Anti-Gal Binds to Pili of *Neisseria meningitidis*: the Immunoglobulin A Isotype Blocks Complement-Mediated Killing†

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α1,3-Galactosyl antibodies (anti-Gal) are ubiquitous natural human serum and secretory polyclonal antibodies that bind to terminal galactose-α1,3-galactose (α-galactosyl) residues. Serum immunoglobulin G (IgG) anti-Gal can block alternative complement pathway-mediated lysis of representative gram-negative enteric bacteria that bind it to lipopolysaccharide α-galactosyl structures, thereby promoting survival of such bacteria in the nonimmune host. We wanted to know whether anti-Gal also could bind to the lipooligosaccharides (LOS) of *Neisseria meningitidis*. To our surprise, we found that serum and secretory anti-Gal bound to pili but not to LOS of certain strains. This suggested the presence of an immunogenic pilus carbohydrate epitope. Mild periodate oxidation of sodium dodecyl sulfate-polyacrylamide gel electrophoresis-separated outer membrane preparations from strains that bound anti-Gal followed by labeling of the neoaldehyde groups resulted in the labeling of bands that corresponded to pilin and LOS, confirming that pilin contains carbohydrate structures. A *Bandeiraea simplicifolia* lectin that also binds terminal α1,3-galactosyl residues also bound to pilin. Serum IgG, IgA, and IgM anti-Gal as well as colostral secretory IgA anti-Gal bound to pilin, as judged by immunoblotting, and to the pili of intact piliated organisms, as judged by immunoelectron microscopy. Total serum anti-Gal (IgG, IgA, and IgM) and purified serum IgA1 anti-Gal, but not its purified IgG isotype, blocked complement-mediated lysis of a piliated meningococcal strain that bound anti-Gal to its pili. Colostral anti-Gal secretory IgA blocked killing of the same strain. Thus, anti-Gal IgA may promote disease when it binds to the pili of *N. meningitidis* strains.

α1,3-Galactosyl antibodies (anti-Gal) are ubiquitous natural human polyclonal antibodies that bind to terminal galactose-α1,3-galactose (α-galactosyl) residues (15). Serum anti-Gal was thought to be predominantly immunoglobulin G (IgG) and to constitute around 1% of total circulating IgG (16). We have recently purified anti-Gal IgM and anti-Gal IgA antibodies from normal human serum (NHS) in addition to the previously described anti-Gal IgG (24). We have also described secretory antibodies with α-galactosyl specificities similar to those of serum anti-Gal in human colostrum, milk, saliva, bile, and vaginal washings (24).

Enteric gram-negative bacteria that cause sepsis are more likely to bind serum anti-Gal than are bacteria of the same species that are isolated from the stools of well persons (25). This may be because serum IgG anti-Gal can block alternative complement pathway-mediated lysis of representative bacteria that bind it to lipopolysaccharide (LPS) α-galactosyl structures, thereby promoting survival of such bacteria in the nonimmune host (25).

We were interested in examining whether anti-Gal binds to the lipooligosaccharides (LOS) of *Neisseria meningitidis*, analogous to its binding to LPS of enteric gram-negative bacteria. To our surprise, we found that serum and secretory anti-Gal bound to the pili but not to the LOS of certain *N. meningitidis*

strains. This suggested the presence of an immunogenic carbohydrate epitope on meningococcal pili. Furthermore, we found that serum anti-Gal IgA1 as well as colostral anti-Gal secretory IgA (sIgA) blocked complement-mediated lysis of a meningococcal strain that bound anti-Gal to its pilus.

Pili are found on meningococci and gonococci isolated from human mucosal surfaces (8) and are also found on meningococci isolated from the blood and cerebrospinal fluid of patients with disseminated meningococcal disease (9, 41, 43). The presence of blocking mucosal and serum anti-Gal that bind to meningococcal pili therefore may be of pathogenic importance. Our findings, presented below, support a role for anti-Gal in meningococcal disease.

