SHORT COMMUNICATION

Heterogeneity of Lipopolysaccharide Banding Patterns in *Leptospira* spp.

By MARINA CINCO,¹* ELENA BANFI¹ AND ENRICO PANFILI²

¹ Institute of Microbiology and ² Department of Biochemistry,
Biophysics and Chemistry of Macromolecules, University of Trieste, 34127 Trieste, Italy

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Strains of *Leptospira interrogans* and *Leptospira biflexa*, examined by electrophoresis after whole cell lysis and protein digestion, revealed the presence of 2-keto-3-deoxyoctonate and an heterogeneous lipopolysaccharide electrophoretic banding pattern, which was characteristic of the species.

INTRODUCTION

Since 1956, there have been many studies concerning the possible presence of endotoxins in leptospires (Rothstein & Hiatt, 1956; Gourley & Low, 1962; Areal *et al.*, 1964; Sefer, 1965; Finco & Low, 1967). The results were often equivocal and depended on the methods used to demonstrate endotoxin. When lipopolysaccharides (LPS) were extracted by the classical methods of Westphal and/or Galanos, no clear evidence of endotoxic activity was found (Faine *et al.*, 1974; Johnson & Harris, 1967; Vinh *et al.*, 1984). Hitherto the literature concerning leptospiral LPS has dealt mainly with their antigenic characteristics (Shinagawa & Yanagawa, 1972; Faine *et al.*, 1974) without a detailed description of their chemical and biological features. Hitchcock & Brown (1983) described an improved method for LPS visualization, based on electrophoretic separation and specific silver staining, after protein digestion of whole cell lysates. This method produces LPS patterns identical to those obtained from purified LPS from many genera of bacteria including *Salmonella*, *Neisseria*, *Pseudomonas* and *Campylobacter* (Hitchcock & Brown, 1983; Parr & Bryan, 1984; Darveau & Hancock, 1983; Perez-Perez & Blaser, 1985). Accordingly, in the present study this technique was used to demonstrate and compare putative LPS derived from strains belonging to different serovars of *Leptospira interrogans* and *Leptospira biflexa*.

METHODS

Micro-organisms and media. Pathogenic strains of *Leptospira interrogans* (whose serovar is indicated in parentheses) included Hardjoprajitno (*hardjo*), Mus 127 (*ballum*), Pavia 1 (*bataviae*), Mezzano (*pomona*), Akiyami (*autumnalis*), Dellatte (*copenhageni*), Ballico (*australis*) and Teramo (*icterohaemorrhagiae*); *Leptospira biflexa* saprophytic strains (whose serovar is indicated in parentheses) included Doberdò (*doberdò*), Waz Holland (*holland*), Botanica (*botanica*), Ancona Porto (*ancona*), RPE (*rupino*), Patoc 1 (*patoc*), Basovizza (*basovizza*) and Garcia (*garcia*), together with the halophilic strain Muggia (*muggia*). In addition strain 3705, belonging to *illini* serovar, as a representative of a presumptive new species and genus '*Leptonema*' (Hovind-Hougan, 1983) was examined. All the strains belonging to our reference collection were grown in EMJH medium (Johnson & Harris, 1967) up to 10^8 leptospires ml^{-1} , determined by dark field microscopy in a Thoma-Hawskley counting chamber. For subsequent processing, all the strains were harvested by centrifugation at 10000 g for 60 min, washed once in saline and resuspended to approx. 5×10^9 leptospires ml^{-1} .

Abbreviations: KDO, 2-keto-3-deoxyoctonate; PKD, proteinase K digested.

