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Purification and Immunological Studies of the Cross-Reaction between the 65-Kilodalton Gonococcal Parietal Lectin and an Antigen Common to a Wide Range of Bacteria

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The 65-kDa gonococcal parietal lectin (GPL) has been purified and found to have a lectinlike activity exhibiting both structural and immunological similarities to the common antigen family. Ultrastructural localization of GPL was done by using anti-GPL monoclonal antibodies: GPL is a major component of the outer membrane and is also present in blebs formed by gonococci.

Numerous studies have been carried out on a common antigen protein present in a wide variety of microorganisms. This protein, one of the major components of bacterial whole-cell extracts, is highly immunogenic, has a high native relative molecular mass, and is an oligomer composed of subunits with an M_r varying between 58 and 65 kDa depending on the microorganism from which it has been isolated (12, 13). The common antigen has been shown to cross-react with an equivalent antigen from more than 60 different bacteria, including gram-negative and gram-positive bacteria, treponemes, and even archaebacteria (5, 10, 13).

The development of immunoaffinity electrophoresis methods using immobilized sugars on agarose beads enabled us to identify a protein, gonococcal parietal lectin (GPL), with lectinlike activities in microbial extracts of *Neisseria gonorrhoeae* (9). The present study shows the similarity of this adhesin to the common antigen family and demonstrates that GPL is localized at the cell surface.

N. gonorrhoeae Geizer serotype C (Collection Centre d'Immunochimie Microbienne CIM XIV J_3, Institut Pasteur de Lyon, Lyon, France) was grown on chocolate agar plates containing 1% growth factors (Polyvitex; Biomerieux, Marcy l'Etoile, France) at 37°C in the presence of CO_2 for 24 h. The purified GPL was obtained by extraction of harvested cells (2) in the presence of sterile glass beads on a rotary shaker (New Brunswick Scientific Co., Inc.). After centrifugation and dialysis, 30 mg of extract was applied to an ion-exchange chromatography column (DEAE CL6B; Pharmacia Biotechnology, Uppsala, Sweden) equilibrated with phosphate buffer (pH 5.7). The 65-kDa-component-containing fractions that eluted at 0.5 M NaCl were loaded onto an ACA_{22} gel filtration column (IBF Biothecnic, Villeneuve la Garenne, France) in phosphate buffer (pH 6.8). A total of 1 mg of pure protein antigen was obtained from approximately 30 mg of whole-cell protein extract. The purity of the preparations was assayed by silver staining and sodium

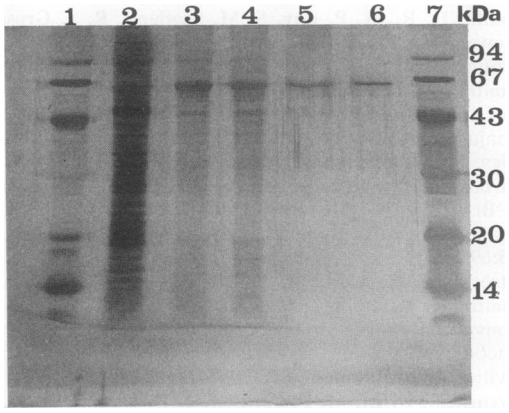

FIG. 1. Purification of the 65-kDa polypeptide of *N. gonorrhoeae*. Samples were taken after each step of the purification procedure and analyzed by SDS–12.5% PAGE followed by silver staining. Lanes: 1 and 7, molecular mass markers; 2, cell extract of *N. gonorrhoeae*; 3 and 4, fractions from the first ion-exchange chromatography; 5 and 6, pooled fractions (6 and 4 μg) from the ACA_{22} gel filtration column.

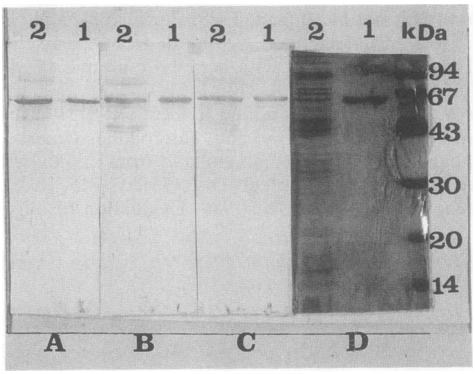

FIG. 2. Western blot (immunoblot) analysis of cross-reactivity between purified (lanes 1) or nonpurified (lanes 2) 65-kDa GPL and rabbit antisera against *L. micdadei* common antigen (A), *P. aeruginosa* common antigen (B), or 57-kDa chlamydial GroEL-like protein (C). The Coomassie blue-stained gel is shown in panel D.