MATERIALS AND METHODS

Bacterial strains. *N. meningitidis* 15515 (C:2a:1.2:L3,7,9) and 15516 (C:2a:1.2:L3,7,9) were isolated from siblings with contemporaneous disseminated meningococcal disease and are probably the same. *N. meningitidis* 7971 (B:2b:1.2.5:L2), 7953 (C:NT:1.13:L2,4), and 8025 (B:2b:1.2.5:L3,4,6,7) were selected from an epidemiologically unbiased sample of meningococci that can cause endemic disease among children that was collected from pediatric patients with disseminated meningococcal disease in Houston, Tex., from February 1977 through March 1978 (2, 5, 11, 20). We have extensively characterized *N. meningitidis* 126E (C:NT:1.2:L1,8) (12) and 120M (A:21:1.10:L9) (49); they are from the collections of the Walter Reed Army Institute of Research (WRAIR).

We used the solid-phase radioimmunoassay inhibition technique with the original polyclonal rabbit antisera developed by Zollinger and Mandrell (48, 49) to serotype by LOS the Houston and WRAIR strains. This system arbitrarily sets 35% of the inhibition of a type-specific antiserum by the homologous prototype strain as the threshold criterion for a serotype designation (48). Brenda Brandt contributed the protein serotypes of the Houston and WRAIR strains; she used monoclonal antibodies. We serotyped by protein and LOS strains 15515 and 15516 by use of established monoclonal antibodies (11, 31, 32).

Outer membrane protein (OMP) complexes were prepared from each strain by methods described previously by our laboratory (31).

When grown at 37°C on laboratory media, meningococci are poorly piliated. We maximized piliation by growing the organisms in gonococcal broth under

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increased CO₂ tension (candle extinction) at 30°C and without shaking (static growth) (3). Under these conditions, the organisms form a pellicle at the surface of the broth. For each batch, organisms were harvested and their piliation was confirmed by electron microscopy (see below). They were then stored at -70°C in skim milk, replated on gonococcal base agar (Difco Laboratories, Detroit, Mich.), and incubated at 37°C when needed; piliation is maintained under these conditions (3).

Purification of meningococcal pili. We used modifications of published methods for the purification of gonococcal pili (4) to purify pili from strain 15515. The organism was grown at 30°C in static gonococcal broth to maximize piliation (3). The basis for the purification procedure for pili was the crystallization of pili under one set of ionic conditions (0.05 M Tris-buffered saline [pH 7.5]) and separation of pilus crystals from soluble impurities by centrifugation, followed by solubilization of the pilus crystals under another set of ionic conditions (0.15 M ethanolamine buffer [pH 10.1]) and removal of particulate impurities by centrifugation. Alternate cycles of crystallization and solubilization allowed for the production of pili with very high purity. Purified pili were visualized by silver staining of reduced and native pili separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (26).

Antibodies. A rabbit polyclonal antibody raised against the cyanogen bromide fragment 2 (CNBr2) of *Neisseria gonorrhoeae* R10T1 pili (44) (rabbit anti-CNBr2) was the kind gift of George F. Brooks, University of California, San Francisco.

Purification of whole serum, serum IgG and IgA1, and colostral sIgA anti-Gal and preparation of anti-Gal-depleted NHS. Total serum anti-Gal (containing IgG, IgM, and IgA) was purified from serum obtained from a laboratory volunteer with no prior history of meningococcal or gonococcal infection. Serum anti-Gal IgG was purified from commercially available pooled gamma globulin (Gamimune N; Miles Inc., Elkhart, Ind.). Colostral anti-Gal sIgA was purified from commercially available pooled colostral sIgA (Sigma Chemical Co., St. Louis, Mo.).

Anti-Gal was purified by affinity chromatography with silica-linked Gal α 1-3Gal β 1-4GlcNAc (SYN 115; Chembiomed, Alberta, Canada), with minor modifications of our previously described method (25). Briefly, serum, Gamimune N, or colostral sIgA was loaded onto the SYN 115 column. The column was washed first with 0.5 M NaCl and then with phosphate-buffered saline (PBS) prior to elution of the bound antibody with 0.1 M glycine HCl (pH 2.6). Antibody preparations were dialyzed extensively against PBS. Serum anti-Gal IgG was concentrated to 100 μ g/ml, which is the concentration of anti-Gal in NHS (15). Colostral anti-Gal sIgA was concentrated also to 100 μ g/ml.

