Characterization of *Haemophilus parainfluenzae* Strains with Low- M_r or Ladder-like Lipopolysaccharides

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Twenty-five *Haemophilus parainfluenzae* strains were characterized for lipopolysaccharide (LPS) profiles, outer membrane protein profiles, serum sensitivity, plasmid profiles and DNA homology. Seventeen strains produced low- M_r LPS that did not contain O-sidechains, while the remaining eight strains contained ladder-like LPS suggestive of O-repeated units. This is the first time in the genus *Haemophilus* that LPS with O-repeated groups has been described. The strains producing the different types of LPS could not be distinguished from each other in outer membrane protein profiles or the other characteristics examined.

INTRODUCTION

Haemophilus parainfluenzae is ubiquitous on all surfaces in the human mouth and pharynx. It may be isolated from the vaginal mucosa and the genito-urinary tract, and has been reported to be detectable in about a quarter of faecal samples (Albritton, 1982; Kilian & Frederiksen, 1980). In most samples from the upper respiratory tract this species constitutes about two-thirds of the total haemophilus flora. On occasion these organisms can cause infection with a similar spectrum of illness as those due to *H. influenzae*, including epiglottitis, otitis media, cellulitis, pneumonia, endocarditis, meningitis (Albritton, 1982) and bacteraemia (Bruun *et al.*, 1984). *H. parainfluenzae* was originally defined on the basis of its requirement for NAD, and other biochemical characteristics. More recently three different biotypes were recognized on the bases of ornithine decarboxylase and urease activity (Kilian, 1976). All *H. parainfluenzae* isolates were originally described as indole-negative, but an indole-positive *H. parainfluenzae* has recently been described (Bruun *et al.*, 1984). Pohl (1980) studied the DNA relatedness among members of the genera *Haemophilus*, *Pasteurella* and *Actinobacillus* and reported that different strains classified as *H. parainfluenzae* were linked by DNA homology to different clusters within this family (the *Pasteurellaceae*). He suggested that the strains labelled *H. parainfluenzae* were a heterogeneous group and did not seem to be a natural species.

More recently, outer membrane protein profiles, plasmid profiles and LPS profiles have been used to distinguish between strains within *H. influenzae* (Inzana, 1983; Roberts *et al.*, 1980) and *H. ducreyi* (Odumeru *et al.*, 1983, 1984) and between species within the genus *Haemophilus*. Therefore, to characterize the species *H. parainfluenzae* further, 25 non-haemolytic, NAD-requiring, oxidase-positive, sucrose- and glucose-positive, porphyrin-producing, indole-

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negative bacteria identified as *H. parainfluenzae* were selected for examination of their LPS profiles, outer membrane protein profiles, serum sensitivity, plasmid profiles and DNA homology.

METHODS

Bacterial strains. The 25 strains used in the study are listed in Table 1.

Media. The media used for the growth of *H. parainfluenzae* have been described previously (Roberts *et al.*, 1980).

LPS extraction. LPS was extracted from plate-grown cells by a microadaptation of the phenol-water method (RIM) as described by Inzana (1984).

Outer membrane protein profiles. Outer membranes were prepared by sodium sarcosinate extraction (Filip *et al.*, 1973).

SDS-PAGE. Outer membrane proteins were run on gels according to the methods of Mietzner *et al.* (1984) and stained with Coomassie blue G250 (Miles Laboratories). LPS samples were analysed on linear gradient gels containing 8–18% (w/v) acrylamide and 0.1% sodium dodecyl sulphate as described previously (Mintz *et al.*, 1984). The gels were stained by the silver staining method of Tsai & Frasch (1982).

Preparation of [³H]thymidine-labelled whole-cell DNA. This was done as described previously (Roberts *et al.*, 1980).

Preparation of unlabelled whole-cell DNA, and DNA-DNA hybridization studies. Purified whole-cell DNA was prepared from broth-grown cultures as described previously (Roberts *et al.*, 1980). Sheared and denatured labelled DNA was reannealed with unlabelled whole-cell DNA extracted from representative strains. The hybridization was done as described previously (Roberts *et al.*, 1980).

