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Characterization of an 18,000-Molecular-Weight Outer Membrane Protein of *Haemophilus ducreyi* That Contains a Conserved Surface-Exposed Epitope

STANLEY M. SPINOLA,[1,2]†* GAIL E. GRIFFITHS,[1] JOHN BOGDAN,[2] AND MARILYN A. MENEGUS[3]

Departments of Medicine[1] and Microbiology,[2] School of Medicine, State University of New York at Buffalo, Buffalo, New York 14215, and Department of Microbiology, University of Rochester, Rochester, New York 14642[3]

Received 24 September 1991/Accepted 7 November 1991

Identification of antigenically conserved surface components of *Haemophilus ducreyi* may facilitate the development of reagents to diagnose and prevent chancroid. A hybridoma derived from a mouse immunized with nontypeable *Haemophilus influenzae* produced a monoclonal antibody (MAb), designated 3B9, that bound to 35 of 35 *H. ducreyi* strains isolated from diverse geographic regions. The MAb 3B9 bound to a non-heat-modifiable *H. ducreyi* outer membrane protein (OMP) whose apparent molecular weight was 18,000 (the 18K OMP), and the 3B9 epitope did not phase vary at a rate of $>10^{-3}$ in *H. ducreyi*. In immunoelectron microscopy, the 3B9 epitope was surface exposed, and there was intrastrain and interstrain variability in the amount of 3B9 labelling of whole cells. The MAb 3B9 cross-reacted with many species of the family *Pasteurellaceae* and bound to the 16.6K peptidoglycan-associated lipoprotein (P6 or PAL) of *H. influenzae*. Unlike P6, the 18K OMP did not copurify with peptidoglycan. In Western blots (immunoblots), five of seven serum samples obtained from patients with chancroid and four of five serum samples obtained from patients with other genital ulcer diseases at the time of presentation contained antibodies that bound to the 18K OMP. In a competition enzyme-linked immunosorbent assay, four of these serum samples inhibited the binding of 3B9 to *H. ducreyi* by more than 50%. We conclude that members of *Pasteurellaceae* expressed a conserved epitope on OMPs that sometimes had different physical characteristics. Patients with chancroid usually have antibodies to the 18K OMP and the 3B9 epitope that may have resulted from infection with *H. ducreyi* or previous exposure to other *Haemophilus* or *Actinobacillus* sp. strains.

Haemophilus ducreyi is a major cause of genital ulcer disease in developing countries and is increasing in prevalence in the United States (21, 32). An important clinical aspect of chancroid is that genital ulcer diseases are independent risk factors for human immunodeficiency virus (HIV) seropositivity (6, 15, 18, 29, 33, 36). HIV has been recovered from genital ulcers in HIV-seropositive prostitutes (17). Genital ulcer disease may enhance HIV transmission by providing an accessible portal of entry or by rendering an HIV-seropositive patient more infectious (33, 36).

To understand the pathogenesis of *H. ducreyi* infections and to develop reagents to diagnose and prevent chancroid, several studies have examined the antigenic properties of *H. ducreyi* surface components. The outer membrane protein (OMP) and lipooligosaccharide profiles of *H. ducreyi* are similar to those of other nonenteric gram-negative organisms (2, 21). Several of these surface structures, including a 4,800-molecular-weight (4.8K) lipooligosaccharide species and a 29K, a 40K, and a 62K OMP, are antigenically conserved among strains (8, 13, 14, 31). There is little information that characterizes these conserved OMPs or their epitopes.

In this study, we describe the presence of a conserved, surface-exposed epitope on an 18K OMP in all *H. ducreyi* strains tested and present preliminary characterization of the 18K protein. We also examine the prevalence of antibodies to the 18K protein in patients who present with genital ulcer diseases.