Serum IgA1 anti-Gal was purified from whole serum anti-Gal by jacalin column chromatography, as described previously (28), and was concentrated to 8 μ g/ml, which is within the NHS concentration of serum IgA1 anti-Gal (24).

NHS depleted of anti-Gal was prepared by repeated passage of this serum over SYN 115 at 0 to 4°C, as reported previously (25).

Binding of anti-Gal to outer membrane complex and pilin as judged by immunoblotting. We studied the binding of serum and colostral sIgA anti-Gal to selected *N. meningitidis* isolates by immunoblotting analysis. Outer membrane complexes that contained LOS and proteins were prepared from each strain, as outlined by Hitchcock and Brown (27); outer membrane complexes and purified pili from *N. meningitidis* 15515 were applied to discontinuous slab gels (3% acrylamide spacer gel, 12.9% resolving gel) and electrophoresed (by SDS-PAGE) (33). These samples were then transferred to nitrocellulose by the Western blot (immunoblot) transfer method of Burnette (6) and incubated for 2 h at room temperature in a 1% casein solution of a 2- μ g/ml concentration of whole serum or colostral sIgA anti-Gal. The transblots were then washed with PBS and incubated with an alkaline phosphatase-labeled goat anti-human IgG, IgA, and IgM (Sigma) secondary antibody mixture for serum anti-Gal and a goat anti-human IgA antibody (Sigma) for colostral sIgA anti-Gal. A naphthol substrate (1 mg/ml) and fast red solution (2 mg/ml; Sigma) in 50 mM Tris buffer (pH 8) were then added, and the development of pink color was assessed visually.

In some experiments, we added either goat anti-human IgG, IgA, or IgM secondary antibody to examine which of the serum anti-Gal isotypes was responsible for binding. Substrate was then added as outlined above.

Detection of carbohydrate residues on meningococcal pilin. We used a glycan detection kit (Boehringer Mannheim, Indianapolis, Ind.) to determine whether pilins have carbohydrate structures. We treated nitrocellulose-immobilized SDS-PAGE-separated OMPs from meningococcal strains with periodate as described in the manufacturer's instructions. This treatment oxidized adjacent hydroxyl groups of sugars to aldehyde groups. We then added digoxigenin, a steroid hapten that covalently bound to these aldehydes via a hydrazide group. Digoxigenin-labeled glycoconjugates were then incubated with sheep anti-digoxigenin Fab fragments linked to alkaline phosphatase and visualized by the addition of substrate, as outlined above.

Binding of Bandeiraea simplicifolia lectin to SDS-PAGE-separated pilin. The *B. simplicifolia* lectin is a plant lectin that binds to cell membrane glycoconjugates bearing terminal Gal α 1-3Gal residues (13). *N. meningitidis* 15515 pilin was prepared, separated by SDS-PAGE, and transferred to nitrocellulose as outlined above. The transblot was incubated for 1 h at room temperature in a 1% casein solution and then for 2 h at room temperature in a 30- μ g/ml solution of biotinylated *B. simplicifolia* lectin I (Vector Laboratories Inc., Burlingame, Calif.) in 1% casein. The transblots were then washed with PBS three times for 5 min each time and incubated with avidin followed by alkaline phosphatase-linked biotin

FIG. 1. Immunoblot of SDS-PAGE-separated OMPs from *N. meningitidis* 15515 (lane 1), 15516 (lane 2), and 7971 (lane 3) reacted with rabbit anti-gonococcal CNBr2 (A) and whole serum anti-Gal (B). Molecular size markers (in kilodaltons) are shown on the left.

(Vectastain ABC-AP kit) as per the manufacturer's instructions (Vector Laboratories). Substrate was then added as outlined above.

