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Antigenic analysis of *Haemophilus ducreyi* by Western blotting

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SUMMARY

Twenty-one strains of *Haemophilus ducreyi* were analysed by Western blotting using two antisera produced in mice. Common antigens of molecular weights 58, 46, 41, 28, 22 and 16 kDa were detected in all the strains. The antigens were protein in nature, since they could not be detected in whole-cell lysates which had been treated with proteinase K. The *H. ducreyi* strains showed antigenic cross-reactivity with strains of *H. influenzae* and *H. parainfluenzae*, but showed minimal or no cross-reactivity with seven other species of bacteria.

INTRODUCTION

Haemophilus ducreyi is the cause of chancroid, a tropical sexually transmitted disease characterized by genital ulceration and frequent associated inguinal lymphadenopathy. Although the organism was first described nearly a hundred years ago, its biological properties including its antigenic composition have not been well characterized. A fuller understanding of the antigenicity of *H. ducreyi* is particularly desirable, as such knowledge should facilitate both epidemiological investigations, such as studies of relapse or re-infection, and studies of the organism's pathogenicity. Indeed, a knowledge of the antigenicity of *H. ducreyi* is an obvious prerequisite if rational attempts are to be made to develop a vaccine.

A preliminary study of the antigenicity of *H. ducreyi* has been reported by Saunders & Folds (1986), who used the technique of Western blotting with rabbit antisera. Their study, however, was complicated by the reactivity of the pre-immunization sera with some *H. ducreyi* antigens. In this study we have also applied the technique of Western blotting to investigate the antigenicity of *H. ducreyi* strains, but have modified the procedure described above by using antisera produced in specific pathogen-free mice to increase the specificity of the reactions.

MATERIALS AND METHODS

Bacteria

Twenty-one strains of *H. ducreyi* were included in the study. Strains designated M (isolated in Munich), 35000 (isolated in Canada), D-14 (isolated in Sheffield) and CIP 542 (obtained from the Pasteur Institute) have been described previously

(Abeck *et al.* 1987; Hammond *et al.* 1978). Of the remaining strains, one was isolated from a patient in the Gambia, and 16 strains were isolated in South Africa. The organisms were cultured on medium consisting of Columbia agar base (BBL) supplemented with 10% (v/v) sterile sheep blood (Tissue Culture Services), 2·5% (v/v) fetal calf serum (Flow Laboratories), 1·5% (v/v) IsoVitalex (BBL) and vancomycin (3 mg/l), in a candle jar at 33 °C.

Other bacteria used were *H. influenzae* (5 strains), *H. parainfluenzae* (5 strains), *Neisseria gonorrhoeae* (3 strains), *Bacteroides ureolyticus* (2 strains), *Gardnerella vaginalis* (3 strains), *Escherichia coli* (2 strains), *Staphylococcus aureus* (3 strains), *Streptococcus agalactiae* (1 strain) and a *Lactobacillus* sp. (1 strain).

Antisera

Antisera to *H. ducreyi* strains M and 35000 were raised in specific pathogen-free TO mice as described previously (Boustouller, Johnson & Taylor-Robinson, 1986), except that the interval between the primary and booster inoculation was 10 days. The mice were exsanguinated 7 days after the booster inoculation, and sera were stored in small samples at −70 °C.

Polyacrylamide gel electrophoresis

The preparation and examination by polyacrylamide gel electrophoresis (PAGE) of whole-cell lysates, isolated sarkosine-insoluble outer membranes or lipopolysaccharide (LPS) were performed as described previously (Abeck *et al.* 1987; Abeck & Johnson, 1987). In a few experiments, gels were stained with a combination of silver and Coomassie Blue, which allowed the simultaneous visualization and differentiation of both proteins and lipopolysaccharide (Hitchcock & Brown, 1983).

Western blotting

Cellular material separated on polyacrylamide gels was transferred electrophoretically in a Trans-Blot cell (BioRad) on to 0·2 μm nitrocellulose filters (Schleicher & Schull), as described by Towbin, Strehelin & Gordon (1979). The filters were then probed with anti-*H. ducreyi* antiserum followed by ^{125}I-labelled rabbit anti-mouse serum, and subjected to autoradiography usually for 12–24 h, as described in detail previously (Boustouller, Johnson & Taylor-Robinson, 1986).