* Corresponding author.
† Present address: Department of Medicine, SUNY Clinical Center, BB109, Erie County Medical Center, Buffalo, NY 14215.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *H. ducreyi* 33921 (Kenya) and 27722 were obtained from the American Type Culture Collection. Strains A75, A76, A77 (Institute Pasteur), and 409 (Kenya) were provided by Alan Ronald and were described previously (26). Isolates CIP 542 (the type strain; Vietnam, 1954), 82-029362 (California, 1982), 84-018676 (West Palm Beach, Fla., 1984), 85-023233 (New York, N.Y., 1985), 35000 (Winnipeg, Canada, 1975), Hd183 (Singapore, 1982), Hd187 (Kenya, 1982), and Hd188 (Kenya, 1982) were provided by William Schalla, Stephen Morse, and Samuel Sarafian of the Centers for Disease Control. Strains NYC5, NYC8, NYC17, NYC19, NYC20, NYC22, and NYC23 were isolated during an outbreak of chancroid in New York, N.Y., during 1989 and provided by Yvonne Faur of the New York City Department of Health; strains R1, R2, and R3 were isolated in Rochester, N.Y., during 1989; strains 41916, 46666, 48617, 68417, 77804, 78650, 5489, 6644, and 7994 were isolated in Boston during 1989 and 1990 and provided by Peter Rice, Boston City Hospital, Boston, Mass.; strains C138 and STD101 were isolated in Dallas in 1990 and provided by Eric Hansen, University of Texas Health Science Center at Dallas. The identity of the strains was confirmed by colonial morphology, Gram stain, requirement for X factor but not V factor, oxidase positivity, catalase negativity, and the inability to ferment dextrose, lactose, and sucrose. The strains were grown in brain heart infusion broth containing 25 μg of hemin per ml, 1% IsoVitaleX, and 5% fetal bovine serum or chocolate agar supplemented with 1% IsoVitaleX at 35°C in a 5% CO_2 atmosphere.

Nontypeable *Haemophilus influenzae* 3198 and 1479 were obtained from Timothy Murphy of the State University of

New York at Buffalo and were described previously (23). *Haemophilus aegyptius* strains were provided by Alan Lesse of the Buffalo Veterans Administration Hospital, and *Bordetella pertussis* strains were provided by David Dyer of the State University of New York at Buffalo. Other *Haemophilus*, *Neisseria*, *Actinobacillus*, and *Branhamella* sp. strains and gram-negative strains were obtained from our collection in the Division of Infectious Disease at the State University of New York at Buffalo. *Bordetella* strains were grown on Stainer-Scholte agar with 10% defibrinated sheep blood at 37°C in a 5% CO_2 atmosphere. All other strains were grown on chocolate agar at 35°C in a 5% CO_2 atmosphere.

Escherichia coli JM109 transformed with the recombinant plasmid pBUD5 that encodes *H. influenzae* protein 6 (P6) was supplied by Timothy Murphy and maintained on LB medium with 50 μg of ampicillin per ml (25).

Isolation of outer membranes. Whole membranes were prepared from French pressure cell lysates of *H. ducreyi* and separated into inner and outer membrane fractions by Sarkosyl extraction as described previously (35).

Isolation of peptidoglycan-associated proteins. Isolation of peptidoglycan-associated proteins was accomplished through a modification of the methods of Munson and Granoff and Murphy et al. (22, 23). Bacteria were scraped from plates and incubated in a buffer containing 1% sodium dodecyl sulfate (SDS), 0.1 M Tris HCl, 0.5 M NaCl, and 0.1% 2-mercaptoethanol (pH 8.0) at 37°C for 30 min. Insoluble material was removed by centrifugation at 21,000 × *g* for 30 min at room temperature. The pellet was suspended in the SDS buffer by sonication, incubated, and centrifuged five times prior to analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Amino acid analysis of the SDS-insoluble material was determined by Audree Fowler (UCLA Protein Microsequencing Facility) by using the Pico-Tag system after the material was hydrolyzed in 6 N HCl.

Monoclonal antibodies (MAbs). BALB/c mice were immunized intraperitoneally with whole cells of nontypeable *H. influenzae* 3198 on days 0 and 28. On day 32, splenic lymphocytes were fused to Sp2/0-Ag14 plasmacytoma cells by a modification (4) of the method of Kennett (16). Hybridomas were screened in an immunodot assay by using whole bacteria as target antigens and tested in a Western blot (immunoblot) assay to confirm their specificity (4).