Binding of anti-Gal to capsular polysaccharides. Group A, B, and C meningococcal capsular polysaccharides were extracted as described in published methods (26). Capsular polysaccharides were diluted in PBS to a final concentration of 100 μ g/ml, and 5 μ l of this solution was placed on a piece of nitrocellulose and air dried. Incubations with anti-Gal, secondary antibody, and substrate were as outlined above.

IEM of serum IgG and colostral sIgA anti-Gal bound to intact bacteria. We followed immunoelectron microscopy (IEM) methods that we published previously (25). Briefly, strain 15515 was grown at 30°C in static gonococcal broth to maximize piliation, pelleted, fixed with 2% paraformaldehyde and 0.1% glutaraldehyde, dehydrated with 2.3 M sucrose, and then sectioned onto copper grids. The grids were either presensitized or not with serum IgG or colostral sIgA anti-Gal (100 μ g/ml) for 30 min at room temperature; they were then washed with PBS, incubated for 30 min at room temperature with a 1:500 solution in PBS of gold-labeled goat anti-human IgG for serum IgG anti-Gal and goat anti-human IgA for colostral sIgA anti-Gal, washed again with PBS, and examined.

Purification of anti-Gal that had bound pilin. We eluted the anti-Gal that had bound to pilin by incubating anti-Gal transblots with 0.1 M glycine HCl (pH 2.6) for 5 min, rapidly neutralizing the eluted antibody with 1 M Tris-HCl (pH 9), and purifying the eluted anti-Gal IgG by protein G affinity chromatography (Pharmacia LKB Biotechnology, Piscataway, N.J.) (28).

RRBC hemagglutinating activity of purified pilin anti-Gal. Purified pilin anti-Gal (see above) was tested for its ability to hemagglutinate rabbit erythrocytes (RRBCs). RRBCs have abundant surface Gal α 1-3Gal β 1-4GlcNAc structures (the cognate epitope for anti-Gal) and have been used extensively to screen for the presence of anti-Gal and to determine its titer (16). Briefly, we mixed twofold serial dilutions of pilin anti-Gal with an equal volume of a 0.5% RRBC suspension in V-shaped wells of a microtiter plate. The diluent was PBS with a pH of 7.4. Agglutination was evaluated after the samples had settled for 1 h at room temperature. Titers were expressed as the reciprocal of the highest dilution that caused complete agglutination.

Bactericidal assay. We used a standard bactericidal assay (25) to study the effect of whole serum anti-Gal (containing IgG, IgM, and IgA), serum IgG anti-Gal, serum IgA1 anti-Gal, and colostral sIgA anti-Gal on complement-mediated killing of strain 15515. We used 20% NHS in all assays. The serum (complement source) was obtained from an individual with no prior history of meningococcal disease and bound only faintly to the pilin of strain 15515 by immunoblot. The concentrations of whole serum anti-Gal used in the reaction mixtures were 100 to 200 μ g/ml, and that of serum IgG anti-Gal was 100 μ g/ml, similar to that reported for this isotype in NHS (15). We used a concentration of colostral sIgA anti-Gal of 100 μ g/ml. Serum IgA anti-Gal was used at a concentration of 8 μ g/ml, which is within the normal concentration of this isotype in NHS (24).

RESULTS

Anti-Gal binds the pilin of certain *N. meningitidis* strains. We were interested in examining whether anti-Gal could bind to OMPs from various *N. meningitidis* strains that had caused disseminated infections. Figure 1 is an immunoblot of SDS-PAGE-separated OMPs from strains 15515 (lane 1), 15516

TABLE 1. Binding of anti-Gal to strains of *N. meningitidis*

Strain	Group	Type		Binding of anti-Gal to SDS-PAGE-separated outer membrane complex
		LOS	Protein	
126E	C	L1,8	NT:1.2	None
7971	B	L2	2b:1.2,5	To a ca. 16-kDa band
7953	C	L2,4	NT:1.13	To a ca. 60-kDa band
8025	B	L3,4,6,7	2b:1.2,5	None
15515 ^a	C	L3,7,9	2a:1.2	To a ca. 14-kDa band
15516 ^a	C	L3,7,9	2a:1.2	To a ca. 14-kDa band
120M	A	L9	21:1.10	None

^a These strains were isolated contemporaneously from the cerebrospinal fluid of siblings and probably are the same.