RESULTS

Analysis of H. ducreyi *strains by Western blotting*

The optimal dilution of the antisera to strains M and 35000 was found to be 1:1500 in each case. When electrophoretically separated antigens of whole-cell lysates of strain M or strain 35000 were blotted with either the homologous or the heterologous antiserum at this dilution, strong bands with molecular weights of 46, 41, 28, 22 and 16 kDa were observed, as shown in Fig. 1. Although weak in Fig. 1, the band at 58 kDa was strongly represented in some other preparations, and the single band seen at 28 kDa could be resolved occasionally into three separate but closely adjacent bands (Fig. 2). Additional faint bands could be

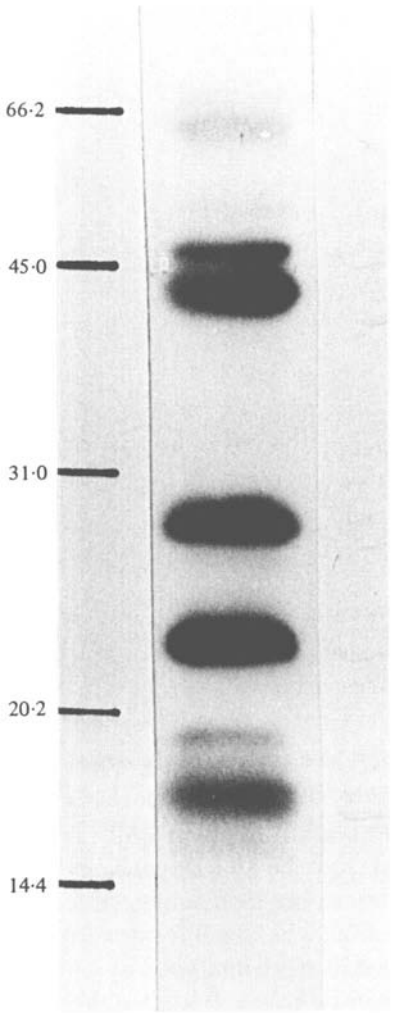

Fig. 1. Western blot analysis of *H. ducreyi* strain M reacted with its homologous antiserum. The banding pattern of proteins of known molecular weights is indicated on the left of the figure.

detected with both antisera if autoradiography was prolonged. Antiserum to strain M was used subsequently to analyse whole-cell lysates of the 21 available strains of *H. ducreyi*. The bands seen in the profile of the homologous strain were also present in preparations of the 20 other strains, indicating a close antigenic relationship between the strains. An autoradiograph obtained for six strains is shown in Fig. 2.

Characterization of common antigens of H. ducreyi

Whole-cell lysates and sarkosine-insoluble outer membranes from all the strains were analysed by Western blotting. With the exception of the 58 kDa protein, the antigens were detected in both whole-cell lysates and outer-membrane protein preparations, indicating their localization in this component of the cell wall.