Serum. Normal human sera (NHS) were obtained from laboratory volunteers with no history of genital ulcer disease and pooled. Serum samples were obtained from one patient with chancroid seen at the Erie County Medical Center, Buffalo, N.Y., and six patients seen at the Monroe County Department of Health Sexually Transmitted Disease Clinic, Rochester, N.Y. Serum samples were collected at the time of presentation and, in one case, 8 months later. Serum samples were also obtained from patients with other genital ulcer diseases (syphilis and genital herpes) at the time of presentation. The diagnosis of chancroid was documented by culture or by an immunofluorescence assay of lesion material done by William Schalla of the Centers for Disease Control (31). Syphilis was diagnosed by a positive direct fluorescent-antibody test of lesion material or by serology, and herpes simplex was diagnosed by culture. No coexisting infections by genital ulcer disease agents were identified in the patients studied.

SDS-PAGE and Western blot assays. Whole bacteria, membrane fractions, and cell wall-associated proteins were solubilized and subjected to SDS-PAGE in 15% acrylamide gels by the method of Laemmli (19) and stained with Coomassie brilliant blue or transferred to nitrocellulose (4). In some experiments, OMPs were treated with proteinase K (25 mg/ml) before solubilization. Western blots were probed with tissue culture supernatants, protein A-peroxidase (Zymed Laboratories, Burlingame, Calif.), and horseradish peroxidase color developer (Bio-Rad Laboratories, Richmond, Calif.) as described previously (34). Western blots probed with 1/500 dilutions of human serum were developed with ^{125}I-labelled protein A (Amersham Corp., Arlington Heights, Ill.) by methods described previously (35).

Immunoelectron microscopy. Bacteria were scraped from plates, suspended in distilled water, placed on Formvar-coated nickel grids (Ladd Research Industries, Burlington, Vt.), and allowed to dry. The grids were probed with 3B9 or Sp2/0 tissue culture supernatants, washed, stained with goat anti-mouse immunoglobulin G (IgG) coupled to 15-nm colloidal gold spheres (Janssen Biotechnology, Piscataway, N.J.), and viewed with a Hitachi H-7000 electron microscope (Hitachi Scientific Instruments, Mountain View, Calif.) at 75 kV as described previously (5). The number of gold particles that bound to the first 100 consecutive cells examined on a grid was recorded. The data were analyzed by determining the distribution of cells that bound various numbers of gold particles (27) and by statistical comparisons between groups. Since we could make no assumptions about the expected distribution of the data, the counts obtained when a strain was probed with MAb 3B9 were compared with those obtained with Sp2/0 tissue culture supernatant in a nonparametric (Mann-Whitney U) test. Comparisons among three or more groups were made in a nonparametric (Kruskal-Wallis) test by using a Macintosh SE computer and a Statview 512+ program (Brainpower, Inc., Ventura, Calif.).

Colony blot assay. Bacteria were applied to nitrocellulose with a toothpick and probed with 3B9 or Sp2/0 tissue culture supernatants, protein A-peroxidase, and horseradish peroxidase color developer as described previously (34). To examine the 3B9 epitope for phase variation, a single 3B9-positive colony of *H. ducreyi* was grown in broth to the mid-log phase and dilutions were spread onto chocolate agar plates at 200 to 300 CFU per plate. The colonies were blotted onto a nitrocellulose disc and probed as described above. The number of colonies that bound 3B9 was compared with the number of colonies present on the plate.

Competition enzyme-linked immunosorbent assay (ELISA). Cell suspensions of *H. ducreyi* were prepared from 24-h-old chocolate agar plates and fixed to microtiter wells exactly as described previously (7). The MAb 3B9 was affinity purified from tissue culture supernatants by passage over a protein A-Sepharose column and diluted in phosphate-buffered saline (PBS)-Tween 20 (0.05%, vol/vol). To generate binding curves for 3B9 to the bacteria, microtiter plates were incubated with increasing concentrations of the antibody overnight at room temperature, washed, and developed with horseradish peroxidase-conjugated goat antibody to mouse IgM and IgG (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and *o*-phenylenediamine substrate (Sigma Chemical Co., St. Louis, Mo.) according to the manufacturer's instructions. Fifty percent saturation of 3B9 binding was reproducibly achieved at an antibody concentration of 25 μg/ml.

