Demonstration of lectin-like activity for the common antigen of *Neisseria gonorrhoeae* by quantitative immunoelectrophoretic techniques

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(Received 18 December 1992; revision received 11 February 1993; accepted 16 February 1993)

Abstract: To overcome problems associated with Western blotting of denatured proteins, we have used quantitative immunoelectrophoretic techniques to perform functional analysis of the *Neisseria gonorrhoeae* common antigen. Using these techniques, we show (a) that *Neisseria gonorrhoeae* expresses an antigen that is cross-reactive with the common antigen of *Pseudomonas aeruginosa* and *Legionella micdadei* and with the GroEL-like protein of *Chlamydia*, and (b) that this *N. gonorrhoeae* common antigen has lectin-like activity and can be precipitated with three different sugars immobilized on agarose beads: α-D-glucosamine, maltose and fucose.

Key words: *Neisseria gonorrhoeae*; Common antigen; Heat shock protein; Lectin adhesin

Introduction

Many pathogenic bacteria possess lectins which facilitate adherence to host cells. This bacterial attachment contributes to microbial persistence at mucosal surfaces and may be a prerequisite for infection [1]. Some outer membrane components have been implicated in the adhesion of gonococci. In effect, adherence of *Neisseria gonorrhoeae* to host cells is facilitated by pili [2,3]. However, the exact nature of the pathogen and host cell factors involved in epithelial invasion have not been identified. Microscopy studies indicate that tight attachment of the pathogen to the epithelial membrane, probably conferred by outer membrane adhesins, is essential for the initiation of the invasion process [4,5]. Several adhesins have been detected on the gonococcal surface, the best studied example of which is a 36 kDa adhesin with binding properties for gangliotetrasylceramide, a glycolipid abundant in a variety of tissues [6]. In addition, the presence of outer membrane protein PII, or Opa proteins (Opa), on the gonococcal surface have been correlated with the attachment of *N. gonorrhoeae* to many cell types, including urogenital cells, conjunctival cells and buccal epithelial cells [7–10]. However, it is difficult to associate the function of attachment (which is of great importance with regard to
virulence) with the surface antigens of *Neisseria gonorrhoeae* as presently known. We have recently demonstrated that an additional bacterial lectin molecule is present in *N. gonorrhoeae*: gonococcal parietal lectin (GPL) which is a member of the common antigen (CA) family [11]. The CA is a protein which is present in many species of bacteria, including Gram-negative, Gram-positive, treponemes and even archaebacteria [12-14]. The prototype of this family is the GroEl protein of *Escherichia coli*, which is a tetradecamer of subunits, each subunit having a *M* subunit of about 65000 [15,16]. The present study was undertaken to show the relationship between the native gonococcal parietal lectin and the CA family. Using quantitative immunoelectrophoretic techniques (absorption in situ of antibodies and immobilized sugars), we demonstrate the lectin-like activity of the purified protein and characterize its sugar specificity.

**Materials and Methods**

**Antigens**

*Neisseria gonorrhoeae* Geizer serotype C (Collection Centre d’Immunochimie Microbiennne, CIM XIV J3, Institut Pasteur de Lyon, Lyon, France) was grown on chocolate agar plates containing 1% growth factors (Polyvitex Biomerieux, Marcy l’Etoile, France) incubated at 37°C in the presence of CO2 for 24 h. GPL was purified as previously described [11].

**Antisera**

Polyclonal hyperimmune serum was produced by immunizing rabbits with an extract of *N. gonorrhoeae* Geizer serotype C. Immunization and the titration of serum antibodies were performed as previously described [17]. The three monospecific antisera (MAS), MAS 3974 against *Legionella micdadei* common antigen, MAS 5343 against *Pseudomonas aeruginosa* common antigen were kindly provided by N. Hoiby, and a MAS against the 57-kDa *Chlamydia* GroEl-like protein was generously provided by R.P. Morrison.