(lane 2), and 7971 (lane 3), with rabbit CNBr2 antibody (Fig. 1A) and whole serum anti-Gal (Fig. 1B). Anti-Gal and the rabbit CNBr2 antibody bound to identical bands from these strains, indicating that anti-Gal bound to the pilin of all three organisms. Proteinase K treatment of the OMP samples abolished binding of anti-Gal to them (data not shown).

Anti-Gal bound to pilus proteins made by the L3,7,9 strains, 15515 and 15516, and by an L2 and an L2,4 strain; they did not bind to pilus proteins made by an L1,8, an L3,4,6,7, or an L9 strain (Table 1).

All three isotypes of serum anti-Gal bind pilin. We next examined which of the three isotypes of circulating anti-Gal bound to pilin. Figure 2 is an immunoblot of SDS-PAGE-separated OMPs from strains 15515 (lane 1), 7971 (lane 2), and 15516 (lane 3) and purified pilin from strain 15515 (lane 4) reacted with whole serum anti-Gal and then goat anti-human IgG (Fig. 2A), goat anti-human IgA (Fig. 2B), and goat anti-human IgM (Fig. 2C). All three anti-Gal isotypes bound pilin from these strains.

Colostrals sIgA anti-Gal binds pilin. Anti-Gal has been detected in human secretions, including human milk, colostrum, saliva, and bile (24). We wanted to know whether secretory anti-Gal also could bind pilin. Pilin made by strain 15515 had an estimated M_r of between 14,000 and 21,000 by SDS-PAGE (Fig. 3A). Both serum anti-Gal and colostrals sIgA anti-Gal bound this pilin, as judged by Western blot (Fig. 3B, lanes a and b, respectively).

Pilin of strains 15515, 15516, and 7971 have carbohydrate structures. We used a glycan detection kit to find out whether pilin of *N. meningitidis* 15515, 15516, and 7971 contained sugar structures. Figure 4 shows that only LOS and pilin were visu-

FIG. 2. Immunoblot of SDS-PAGE-separated OMPs from *N. meningitidis* 15515 (lane 1), 7971 (lane 2), and 15516 (lane 3) and purified pilin from *N. meningitidis* 15515 (lane 4) reacted first with whole serum anti-Gal and then with goat anti-human IgG (A), goat anti-human IgA (B), or goat anti-human IgM (C). Molecular weight markers are shown on the left. k, thousand.

FIG. 3. Binding of whole serum and colostrals sIgA anti-Gal to purified pilin from *N. meningitidis* 15515. (A) Silver stain of SDS-PAGE-separated purified pilin from *N. meningitidis* 15515. The arrowhead indicates the location of the pilin. Molecular size markers (in kilodaltons) are shown in the left lane. (B) Immunoblot of SDS-PAGE-separated pilin from *N. meningitidis* 15515 reacted with whole serum anti-Gal (lane a) or colostrals sIgA anti-Gal (lane b). The arrowhead indicates the location of the pilin. Molecular size markers (in kilodaltons) are shown in the first lane on the left.

alized by the detection method. This confirmed that pilin from these strains contained carbohydrate structures.

Binding of *B. simplicifolia* lectin to pilin. The *B. simplicifolia* lectin is a plant lectin that binds to cell membrane glycoconjugates bearing terminal Gal α 1-3Gal residues (13). We used this lectin to confirm that pilin from strain 15515 contained Gal α 1-3Gal structures. Figure 5 shows the binding of the *B. simplicifolia* lectin to purified 15515 pilin (lane B). Lanes A and C show the binding to pilin of anti-Gal and rabbit CNBr2 antibodies, respectively. Anti-Gal, *B. simplicifolia* lectin, and the rabbit antibodies all bound to pilin from this strain. Anti-Gal and the *B. simplicifolia* lectin are both specific for Gal α 1-3Gal residues, thus confirming the presence of this structure on *N. meningitidis* pilin.