The ability of serum to inhibit the binding of 3B9 to *H. ducreyi* was determined in a competition ELISA exactly as described by Black et al. (7) with the following modifications. Wells coated with bacteria were incubated in triplicate with PBS-Tween containing 3B9 (25 μg/ml) and serial dilu-

TABLE 1. Binding of MAb 3B9 to whole cells of members of the family *Pasteurellaceae* in colony blots

Species	No. of strains	
	Tested	Positive
Haemophilus ducreyi	35	35
Haemophilus influenzae	14	14
Haemophilus aegyptius	5	5
Haemophilus aphrophilus	3	3
Haemophilus parahaemolyticus	1	1
Haemophilus parainfluenzae	2	0
Haemophilus paraphrophilus	1	1
Haemophilus paraphrohaemolyticus	1	1
Haemophilus segnis	1	1
Actinobacillus actinomycetemcomitans	2	2

tions of human sera (from 1/50 to 1/800) overnight at room temperature. Control wells were incubated with PBS-Tween, 3B9, or dilutions of human sera alone. The microtiter plates were washed and developed with goat anti-mouse IgM and IgG as described above. Optical density readings were determined at 490 nm and corrected for background binding (A_{490} of wells incubated with human serum alone). Percent inhibition of 3B9 binding was calculated for each serum sample by comparing test values with those of controls that were probed with 3B9 alone.

RESULTS

Binding of the MAb 3B9 to *H. ducreyi* and other members of the family *Pasteurellaceae*. In colony blots, the MAb 3B9 (isotype IgG 2a) bound to 35 of 35 *H. ducreyi* strains tested and to many members of the family *Pasteurellaceae* (Table 1). There was no detectable binding of 3B9 to colonies of 4 *Neisseria*, 3 *Branhamella*, and 2 *Bordetella* sp. strains or to colonies of 14 other gram-negative (*Escherichia*, *Salmonella*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Serratia*, *Enterobacter*, and *Morganella* sp.) strains tested.

In Western blots of solubilized *H. ducreyi* whole cells, 3B9 bound to a protein whose apparent molecular weight was 18K in 35 of 35 strains (Fig. 1). The MAb cross-reacted with proteins whose apparent molecular weights ranged from 16K to 19K in other *Haemophilus* and *Actinobacillus* sp. strains (Fig. 1). The MAb bound to a 16K OMP of *H. influenzae* and to a 16K protein in whole-cell lysates of *E. coli* transformed with a recombinant plasmid (pBUD5) that encodes the *H. influenzae* peptidoglycan-associated lipoprotein, designated P6 or PAL (25). Although 3B9 did not bind to *E. coli* K-12 derivatives in colony blots, there was variable binding of 3B9 to a 35K protein in whole-cell lysates of *E. coli* in Western blots (data not shown).

Characterization of the *H. ducreyi* 18K protein. To determine the cellular localization of the 18K protein, Sarkosyl extraction was used to fractionate outer and inner membranes. The 18K protein was present in the Sarkosyl-insoluble fractions prepared from strain 85-023233 and seven other *H. ducreyi* strains tested (Fig. 2 and data not shown). The 18K protein was not heat modifiable, and binding of 3B9 to its epitope was destroyed by digestion of the OMPs by proteinase K (Fig. 2).

To test whether the *H. ducreyi* 18K OMP and *H. influenzae* P6 had similar physical characteristics, peptidoglycan-associated proteins were prepared from *H. ducreyi* 85-023233 and an *H. influenzae* control (22, 23). Bacteria were lysed in a buffer containing 1% SDS, and SDS-insoluble material was obtained by repeated cycles of solubilization and centrifugation. Analysis of the *H. ducreyi* SDS-insoluble material showed that it contained amino acids present in peptidoglycan (36% alanine, 32% diaminopimelic acid, 22% glutamic acid, and 3% lysine). The *H. ducreyi* 18K OMP was soluble in SDS (Fig. 2), and a 25K protein that did not bind 3B9 was precipitated by this procedure. The majority of P6 was precipitated from the *H. influenzae* control (Fig. 2).