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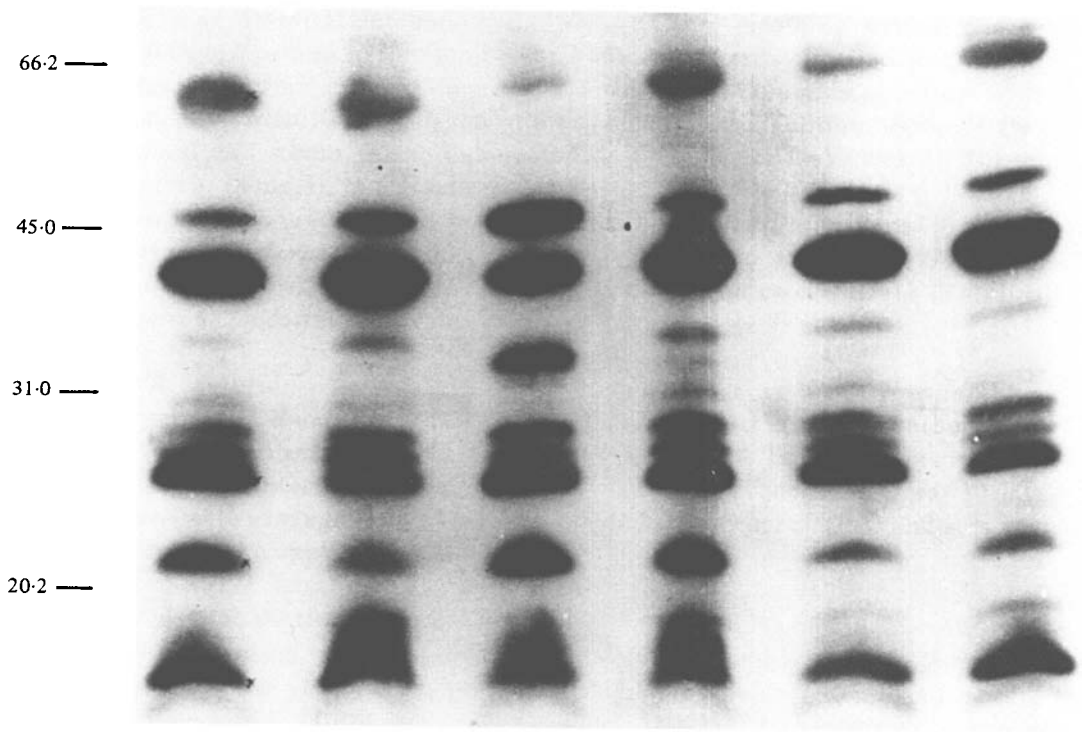

Fig. 2. Western blot analysis of six strains of *H. ducreyi*.

Staining of gels with a combination of silver and Coomassie Blue, which stained proteins and LPS, indicated that the 16 kDa antigen banded in a region of the gel which contained LPS. To determine if this antigen was LPS or protein in nature, whole-cell lysates, together with whole-cell lysates which had been de-proteinated by treatment with proteinase K, were analysed by blotting. In each case, antigens were detected only in untreated whole-cell lysates (Fig. 3), indicating that they are composed either of protein or protein complexed with LPS or carbohydrate.

Antigenic cross-reactivity between H. ducreyi *and other bacterial species*

The ability of antiserum to *H. ducreyi* strain M to detect antigens of nine other bacterial species was examined by Western blotting. *H. ducreyi* was found to cross-react with strains of *H. influenzae* and *H. parainfluenzae* (Fig. 4), but showed minimal or no antigenic cross-reactivity with *N. gonorrhoeae*, *E. coli*, *G. vaginalis*, *B. ureolyticus*, *S. aureus*, *Strep. agalactiae* or *Lactobacillus* spp. Representative results are shown in Fig. 4.

DISCUSSION

The results presented here indicate that bacteria belonging to the species *H. ducreyi* constitute a highly antigenically related group of organisms, as evidenced by the presence of a number of common antigens in strains isolated in different parts of the world. The organisms also show cross-reactivity with other members