Binding of anti-Gal to meningococcal capsular polysaccharides. Anti-Gal did not bind to purified group A, B, or C capsular polysaccharides in a dot blot assay (results not shown); thus, the cognate epitope for anti-Gal was not present on the capsule of these strains.

IEM showed that anti-Gal bound to intact pili. We next studied whether the pilin epitope for anti-Gal was surface

FIG. 4. Binding of *B. simplicifolia* lectin to SDS-PAGE-separated *N. meningitidis* 15515 pilin by immunoblot. *N. meningitidis* 15516 pilin was separated by SDS-PAGE and then reacted with anti-Gal (lane A), the *B. simplicifolia* lectin (lane B), or the rabbit anti-CNBr2 (lane C). Molecular weight markers are shown in the first lane on the left. k, thousand.

exposed on intact organisms. This was done by IEM; the results are shown in Fig. 6. *N. meningitidis* 15515 exhibited alpha-type, or aggregated, pili; this pilus arrangement has been associated with organisms that cause disseminated disease. Both serum IgG and colostral sIgA anti-Gal bound exclusively to the pili of this strain, confirming that the epitope for anti-Gal is found on intact pili and is surface exposed.

Binding of NHS and anti-Gal-depleted NHS to *N. meningitidis* OMP, as judged by immunoblot. We tested the ability of synthetic Gal α 1-3Gal structures linked to silica bead columns to absorb the pilin-binding activity of NHS. These results are shown in Fig. 7 (with emphasis on areas corresponding to pilin and LOS). Lane A shows anti-Gal binding to pilin by immunoblot; this marks the location of pilin (arrow). Note that serum from a normal adult contained antibodies that bound to pilin as well as those that bound to LOS (Fig. 7, lane B). Pilin-binding antibodies in NHS could be absorbed completely by passing the serum over silica-linked Gal α 1-3Gal β 1-4GlcNAc (anti-Gal-depleted NHS), as evidenced by the loss of binding to pilin, but not to LOS, on an immunoblot (Fig. 7,

FIG. 5. Carbohydrate stain of SDS-PAGE-separated OMPs from *N. meningitidis* 15515 (lane A), 15516 (lane B), and 7971 (lane C). Areas corresponding to LOS and pilin are labeled accordingly. Molecular size markers (in kilodaltons) are indicated on the left.

FIG. 6. IEM of *N. meningitidis* 15515 reacted with whole serum anti-Gal and then gold-labeled anti-IgG (a) or reacted with colostral sIgA anti-Gal and then gold-labeled anti-IgA (b).

lane C). These results confirmed that anti-Gal bound Gal α 1-3Gal structures on pilin.

Agglutination of RRBCs by anti-Gal eluted from nitrocellulose-bound pilin. RRBCs express abundant Gal α 1-3Gal surface structures and have been used to screen for anti-Gal and determine its titer in serum and in secretions (16, 24). We wanted to know whether anti-Gal that bound to meningococcal pilin had the same specificity as that of the previously studied circulating and secretory anti-Gal.

We bound anti-Gal to nitrocellulose-immobilized SDS-PAGE-separated OMP preparations from strains 15515, 15516, and 7971. We eluted the bound anti-Gal from each, purified anti-Gal IgG, and used this eluted antibody in a hemagglutination assay with RRBCs. Anti-Gal eluted from pilin made by all three meningococcal strains agglutinated RRBCs to a titer of 1:8, indicating that the Gal α 1-3Gal specificity of pilin-binding anti-Gal was the same as that of total serum and secretory anti-Gal.

Role of anti-Gal in complement-mediated killing of *N. meningitidis*. We examined the effect of whole serum, serum IgG, and serum IgA1 anti-Gal and colostral sIgA anti-Gal on the complement-mediated killing of a group C *N. meningitidis*, strain 15515, that was grown under conditions that would either maximize or minimize piliation. Serum anti-Gal that contained all three isotypes (IgG, IgA, and IgM) blocked complement-mediated lysis of *N. meningitidis* 15515 ($P = 0.009$) (Fig. 8). Serum anti-Gal IgG alone had no effect on the killing of this organism (Fig. 9), whereas purified serum anti-Gal IgA1 blocked lysis of strain 15515 ($P = 0.04$) (Fig. 9). This demonstrated that IgA was the blocking anti-Gal isotype in serum. Colostral anti-Gal sIgA also blocked complement-mediated lysis of *N. meningitidis* 15515 ($P = 0.007$) (Fig. 9).