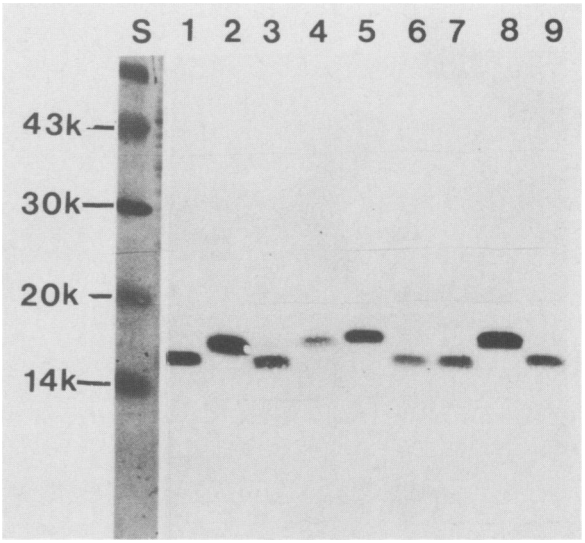

FIG. 1. Western blot of whole-cell preparations probed with 3B9. Lane S contains molecular weight standards. Lanes: 1, nontypeable *H. influenzae*; 2, *H. ducreyi*; 3, *H. aegyptius*; 4, *Actinobacillus actinomycetemcomitans*; 5, *Haemophilus aphrophilus*; 6, *Haemophilus parahaemolyticus*; 7, *Haemophilus paraphrohaemolyticus*; 8, *Haemophilus paraphrophilus*; 9, *Haemophilus segnis*.

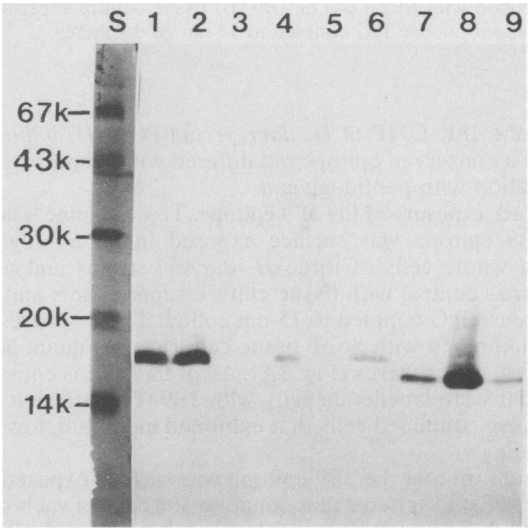

FIG. 2. Western blot of OMPs, whole cells, and peptidoglycan-associated proteins from *H. ducreyi* 85-023233 (lanes 1 to 6) or *H. influenzae* 1479 (lanes 7 to 9) probed with 3B9. Lanes: 1, OMPs solubilized at 37°C; 2, OMPs solubilized at 100°C; 3, OMPs treated with proteinase K; 4 and 7, whole cells before SDS treatment; 5 and 8, SDS-insoluble material; 6 and 9, SDS-soluble material. Molecular mass markers are shown in lane S.

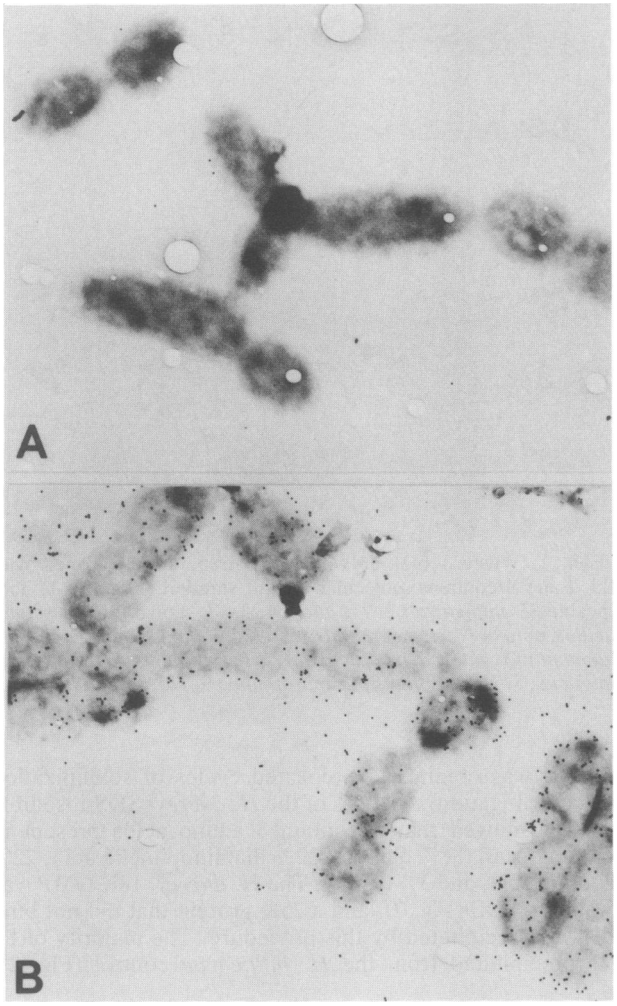

FIG. 3. Electron micrographs of *H. ducreyi* 82-029362 whole cells probed with SP2/0 (A) or 3B9 (B) tissue culture supernatants and goat anti-mouse IgG coupled to 15-nm gold spheres.