**Native polyacrylamide gel electrophoresis (PAGE)**

Native PAGE was performed in a Pharmacia Phast System flat-bed apparatus according to the procedures described in the Phast System manual [18].

**Immobilized sugars**

Seven sugars immobilized on agarose beads were used: α-D-glucosamine (Pharmacia, Uppsala, Sweden); fucose, β-D-glucose, lactose, d-N-acetyl-glucosamine (Pierce Chemical Co., Rockford, USA) and α-D-galactosamine, maltose (Sigma Chemical Co., St. Louis, USA).

**Quantitative immunoelectrophoretic methods**

*Line immunoaffinoelectrophoresis (LIAE).* LIAE was performed as described by Kroll [19]. The purified GPL (10%) was mixed with 0.8% buffered agarose and poured to obtain a gel (13.5 × 0.5 × 0.13 cm) on gel-bound film. A blind gel (agarose alone) was poured onto part of the gel-bound film (13.5 × 1.5 × 0.13 cm) then wells (2.5 mm diameter) were punched in the blind gel between the antigen gel strip and the antiserum-containing gel (13.5 × 5.5 × 0.1 cm) and filled with 7 μl of agarose-immobilized sugars.

*Crossed immunoelectrophoresis (CIE).* CIE was performed by the method of Weeke [20], using a 0.8% agarose gel (Biorad, Richmond, USA) in 0.025 M Tris barbital buffer (pH 8.6). First dimension electrophoresis of purified GPL (10 μl) was performed at 15°C applying 10 V/cm for 70 min, with a bromphenol blue albumin marker, followed by a second dimension electrophoresis performed at 15°C by applying 2 V/cm for 18 h through a gel containing 10 μl of antiserum per cm². In the first dimension electrophoresis, the plates were 11 cm long and 9 cm wide, and the thickness of the gel was 0.15 cm. In the second dimension electrophoresis, the plates were 8 cm long, 8 cm wide and 0.1 cm thick. After the first dimension, a slab (8 × 2 cm) was transferred to a squared-gel bound film (8 × 8 cm). A blind gel (8 × 2 × 0.13 cm) was poured along the first dimension electrophoresis gel. The antibody-containing gel was formed by pouring 5 ml of agarose containing *N. gonorrhoeae* antiserum into the remaining part of the plate.
**CIE with intermediate gel.** After freezing, a gel strip (8 × 0.5 × 0.13 cm) was cut from the blind gel and replaced with a gel strip containing different antisera.

**Crossed immunoaffinoelectrophoresis (CIAE).** The casting of the gels and the electrophoresis were carried out essentially as previously described [21,22]. After freezing, a gel strip (8 × 0.5 × 0.13 cm) was cut from the blind gel and replaced with a strip of intermediate gel containing immobilized sugars on agarose-beads.

After electrophoresis non-precipitated proteins were removed by pressing the gel and washing twice in buffered saline (1 h) followed by distilled water (15 min). Plates were stained for 5 min in 0.5% (w/v) Coomassie brilliant blue R in ethanol-acetic acid-water (45:10:45). The excess dye was removed by several washes in ethanol-glacial acetic acid-water (25:10:45).

**Results and Discussion**

On a native polyacrylamide gel after silver staining, the purified GPL protein resolved into a single band with an apparent $M_r$ between 669 kDa and 900 kDa (Fig. 1).

To determine which sugars may function as receptors for purified GPL, LIAE using sugars immobilized on agarose beads was employed. The

![Fig. 1. Silver-stained native polyacrylamide gel (Phast-gel gradient 4-15). (a,d) High molecular mass markers; (b) purified rat IgM; (c) purified gonococcal parietal lectin (GPL).](https://academic.oup.com/femsle/article-abstract/109/1/101/491858)

precipitin line was abolished only with $\alpha$-d-glucosamine, maltose and fucose beads (Fig. 2). The results obtained in screening by LIAE were confirmed by CIAE. Figure 3 shows that only $\alpha$-d-glucosamine, maltose and fucose were able to modify the GPL precipitation peak.