FIG. 7. Binding of NHS and anti-Gal-depleted NHS to *N. meningitidis* 15516 SDS-PAGE-separated OMP by immunoblot. Shown are areas corresponding to LOS and pilin only. Lanes: A, binding of anti-Gal to SDS-PAGE-separated OMP from *N. meningitidis* 15515, marking for the location of the pilin (arrowhead); B, binding of NHS; C, binding of anti-Gal-depleted NHS to this OMP.

FIG. 8. Effect of whole serum anti-Gal on the complement-mediated killing of *N. meningitidis* 15515. The bar graph on the left (–) depicts killing by NHS, which contains normal concentrations of anti-Gal of all three serum isotypes, in the absence of added anti-Gal. The bar graph on the right (+) shows how the addition of excess anti-Gal that had been purified from whole serum and contained all three serum isotypes abolished killing by NHS. Values represent the mean percentage of killed organisms ($\pm$ standard deviation), as compared with the control, for at least two independent experiments.

For the most part, the lytic blocking effect of serum IgA1 anti-Gal and colostral sIgA anti-Gal was seen only when the organisms were grown under conditions that maximized piliation (Fig. 9). Also note that piliated organisms that would have bound anti-Gal in NHS were less sensitive to complement-mediated lysis than nonpiliated organisms that would not have bound these antibodies (Fig. 9).

DISCUSSION

Our study shows that some meningococci make pili that have α -galactosyl structures. These α -galactosyl pilus structures bind anti-Gal, an abundant circulating and secretory antibody (16, 24). When bound to pili, the IgA isotype of serum anti-Gal, as well as sIgA anti-Gal, blocks complement-mediated killing of meningococci. This suggests that piliated organisms that bind anti-Gal may be more virulent because they evade host immune lysis.

The group A, B, and C meningococcal capsules are derived from mannosamine and do not contain galactose; they did not bind anti-Gal. Gal α 1-3Gal structures have not been found on neisserial LOS, and we were not able to detect the binding of anti-Gal to the LOS of any of the strains we examined (representing L types 1 to 4 and 6 to 9). The failure of anti-Gal to bind LOS was not due to sialylation of LOS, since only the L1 and L3,7 LOS structures are known to be sialylated (19, 34, 47), and sialylation of these LOS structures is incomplete (11).

Early analyses of gonococcal pili by qualitative gas chromatography found that these pili have galactose and traces of glucose but no detectable heptose or amino sugars (38). Interestingly, periodate or galactosidase treatment of gonococcal pili or their cyanogen bromide-1 fragments markedly reduced attachment (22), suggesting the importance for their attachment function of the galactosyl structures on pili.

More recently, Virji et al. (46) proposed that meningococcal pili are glycosylated on the basis of the increased electrophoretic mobility of chemically deglycosylated pili, labeling of pili by a carbohydrate-specific labeling procedure, and the demonstration of putative potential N-glycosylation sites on pilin. Meningococcal pili appear to increase bacterial adher-

ence to human epithelial and endothelial cells (42, 43), and Virji et al. suggested that modulation of the adherence phenotype of *N. meningitidis* may be due in part to changes in the glycosylation status of pilin (46).

Glycosylated pili also may contribute to the cytotoxicity of meningococci for endothelial cells in vivo (46). Meningococci isolated from disseminated infections are predominantly encapsulated and piliated (46). Pili that occur in bundles are known as alpha-type pili and are associated with organisms that cause disseminated disease (17). The strain we studied had alpha-type pili as seen by IEM.