Thus, the 18K OMP of *H. ducreyi* and P6 of *H. influenzae* shared a conserved epitope but differed with respect to their association with peptidoglycan.

Surface exposure of the 3B9 epitope. To determine whether the 3B9 epitope was surface exposed in *H. ducreyi*, we probed whole cells of three *H. ducreyi* strains and an *H. influenzae* control with tissue culture supernatants and goat anti-mouse IgG coupled to 15-nm colloidal gold spheres. All bacteria probed with Sp2/0 tissue culture supernatant bound no or few gold spheres (Fig. 3A). All of the strains contained cells that were labelled heavily with 3B9 (Fig. 3B), but each strain also contained cells that exhibited moderate, low, and no binding.

To confirm that the 3B9 epitope was surface exposed, the number of gold spheres that bound to 100 cells of each strain after incubation with 3B9 was statistically compared with the number obtained with an Sp2/0 control. The distribution of cells that bound 0, 1 to 5, 6 to 10, 11 to 25, 25 to 50, and >50 gold particles are shown in Table 2. For the four strains tested, there was intrastrain variation in the level of 3B9 surface labelling, and the level of 3B9 binding was significantly greater than that of the Sp2/0 control (Mann-Whitney U test; all P values = 0.0001). Since the level of binding in all of the controls was essentially 0, the levels of 3B9 binding among the strains were compared, and there was significant interstrain variation in the amount of 3B9 surface labelling among the four strains (Kruskal-Wallis test; $P = 0.0001$). We conclude that the 3B9 epitope was probably surface exposed in *H. influenzae* and *H. ducreyi* and that there was variation in the surface exposure of the epitope among strains under the conditions tested.

TABLE 2. Surface labelling of bacteria with 3B9

Strain	Probe	Distribution of cells in the scoring classes						Median particles/cell
		0[a]	1–5	6–10	11–25	25–50	>50	
3198	Sp2/0	51	49					0
	3B9	10	22	10	16	20	22	16
82-029362	Sp2/0	71	29					0
	3B9	3	24	20	35	16	2	12
R3	Sp2/0	56	44					0
	3B9	18	45	17	17	3		3
85-023233	Sp2/0	56	44					0
	3B9	22	56	15	6	1		2

[a] Number of gold particles bound.

Phase variation of the 3B9 epitope. Because of the disparity in 3B9 surface labelling, a strain that exhibited low binding (85-023233) was examined for phase variation of the epitope. A single 3B9-positive colony was grown in broth, dilutions were spread onto plates, and colonies were blotted onto nitrocellulose discs and probed with 3B9. All 3,459 colonies tested bound 3B9. Thus, the 3B9 epitope did not phase vary in colonies of *H. ducreyi* at a frequency of $>10^{-3}$.

Prevalence of antibodies to the 18K OMP in patients with genital ulcer diseases. To determine whether sera from patients with genital ulcer diseases contained antibodies of the IgG class to the 18K OMP, *H. ducreyi* OMPs were probed with 3B9 and patients' sera in Western blots (Fig. 4). Although all chancroid cases had been confirmed by culture or immunofluorescence, many of the strains did not survive storage. In SDS-PAGE, the remaining strains (R1, R2, R3) had identical OMP profiles; OMPs prepared from R3 were used in these assays. Serum samples obtained at the time of presentation from five of seven patients with chancroid contained antibodies to a band that comigrated with the 18K OMP. Serum samples from two of three patients with syphilis and two of two patients with genital herpes also contained antibodies to a band comigrating with the 18K OMP, as did pooled NHS (data not shown). Thus, patients with chancroid or other genital ulcer diseases and NHS frequently had antibodies to the 18K OMP.