Bactericidal assay. Susceptibility of *H. parainfluenzae* to killing by normal human serum was determined as described by Morse *et al.* (1983).

RESULTS AND DISCUSSION

Seventeen of the *H. parainfluenzae* strains produced LPS that contained multiple, low-*M_r* bands (rough LPS). The electrophoretic profiles contained between two and five bands (Fig. 1). Although similarities existed between the LPSs, each exhibited a distinct banding pattern. There was no evidence for the presence of an O-repeated unit in any of these LPSs. In contrast, the remaining eight strains produced LPS that appeared to contain O-side chains (smooth LPS) (Fig. 2). The ladder-like appearance of these LPS profiles is suggestive of differences in the number of O-repeat units in the nascent LPS molecules (Goldman & Lieve, 1980; Hitchcock & Brown, 1983). The banding pattern of LPS from each of the eight strains was observed each time the extractions were prepared and thus appeared to be a stable characteristic. To ensure that the observed banding pattern represented LPS rather than protein contamination, LPS samples were treated with Proteinase K (Sigma) for 60 min. The treatment did not alter the LPS banding pattern (Fig. 2).

Three of the eight strains (KC469, KC470, P136) producing smooth LPS and four other strains (P160, 78-2257, R740, KC471) carried typical R plasmids (> 20 Mda) found only in the genus *Haemophilus* (Roberts *et al.*, 1980). Two strains, RSF0855 and 79-23164, carried smaller (< 10 Mda) plasmids encoding TEM β -lactamase, and four strains carried small cryptic plasmids.

Two of the strains, KC469 and KC470, isolated in Atlanta, had identical LPS profiles but differed in biotype, plasmid profile and outer membrane protein pattern. Strains P131 and P136, both isolated in Vancouver, and the three Seattle isolates with smooth LPS, GU1, 1154 and 1175, differed in LPS patterns as well as other measurable characteristics.

Structural differences in LPS have been found to affect the protein composition of the outer membrane of several Gram-negative bacteria (Gmeiner & Schlecht, 1979). The outer membrane protein profiles of the *H. parainfluenzae* were similar to those of *H. influenzae* and *H. ducreyi* (Odumeru *et al.*, 1983) but could generally be distinguished from them. A diverse range of profiles was found (Fig. 3). There appeared to be no association between outer membrane protein profiles and LPS profile.

Differences in LPS structure may also influence the susceptibility of Gram-negative bacteria to killing by normal serum (Taylor, 1983; Odumeru *et al.*, 1984). Twenty-two of the twenty-five strains were resistant to killing (> 90% survival) in normal human serum. The three susceptible

Fig. 1

Fig. 2

Fig. 1. Silver-stained SDS-PAGE gel of *H. parainfluenzae* rough LPS. Lane 1, strain UB2832; lane 2, strain KC471; lane 3, strain R832.

Fig. 2. Silver stained SDS-PAGE gel of *H. parainfluenzae* smooth LPS. Lane 1, strain P131; lane 2, strain P131 treated with Proteinase K; lane 3, strain P136; lane 4, strain P136 treated with Proteinase K.

strains were the one cerebrospinal fluid isolate; and one throat and one sputum isolate (Table 1). We found no correlation between the type of LPS produced and apparent serum susceptibility.

Pohl (1980) suggested that the species *H. parainfluenzae* represents a genetically heterogeneous group, therefore it was of interest to examine some of the strains more closely to determine the genetic relationship between strains with smooth LPS and those with rough LPS. As probe strains we selected ATCC 7901 with a rough LPS, isolated in 1940 (one of the first strains of *H. parainfluenzae* described) and a recent isolate P136, with a smooth LPS. The use of two different LPS types, isolated 40 years apart, as probes would allow the genetic relationship between the two LPS types to be determined. The results are presented in Table 2. Seven of the eleven test strains had greater than 60% homology with ATCC 7901; in contrast, only four strains showed greater than 60% homology with P136. This heterogeneity is similar to that described by Pohl (1980). The four strains with the smooth LPS profiles did not seem to form a genetic cluster distinct from the other *H. parainfluenzae* strains in the study, suggesting that they do not represent a separate group within the species as it is presently defined. There also did not seem to be genetic clustering based on time of isolation in the strains examined.