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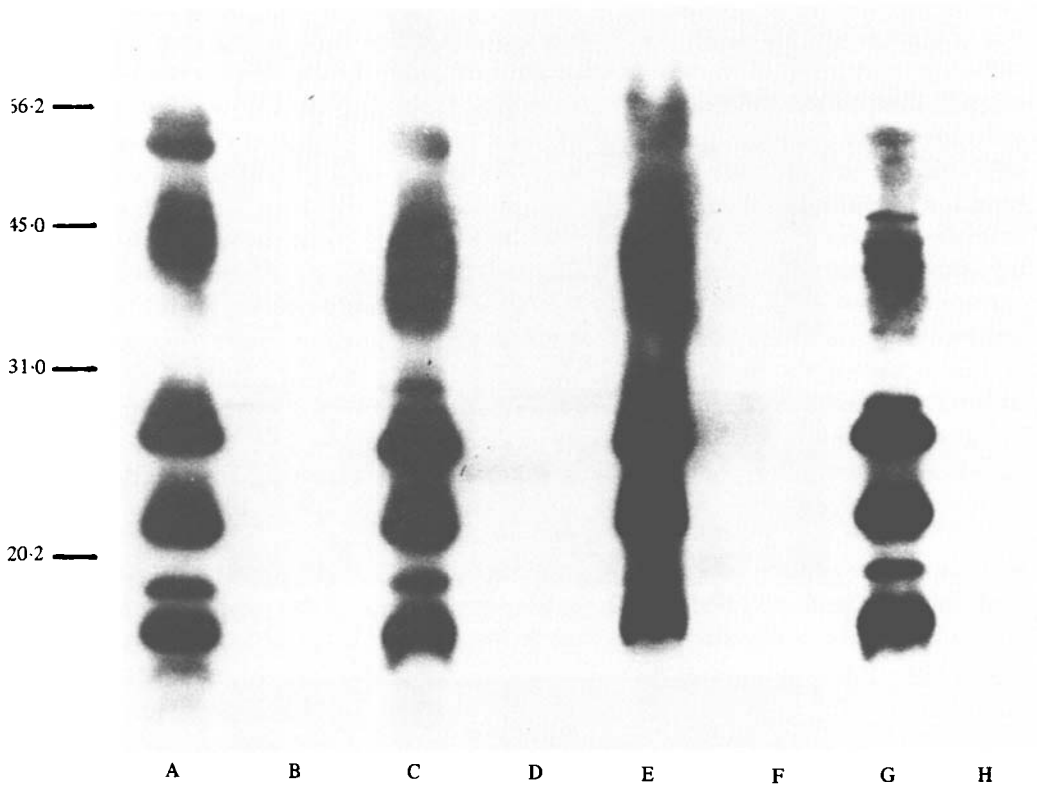

Fig. 3. Western blot analysis of untreated whole-cell lysates (lanes A, C, E and G) and proteinase K-treated whole-cell lysates (lanes B, D, F and H) of four strains of *H. ducreyi*. The bands seen in lanes A, C, E and G are very intense, as the autoradiograph was subjected to prolonged exposure. Despite the prolonged exposure, antigens were not detected in the proteinase K-treated samples.

of the same genus, but appear antigenically unrelated to taxonomically distinct micro-organisms. The common antigens appear to be protein in nature, as shown by their disappearance following treatment with proteinase K. However, the possibility that some of the antigens detected consisted of lipopolysaccharide complexed with protein cannot be excluded at present. Indeed, such a possibility may explain the somewhat surprising finding that the two antisera tested did not appear to contain antibodies to lipopolysaccharide.

The results of the present study agree in part with those reported by Saunders & Folds (1986), who used rabbit antisera to analyse the antigenicity of *H. ducreyi*. The antigens of molecular weight 41 and 22 kDa that we have noted are probably the same as those of 42 and 22·5 kDa described by Saunders & Folds (1986). However, the molecular weights of the other antigens described in the two studies do not correspond. These differences could possibly be due to the use either of different bacterial strains or of different species of laboratory animal for the production of antisera. Further work will be needed to clarify this point.

The role, if any, that the common antigens described here have as determinants of virulence is unknown. In a previous study we reported that two proteins of molecular weights 39 and 27–30·5 kDa, respectively, were the predominant