Competition ELISA. To determine if serum antibodies to the 18K protein were directed against the 3B9 epitope, *H. ducreyi* R3 was fixed to microtiter plates and probed with dilutions of human serum mixed with a constant concentration (25 μg/ml) of 3B9. Percent inhibition of 3B9 binding was calculated for each serum sample by comparing test values with those of controls that were probed with 3B9 alone. At a dilution of 1/50, all serum samples tested inhibited 3B9 binding to *H. ducreyi* by more than 50%: serum sample no. 4, from a subject with chancroid, exhibited 67.0% ± 6.1% inhibition; serum sample no. 7, from a subject with chancroid, exhibited 62.3% ± 3.5% inhibition; serum sample no.

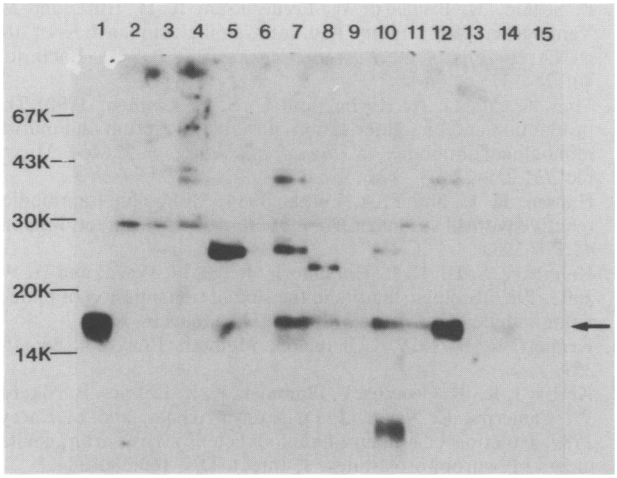

FIG. 4. Western blot of OMPs isolated from *H. ducreyi* R3 and probed with MAb 3B9 or sera from patients with genital ulcer diseases. Lane 1 was probed with 3B9; lanes 2 to 4 and 6 to 10 were probed with sera from patients with chancroid; lanes 11 and 12 were probed with sera from patients with genital herpes; lanes 5, 13, and 14 were probed with sera from patients with syphilis. In lane 15, serum was omitted from probing. Serum samples were obtained from one of the chancroid patients at presentation (lane 2) and 8 months later (lane 3); the remainder of the serum samples were obtained at the times of presentation. The relative migration of the molecular mass markers is shown at the left. The autoradiogram was developed after 36 h of exposure. Note that 3B9 bound to the 18K OMP (designated by the arrow) and that many serum samples contained antibodies to a band that comigrated with the 18K OMP.

13, from a subject with syphilis, exhibited 58.3% ± 1.4% inhibition; and the pooled NHS sample exhibited 58.6% ± 5.5% inhibition. (Values were derived from three separate experiments. Serum sample numbers correspond to lane numbers in Fig. 4.) No inhibition was achieved at serum dilutions greater than 1/800. Thus, serum samples from patients with genital ulcer diseases and pooled NHS contained antibodies that either bound to the 3B9 epitope or sterically hindered the binding of 3B9 to its epitope.

DISCUSSION

The MAb 3B9 recognized an epitope present on 35 of 35 *H. ducreyi* strains tested. In colony blots, 3B9 also bound to many members of the family *Pasteurellaceae*. Taxonomic and evolutionary relationships among members of the family *Pasteurellaceae* are not fully understood. *H. ducreyi* is classified in a distinct branch of rRNA superfamily I and is only remotely related to the core of the *Pasteurellaceae* by a variety of criteria including rRNA hybridization, DNA hybridization, ability of DNA to compete for transformation, and isoprenoid quinone content (3, 10–12). *H. ducreyi* had been originally classified as a member of the genus *Haemophilus* because of its growth requirements, biochemical properties, and antigenic relatedness to other *Haemophilus* species (1, 10, 21, 30, 31). The conservation of the 3B9 epitope may account for some of the observed immunologic cross-reactivity and may represent evolutionary convergence rather than evolutionary relatedness between *H. ducreyi* and other *Haemophilus* sp. strains.