IgG anti-Gal binds to senescent human erythrocytes and promotes their removal by macrophages (14). We have reported that serum IgG anti-Gal can block alternative complement pathway-mediated lysis of representative enteric gram-negative bacteria that bind it to α -galactosyl LPS structures, thereby promoting survival of such bacteria in the nonimmune host (25). We hypothesized that the blocking of ACP activation by anti-Gal may serve to protect humans from the continuous activation of complement on senescent erythrocytes. Meningococci also could take advantage of this protective mechanism, but the IgG isotype of anti-Gal had no effect, positive or negative, on immune lysis of the meningococcal strain we tested.

IgA blockade of immune lysis has been reported extensively. It originally was described with *Salmonella typhimurium* as the target cell (10) and was subsequently found with many additional targets (1, 21, 23, 36, 39, 40). It has been shown by use of polymeric sIgA (7, 10, 36, 40) and monomeric serum IgA (1, 18, 23) and for both pathways of complement activation (7, 30). IgA binding also can suppress chemotaxis and block opsonophagocytosis by polymorphonuclear leukocytes (36, 45).

Piliation is correlated with resistance of gonococci to phagocytosis by human polymorphonuclear leukocytes (37) and with their resistance to complement-mediated killing by human serum (35), but the molecular basis for either phenomenon has not been reported. It is of note, then, that piliated meningococci that would bind anti-Gal, including its IgA isotype, were more resistant to the complement-mediated killing of NHS than their nonpiliated counterparts that would not bind these antibodies. The binding to neisserial pili of the IgA isotype of

FIG. 9. Effect of serum IgG anti-Gal, serum IgA1 anti-Gal, and colostral IgA anti-Gal on the complement-mediated killing of *N. meningitidis* 15515 grown under conditions that minimize its piliation (open bars) or that maximize its piliation (hatched bars). Bar graphs that are labeled with a minus represent reactions with no added anti-Gal; those that are labeled with a plus represent reactions with added anti-Gal. Results are expressed as the mean percentage of killed organisms ($\pm$ standard deviation), as compared with the control, and for at least two independent experiments for each. *, $P = 0.04$; **, $P = 0.007$.

anti-Gal that is ubiquitous in NHS could explain the relative resistance of piliated organisms to both clearance mechanisms.

The fact that the reduction in the percent kill of piliated organisms with whole serum anti-Gal was greater than that with purified anti-Gal IgA1 could be due to various factors. Whole serum anti-Gal presumably contains both anti-Gal IgA1 and IgA2, the latter of which may contribute to blockade. In addition, binding of anti-Gal IgG to the pilus may increase the binding of anti-Gal IgA1 in a manner analogous to the increase in binding of IgA1 to the group C meningococcal capsule in the presence of capsular IgG, as compared with maximal IgA1 binding in the absence of IgG (29). Assuming that blockade is a function of the amount of anti-Gal IgA1 bound to the pilus, an increase in anti-Gal IgA1 binding in the presence of anti-Gal IgG would result in a greater reduction in the percent kill of piliated organisms than would be seen in the absence of IgG.

Serum blocking meningococcal IgA has been described in epidemic meningococcal disease (18), but its specificity has been thought to be restricted to the capsule of the organisms. This study describes an additional specificity for blocking meningococcal IgA, namely pilus α -galactosyl structures. We have not compared the relative blocking efficiencies of the two specificities of IgA, but the amount of anti-Gal IgA in NHS is sufficient to reduce the killing of piliated organisms, whereas sufficient capsular IgA to block lysis is found only during epidemic conditions. This difference could reflect the fact that capsular IgG is an efficient activator of immune lysis and competes with IgA on a molar basis (21), whereas pilus IgG anti-Gal is a null antibody that does not counter the blocking effect of IgA anti-Gal.

Our study confirms the presence of carbohydrate structures on meningococcal pili and describes a functional role for pilus glycosylation. We propose that some pili contain Gal α 1-3Gal structures on the basis of their binding of anti-Gal and the *B. simplicifolia* lectin. When these pili bind circulating anti-Gal in vivo, they confer on the organism the ability to evade complement-mediated lysis. This argues for a role for anti-Gal in promoting meningococcal dissemination.

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