The cross-reactivity of native GPL with the common antigen of *L. micdadei* and *P. aeruginosa* and with GroEl-like protein was also examined using CIE techniques. CIE of purified GPL using polyclonal rabbit antibody against *N. gonorrhoeae* (Gono Geizer serotype C) revealed only one peak corresponding of GPL (Fig. 4A). When a monospecific antiserum to the common antigen of *P. aeruginosa* or *L. micdadei* and the monospecific antiserum against the 57 kDa Chlamydia GroEl-like protein were added to the intermediate gel, the well-defined precipitate of GPL was retained (Fig. 4B, C and D). This indicated strong cross-reactivity between the CA of *P. aeruginosa*, the CA of *L. micdadei* and the 57-kDa Chlamydia GroEl-like protein with the antigen of the retained precipitate which was therefore designated the CA of *N. gonorrhoeae*. We have confirmed these results in LIE (Fig. 5): the immobilized p-glucosamine, maltose or fucose and the antisera against the CA of *P. aeruginosa* or *L. micdadei* and against GroEl Chlamydia protein were able to absorb the precipitation
line of GPL. No absorption was detected with a normal rabbit serum.

Previous investigation of the *N. gonorrhoeae* GPL using immunoblotting showed that GPL exhibited both structural and immunological similarities to the CA family [11]. The CA of *N. gonorrhoeae* has been purified and characterized as a high molecular mass protein consisting of 65 kDa subunits. However, studies made using Western blotting techniques are limited because the protein is in a denatured state. The immunoelectrophoretic techniques used in the present work permitted the study of the protein in its native state. Our report provides evidence that a lectin-like molecule (GPL) enables *N. gonorrhoeae* to attach to ϑ-glucosamine-maltose- and fucose-receptors. Certain outer membrane components have been previously implicated in gonococcal adhesion to surfaces: pili and protein II [23]. However, gonococcal attachment does not correlate with the surface antigens of *N. gonorrhoeae* as currently known. We therefore speculate that the GPL common antigen may be involved in the pathogenesis of gonococcal infections via its lectin-activity which promotes gonococcal adhesion to epithelial cells. Further studies are currently being undertaken to characterize this lectin more fully, and in particular to examine its role in gonococcal-host cell interactions.

Acknowledgements

We thank N. Hoiby, Department of Clinical Microbiology at Rigshospitalet, Statens Seruminstitut, Copenhagen, Denmark, and P.R. Morrison, Rocky Mountain Laboratories, Hamilton, MT, for the gift of the antisera directed against
Fig. 4. CIE of purified GPL against polyclonal rabbit antiserum to *N. gonorrhoeae* (a-Ng) with Blank intermediate gel (A). B, C and D are the same as A, except that 50 μl (10%) of antisera against *P. aeruginosa* CA (B), *L. micdadei* CA (C) or 57-kDa *Chlamydia* GroEl-like protein (D) were included in the intermediate gel.

Fig. 5. Demonstration of lectin-like activity for the CA by LIE with polyvalent anti-*N. gonorrhoeae* antiserum used with purified GPL. (A) The wells were loaded with: β-β-glucose (1), α-β-glucosamine (2), maltose (3), fucose (4) and lactose (5). (B) The wells were loaded with: antiserum anti-*P. aeruginosa* CA (6), antiserum anti-*L. micdadei* CA (7), antiserum anti-57-kDa *Chlamydia* GroEl-like protein (8) and normal rabbit serum (9).

the CAs of *Pseudomonas aeruginosa*, *Legionella micdadei* and against the 57-kDa *Chlamydia* GroEl-like protein. We thank G. Joly for photography and C.B. Bluink and S. Bruneau, Institut Pasteur de Lyon, Lyon, France, for their encouragement and advice.

References