Fig. 1. Electrophoresis of PKD leptospirae and of standard LPS from *E. coli*; specific LPS silver staining was done according to Hitchcock & Brown (1983). Lane 1, LPS from *E. coli*. Lanes 2 to 9, pathogenic *Leptospira* PKD strains: Hardjoprajtino (2), Mus 127 (3), Pavia 1 (4), Mezzano (5), Akiyami (6), Dellatte (7), Ballico (8) and Teramo (9). Lanes 10 to 19, non-pathogenic *Leptospira* PKD strains: Doberdo (10), *L. illini* 3705 (11), Waz Holland (12), Botanica (13), Ancona Porto (14), RPE (15), Muggia (16), Patoc 1 (17), Basovizza (18) and Garcia (19). Positions of molecular mass markers are indicated on the left. Electrophoresis was done four times using varying amounts of materials and resulting in no change in electrophoretic mobilities.

Lysis of bacteria. The suspensions of leptospires were lysed and digested with proteinase K (Sigma) as described in the improved method of Hitchcock & Brown (1983).

Electrophoresis. The proteinase-treated materials were examined by SDS-PAGE as described by Laemmli (1970) by using about 3 µl of digested samples. A mixture of standard proteins (bovine serum albumin, ovalbumin, chymotrypsinogen, soybean trypsin inhibitor and cytochrome c; Boehringer) was used as molecular mass references. Proteins were normally visualized with (0.125%, w/v) Coomassie brilliant blue. Proteinase-resistant material was silver stained by the improved method of Hitchcock & Brown (1983).

Lipopolysaccharide (LPS). Standard LPS from *Escherichia coli* O111:B4 (Difco) was used. LPS was assayed by determining the amount of 2-keto-3-deoxyoctonate (KDO) in proteinase K digested (PKD) materials by the method of Kharkhanis *et al.* (1978).

RESULTS AND DISCUSSION

The electrophoretic patterns of the 18 PKD strains of leptospires and *E. coli* purified LPS preparation were compared using the specific LPS silver stain procedure (Fig. 1). The scant amount of routine material used did not allow examination by Coomassie brilliant blue staining. All the electrophoretic profiles exhibited different patterns, each strain containing two or three major bands. The mobility of the fastest bands corresponded to a molecular mass of approx. 10 kDa, whereas that of the second fastest group of bands corresponded to approx. 20 kDa. The pattern given by purified LPS from *E. coli* revealed only two bands, both in the 10 kDa region, which are common in the rough LPS profiles of *Enterobacteriaceae* (Fig. 1, lane 1). This feature is completely different from that exhibited by the leptospires. Although there were individual differences among the serovars, it is evident that the pathogenic leptospires (Fig. 1, lanes 2 to 9) have banding patterns which differ from those of the non-pathogenic ones. The bands visible in the region corresponding to 20 kDa were absent from the profiles of saprophytic leptospires. This could reflect the species distinction between *L. interrogans* and *L. biflexa* respectively. The evidence of a faint repeating band in PKD *Leptospira illini* (Fig. 1, lane 11), not detectable in other electrophoretic profiles, may correlate with the different taxonomic position of this leptospire, which may belong to a different species of the genus '*Leptonema*' (Hovind-Hougan, 1983). We cannot allocate these LPS profiles to a smooth-type LPS or to a rough-type LPS, as commonly found among *Enterobacteriaceae*. However, it is noteworthy that the pathogenic leptospires were serum resistant and feebly complement activating (Cinco & Banfi, 1983), while the non-pathogenic ones were serum sensitive; LPS heterogeneity among the two groups could therefore partially reflect the different behaviour towards serum killing, rather than a strict species correlation.

The presence of LPS in leptospiral preparations is suggested also by the presence of KDO, a unique component of LPS core. Preliminary determination of KDO content revealed the presence of this molecule in all the leptospiral extracts for the first time in this genus. The amount was in the range 0.2 to 2.8 µg (100 µg protein)⁻¹ before digestion with proteinase K, being more consistent in the *L. interrogans* strains. The evidence of this specific LPS component together with the specifically silver stained banding patterns suggest that leptospiral LPS may have an architecture comparable to that of well defined enterobacterial LPS. Studies are in progress to characterize leptospiral LPS further, by chemical and biological means.

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