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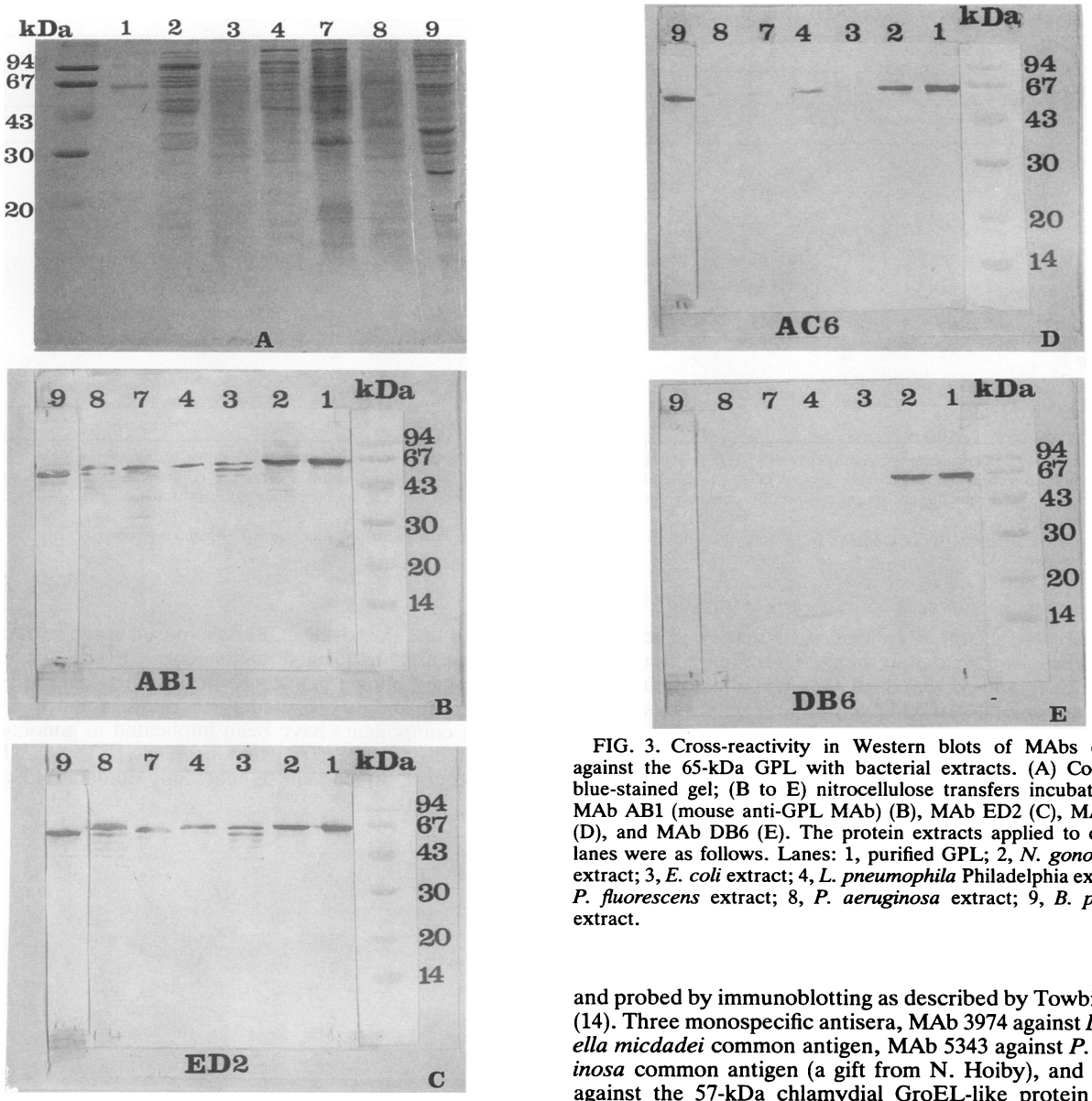