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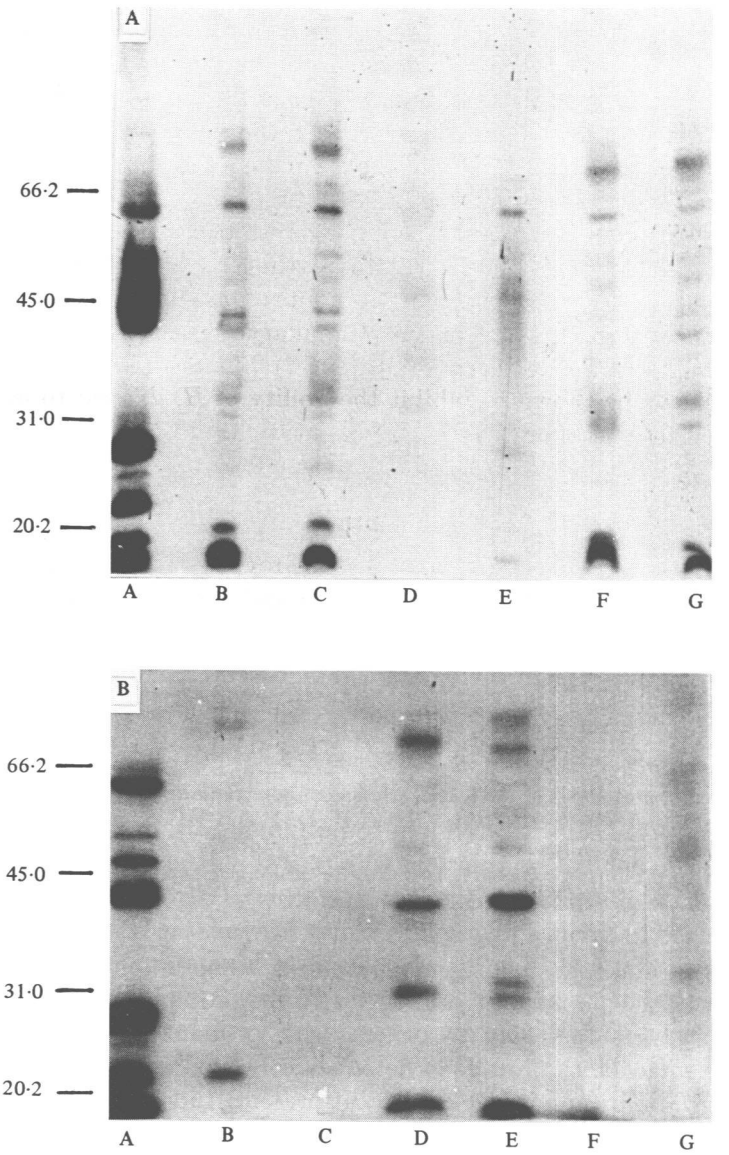

Fig. 4. (A) Western blot analysis of *H. ducreyi* (lane A) and other bacterial species including (*H. parainfluenzae* (lanes B and C), *Neisseria gonorrhoeae* (lane D). *Straphylococcus aureus* (lane E) and *H. influenzae* (lanes F and G). (B) Western blot analysis of *H. ducreyi* (lane A) and other bacterial species including *Gardnerella vaginalis* (lanes B and C), *H. influenzae* (lane D), *H. parainfluenzae* (lane E). *Lactobacillus* spp. (lane F), *N. gonorrhoeae* (lane G).

protein antigens exposed at the surface of *H. ducreyi* (Abeck & Johnson, 1987). We feel it is likely that the 39 kDa antigen described previously is the same as the 41 kDa antigen described in the present study, the antigen, in fact, being the common major outer-membrane protein seen on polyacrylamide gels (Odumeru, Ronald & Albritton, 1983; Taylor *et al.* 1985). The minor discrepancy between our

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two studies in the reported molecular weight is within the range of experimental error that may be expected when making measurements on autoradiographs, and is compatible with the variation in the molecular weight of this antigen as reported by other workers (i.e. 39·4–40 kDa) (Odumeru, Ronald & Albritton, 1983; Taylor *et al.* 1985). For the same reason, we also feel that the possibility exists that the 28 kDa common antigen described here may be the same as the 27–30·5 kDa proteins described previously. Assuming that one or more of the common antigens is indeed surface-exposed, it will be of some interest to determine the effect of antibodies to these antigens. Studies with other pathogenic organisms have shown that surface antigens frequently act as determinants of pathogenicity, for example by promoting the adherence of organisms to eukaryotic cells, or by mediating resistance to killing by host defences (Smith, 1977). Should antibodies to any of the common antigens be shown to inhibit the ability of *H. ducreyi* to adhere to cells, or to promote killing of organisms by phagocytic leukocytes or complement, such antigens would then be candidates for inclusion in any experimental vaccines against chancroid.

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