The MAb 3B9 was raised by immunization with *H. influenzae* and bound to an SDS-insoluble 16K protein of *H. influenzae*, designated P6 or peptidoglycan-associated lipoprotein (PAL). The MAb 3B9 bound to an 18K OMP in *H. ducreyi* and to proteins with apparent molecular weights of 16,000 to 19,000 in many *Haemophilus* and *Actinobacillus* sp. strains. Unlike *H. influenzae* P6, the 18K OMP of *H. ducreyi* did not copurify with peptidoglycan. The MAbs 4G4 and 7F3, which bind to P6 (23, 24), did not bind to the 18K OMP (data not shown). In Southern blots and colony hybridization assays done under high-stringency conditions, a 550-bp *Bam*HI restriction fragment that consisted almost entirely of P6 coding sequence did not bind to *H. ducreyi* (25) (data not shown). The N-terminal amino acid sequence of an 18K band purified by preparative SDS-PAGE and transferred to an Immobilon membrane had no homology to the predicted sequence of mature P6 (data not shown). Thus, *H. ducreyi* and *H. influenzae* contained OMPs that shared a conserved epitope and had similar apparent molecular weights but that were distinct by a number of criteria.

The MAb 3B9 bound to an epitope that did not phase vary in colonies of *H. ducreyi* at a frequency of $>10^{-3}$. In immunoelectron microscopy experiments, the immunizing *H. influenzae* strain and three *H. ducreyi* strains probed with 3B9 contained cells that stained with goat anti-mouse IgG coupled to colloidal gold spheres. Although all strains contained bacteria that were labelled heavily, there was variation in the amount of labelling of individual cells of the same strain and interstrain differences in labelling. The data suggest that the 3B9 epitope was surface exposed in *H. influenzae* and *H. ducreyi*. Similar variability in antibody binding in immunoelectron microscopy experiments has been observed for MAbs raised to gonococcal and meningococcal OMPs (27) including the H.8 MAb, which is apparently surface exposed by a number of criteria (9). Demonstration of antibody binding in immunoelectron microscopy may have been affected by fixation of the cells to a hydrophobic surface, viability or drying of the cells, or phase variation of other bacterial surface components; however, the cause of the variability in 3B9 surface labelling was unclear.

There is little information characterizing the human immune response to *H. ducreyi* infection (21). Our data showed that sera from five of seven patients with chancroid, four of five patients with other genital ulcer diseases, and pooled NHS contained antibodies of the IgG class to the 18K OMP. Four of the serum samples tested inhibited the binding of 3B9 to *H. ducreyi* by more than 50%, suggesting that some of the serum antibodies were directed against the 3B9 epitope or sterically hindered the binding of 3B9 to its epitope. The sera had been collected from the patients at the time of presentation, and most of the patients with chancroid had experienced symptoms for 1 week to 1 month before seeking medical attention. Thus, sera from patients with chancroid contained antibodies to the 18K OMP that may have resulted from infection with *H. ducreyi* or previous exposure to the cross-reacting 3B9 epitope found in other *Haemophilus* and *Actinobacillus* sp. strains. Since 3B9 variably bound to a 35K protein of *Escherichia coli* in Western blots, colonization with *E. coli* may have also led to the development of cross-reacting antibodies. Similarly, sera from healthy children and adults and convalescent-phase sera from patients with invasive nontypeable or *H. influenzae* type b disease contain antibodies to *H. influenzae* P6 (20, 24).

H. ducreyi is a fastidious organism, and many investigators have focused on the development of diagnostic probes for chancroid (13, 14, 28, 31). MAb 3B9 cross-reacted with many *Haemophilus* and *Actinobacillus* sp. strains and will probably not be a useful diagnostic reagent. Since *H. ducreyi* is not closely related to other members of the family *Pas-*

teurellaceae and the 18K OMP differed from *H. influenzae* P6 by a number of criteria, the gene or portions of the gene encoding the 18K OMP may have the potential to be the basis of an *H. ducreyi*-specific probe.

These studies demonstrate that OMPs isolated from *H. ducreyi* and other *Haemophilus* sp. strains shared a conserved, surface-exposed epitope. Although the *H. ducreyi* 18K OMP and *H. influenzae* P6 shared an epitope and had similar apparent molecular weights, these proteins differed in their primary structure and physical characteristics. The conservation of the 3B9 epitope in the members of the family *Pasteurellaceae* suggests that the 3B9 epitope may be important in bacterial survival on mucosal surfaces. Future studies will be directed towards examining the role of the 3B9 epitope and the 18K OMP in *H. ducreyi* pathogenesis.

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