FIG. 3. Cross-reactivity in Western blots of MAbs directed against the 65-kDa GPL with bacterial extracts. (A) Coomassie blue-stained gel; (B to E) nitrocellulose transfers incubated with MAb AB1 (mouse anti-GPL MAb) (B), MAb ED2 (C), MAb AC6 (D), and MAb DB6 (E). The protein extracts applied to different lanes were as follows. Lanes: 1, purified GPL; 2, *N. gonorrhoeae* extract; 3, *E. coli* extract; 4, *L. pneumophila* Philadelphia extract; 7, *P. fluorescens* extract; 8, *P. aeruginosa* extract; 9, *B. pertussis* extract.

dodecyl sulfate–12.5% polyacrylamide gel electrophoresis (SDS–12.5% PAGE) as described by Laemmli (8) (Fig. 1).

Nine monoclonal antibodies (MAbs) against the purified 65-kDa GPL were produced, purified, and characterized as previously described (1).

In order to confirm the ultrastructural location of the 65-kDa GPL on the surface of intact gonococci, we used immunogold probes (11) to visualize antibody binding to the surface of whole bacteria. For this study, we used a murine anti-GPL MAb obtained by using a slight modification (3) of the method of Kôhler and Milstein (7).

For studies of the cross-reactivity of the 65-kDa GPL with the common antigen, the SDS lysates of several gram-negative bacteria (*N. gonorrhoeae*, *Escherichia coli* JM109, *Legionella pneumophila* Philadelphia strain ATCC 33152, *Mycobacterium tuberculosis* H37Ra, *Pseudomonas aeruginosa* PAC 605, *Pseudomonas fluorescens*, and *Bordetella pertussis*) were electrophoresed on SDS–12.5% PAGE (8) and probed by immunoblotting as described by Towbin et al. (14). Three monospecific antisera, MAb 3974 against *Legionella micdadei* common antigen, MAb 5343 against *P. aeruginosa* common antigen (a gift from N. Hoiby), and a MAb against the 57-kDa chlamydial GroEL-like protein (a gift from R. P. Morrison) reacted with both the purified 65-kDa GPL and the same protein in *N. gonorrhoeae* whole-cell extracts (Fig. 2). A positive cross-reaction was also found with antisera against *M. tuberculosis* H37Ra, *L. pneumophila* Philadelphia, and *Mycobacterium bovis* BCG (DAKO): these antisera revealed both the purified 65-kDa GPL and the same protein in *N. gonorrhoeae* whole-cell extracts (Table 1). In the same manner, we tested for the presence of antibodies against the common antigen of several bacteria in polyclonal antibodies and MAbs directed against the 65-kDa GPL (Fig. 3). The four groups of anti-GPL MAbs did not have the same reactivities against the different bacterial extracts: one recognized the common antigen in all the bacterial extracts tested, but the DB6 MAb seemed to recognize a specific GPL epitope (Table 1). Our results provide strong evidence that the GPL is related to the 60- to 65-kDa major antigenic protein or common antigen of several bacteria. The GPL exhibits both structural and immunological similarities to the GroEL family of proteins.

At present, the localization of the common antigen in the

TABLE 1. Summary of cross-reactivity studies with various antibodies

Antibody[a]	Cross-reactivity with:							
	65-kDa polypeptide GPL	64- to 65-kDa polypeptide in extract from[b]:						
		Ng	Ec	Lp	Pf	Pa	Bp	Mt
Antibodies								
Poly-anti GPL	+	+	+	+	NT[c]	NT	NT	+
Poly-anti H37Ra	+	+	+	+	NT	NT	NT	+
Poly-anti Lpp	+	+	+	+	NT	NT	NT	+
Mono-anti Pa	+	+	NT	NT	NT	NT	NT	NT
Mono-anti Lm	+	+	NT	NT	NT	NT	NT	NT
Mono-anti Ch	+	+	NT	NT	NT	NT	NT	NT
Anti-GPL MAbs								
Group 1	+	+	+	+	+	+	+	−
Group 2	+	+	+	+	−	−	+	−
Group 3	+	+	−	+	−	−	+	−
Group 4	+	+	−	−	−	−	−	−

[a] Poly-anti GPL, polyspecific antiserum against 65-kDa gonococcal protein; Poly-anti H37Ra, polyspecific antiserum against *M. tuberculosis*; Mono-anti Lpp, monospecific antiserum against 65-kDa protein of *L. pneumophila*; Mono-anti Pa, monospecific antiserum against the common antigen of *P. aeruginosa*; Mono-anti Lm, monospecific antiserum against the common antigen of *L. micdadei*; Mono-anti Ch, monospecific antiserum against the 57-kDa chlamydial GroEL-like protein.

