracheas from Lewis rats. In one experiment, tracheal rings from a Lewis rat were found to be susceptible to damage by LOS from *H. influenzae* strain 1060; there was $> 60\%$ decrease in ciliary activity after observation for 4 days (data not shown). Thus it appeared that tracheal tissue from both Sprague-Dawley and Lewis rats was susceptible to the toxic effect of LOS.

A second group of experiments was then performed in which the effect of LOS from *H. influenzae* strain S-2 on the ciliary activity of tracheal rings was assessed. In these studies, the concentration of LOS was increased to 40 µg/ml in an attempt to shorten the length of time required for loss of ciliary activity to be observed. As in the experiments described above, organ cultures of rat trachea treated with LOS from strain S-2 showed a statistically significant decrease in ciliary activity in comparison to untreated control cultures ($p=0.002$) (figure, B). However, despite the use of a higher concentration of LOS, the time for loss of ciliary activity to become apparent (3–5 days) was similar to that seen in experiments with LOS from strain 1060.

Effect of lipid-free oligosaccharide on ciliary activity

One experiment was performed to test the effect of the oligosaccharide fraction of LOS from strain Eagan on ciliary beating. As shown in the figure (C), oligosaccharide at a concentration of 40 µg/ml had no significant effect on the ciliary activity of tracheal organ cultures up to 5 days after treatment. Six of eight tracheal rings treated with oligosaccharide showed no loss of ciliary beating and the other two rings retained 80% of their original activity.

Discussion

The results presented here show that LOS samples from two strains of *H. influenzae* can produce mucosal damage, manifest as loss of ciliary activity,

in organ cultures of rat trachea. These findings are consistent with the observation that *H. influenzae* produces a heat-labile, non-dialysable product that inhibits ciliary beating in cultures of rat trachea (Denny, 1974). Interestingly, another group of investigators, working with organ cultures of ciliated human nasal epithelium (Wilson *et al.*, 1985) have also recently observed that LOS from *H. influenzae* induces sloughing of epithelial cells (R. Wilson, personal communication).

These findings are consistent with the observation that LOS from *Neisseria gonorrhoeae* produces loss of ciliary activity in organ cultures of fallopian tube (Gregg *et al.*, 1981). The fact that LOS preparations from two different genera of bacteria are both toxic to ciliated mucosal surfaces suggests that there may be value in assessing the ciliostatic potential of LOS from other bacteria which colonise and damage mucosal surfaces, particularly those of the respiratory and upper genital tracts.

The mechanism by which LOS produces mucosal damage is not known, although the finding that gonococcal LOS is toxic for human but not non-human genital tissue (Gregg *et al.*, 1981) implies specific interaction between LOS and the epithelial surface. The finding that rat trachea is susceptible to the toxic effect of LOS from *H. influenzae* is not surprising because rats are known to be susceptible to infection with this organism (Moxon *et al.*, 1974). Studies with gonococcal LOS have indicated that the lipid A portion of the LOS molecule is involved in producing tissue damage (Gregg *et al.*, 1981). The present finding that the oligosaccharide moiety of LOS from *H. influenzae* is non-toxic adds further weight to the idea that lipid A is the active component, although it would be premature to state that the oligosaccharide portion of LOS has no role in the production of tissue damage.

If the ability of LOS from *H. influenzae* to damage ciliated mucosal tissue *in vitro* is also manifest *in vivo* it could constitute an important virulence factor. The mucociliary lining covering the mucosa of much of the respiratory tract provides an important non-specific host defence mechanism, by mechanically inhibiting micro-organisms from readily colonising this site. If LOS acted to reduce mucociliary clearance of organisms by the host it would enhance their ability to colonise and infect the respiratory tract. The sloughing of epithelial cells from the surface of the respiratory tract, which has been observed *in vitro* (Johnson *et al.*, 1983) might also provide a means by which *H. influenzae* can cross the respiratory epithelium to invade the submucosal tissue, with subsequent systemic spread.

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