One of the most interesting observations of our study on strains designated *H. parainfluenzae* is the presence in these bacteria of either a low- M_r or a ladder-like LPS. The low- M_r LPS found in *H. parainfluenzae* is similar to that described for *H. influenzae* (Inzana, 1983) and *H. ducreyi*

Table 1. *Strains of H. parainfluenzae*

Strain	Site of isolation*	Date	LPS type†	Serum sensitivity	Geographical location	Source‡
KC469	throat	1982	s	resistant	Atlanta, Ga.	W. Albritton
KC470	throat	1982	s	resistant		
KC471	throat	1982	R	resistant		
P131	throat	1981	s	resistant	Vancouver, BC	D. Scheifele
P136	throat	1981	s	resistant		
P160	throat	1981	R	resistant		
UB2832	throat	1975	R	sensitive	England	P. Cavanagh
78-2257	sputum	1979	R	sensitive	New Zealand	D. Anderson
79-23164	urethra	1979	R	resistant	Kuala Lumpur	M. Roberts
RSF0885	CSF	1974	R	sensitive	San Francisco	S. Falkow
ATCC 9796	throat	1940	R	resistant	New York, NY	ATCC
ATCC 7901	throat	1940	R	resistant		
R673	eye	1981	R	resistant	Seattle, Wa.	K. K. Holmes
R687	eye	1981	R	resistant		
R740	throat	1981	R	resistant	North Carolina	M. Roberts
R832	epiglottis	1982	R	resistant	Seattle, Wa.	M. Roberts
5F9	throat	1983	R	resistant	Buffalo, NY	M. Apicella
82602	throat	1983	R	resistant		
9460	throat	1983	R	resistant		
4922	throat	1983	s	resistant	Seattle, Wa.	K. K. Holmes
GU1	urethra	1984	s	resistant		
1154	urethra	1979	s	resistant		
1166	urethra	1980	R	resistant		
1170	urethra	1980	R	resistant		
1175	urethra	1980	s	resistant		

* CSF, cerebrospinal fluid.

† s, smooth, ladder-like LPS pattern; R, rough, single to a few LPS bands as determined on SDS-PAGE gels in this study.

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Table 2. *Hybridization of ³H-labelled whole-cell DNA from strains ATCC 7901 and P131 with unlabelled DNA from other H. parainfluenzae strains*

Strain	Relative DNA sequence homology with whole-cell DNA*	
	Source of labelled DNA:	
	ATCC 7901	P136
KC469	70	50
KC470	65	38
KC471	55	21
P131	76	91
P136	89	100
P160	84	100
78-2257	58	37
79-23164	72	51
RSF0885	79	60
ATCC 7901	100	77
R740	50	25

* The actual extent of binding of ³H-labelled ATCC 7901 DNA with unlabelled whole-cell DNA of the parent strain was 90%, and that of ³H-labelled P136 DNA with unlabelled whole-cell DNA of the parent strain was 85%; however, both were set at 100% and all other values were normalized to give relative homology.

Fig. 3. Coomassie blue stained SDS-PAGE gel of outer membrane fraction. Lane 1, M_r standards: 94000; 67000; 43000; 30000 and 20000; lane 2, strain P131; lane 3, strain P136; lane 4, strain UB2832; lane 5, strain GU1; lane 6, strain ATCC 7901; lane 7, strain RSF0885; lane 8, strain R832; lane 9, strain 4922; lane 10, strain 82602.

(Odumeru, 1984) LPSs. All three species have LPS devoid of O-sidechains and exhibit substantial inter-strain structural microheterogeneity. However, eight of our 25 *H. parainfluenzae* strains produced LPS with a ladder-like (smooth) appearance suggestive of O-sidechains. Microheterogeneity was found in this class of LPS also. The ultimate proof of the presence of O-sidechains will require determination of the chemical composition of the LPS. The strains producing the different types of LPS could not be distinguished from each other on the basis of outer membrane protein profiles, serum susceptibility, plasmid profiles or DNA homology. This is the first time that smooth LPS has been described in the genus *Haemophilus*, and the significance of this type of LPS in *H. parainfluenzae* is under investigation.

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