[b] Ng, *N. gonorrhoeae*; Ec, *E. coli*; Lp, *L. pneumophila*; Pf, *P. fluorescens*; Pa, *P. aeruginosa*; Mt, *M. tuberculosis*; Bp, *B. pertussis*.

[c] NT, not tested.

bacterial cell is still a controversial matter. The 65-kDa protein has been located in the cytoplasmic fraction of both *E. coli* K-12 and *M. bovis* BCG (13), but the detection of significant amounts of 60-kDa protein from various *Legionella* species in outer membrane preparations indicates that this protein may not be restricted to the cytoplasm (6). By immunogold labelling of *N. gonorrhoeae*, we have demonstrated that the 65-kDa GPL is exposed at the surface and is present in blebs formed by gonococci (Fig. 4).

We have shown that the 65-kDa gonococcal protein has a lectinlike activity (9), and furthermore some outer membrane components have been implicated in gonococcal adhesion to surfaces: pili and protein II (4). The basis of gonococcal attachment is not fully understood, but we

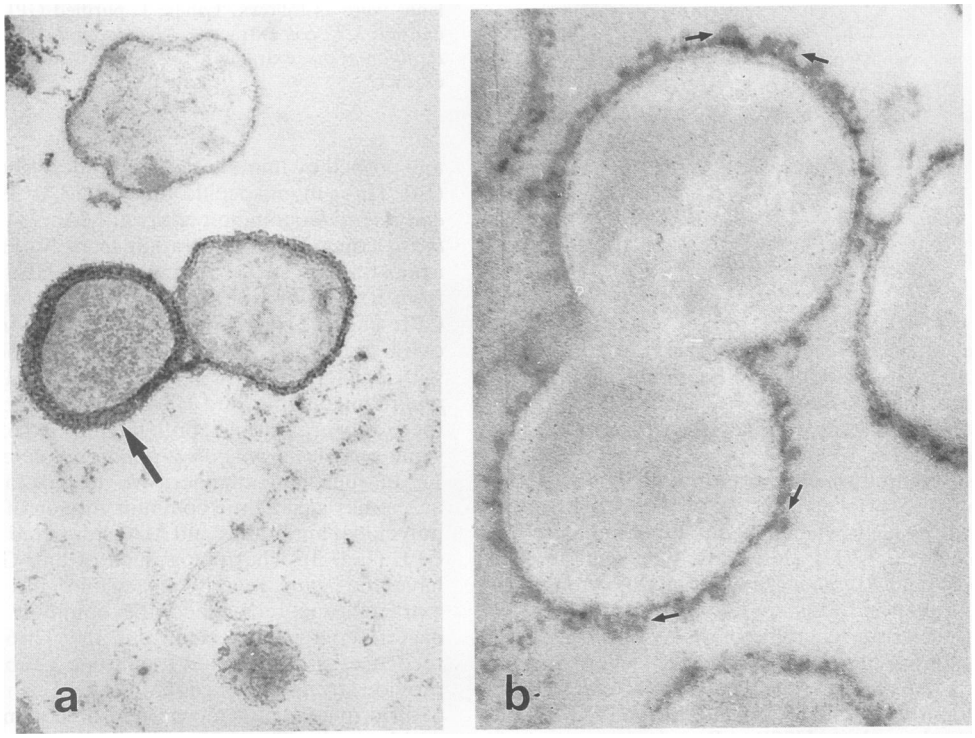

FIG. 4. *N. gonorrhoeae* serotype C Geizer exposed to immunological probes. The gold spheres (diameter, 20 nm) were complexed to a murine anti-GPL MAb. Note the attachment of gold spheres to the surface of the diplococcus (a). Also note the presence of the gold spheres in the outer membrane bleb elaborated by the organism (b). Negative stain was used. Magnification, ×17,000 (a) and ×27,000 (b).

speculate that the surface localization of GPL points to a possible role for this protein in the phenomenon of adhesion. Such lectinlike activity of the common antigen from various microorganisms should be investigated.

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