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Relationship between Colonial Morphology and Adherence of *Streptococcus pneumoniae*

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Phase variants in colonial opacity of pneumococci differ in the ability to colonize the nasopharynx of infant rats. To explain this observation at a cellular level, we compared the ability of opacity variants to adhere to buccal epithelial cells, type II pneumocytes, or vascular endothelial cells and to the glycoconjugates that represent the cognate receptors at each of these sites. The transparent phenotype was associated with enhanced adherence to buccal cells (~100%) and their receptor relative to that of the opaque variants. Only modest differences in adherence (<45%) were demonstrated to resting lung and vascular cells. In contrast, adherence of transparent variants increased by 90% to lung cells stimulated with interleukin-1 and by 130% to endothelial cells stimulated with tumor necrosis factor. In contrast, cytokine stimulation did not influence the adherence of opaque pneumococci. This difference correlated with the unique ability of transparent variants to adhere to immobilized GlcNAc and to cells bearing transfected platelet-activating factor receptors. These results suggest that the mechanism of enhanced colonization of the nasopharynx in vivo by transparent as compared with opaque phase variants involves a greater ability to adhere to both GlcNAcβ1-3Gal on buccal epithelial cells and GlcNAc and PAF receptors on cytokine-activated, as opposed to resting, lung and endovascular cells.

Streptococcus pneumoniae is a gram-positive pathogen which is a major cause of lobar pneumonia, sepsis, and meningitis (11). As a prelude to invasive disease, pneumococci enter the host via the nasopharynx, where they attach to epithelial cells and in some instances persist for several months (2). Pneumococcal isolates undergo spontaneous, reversible phenotypic (phase) variation which is apparent as differences in colonial morphology (17). At least three phase variants have been recognized on the basis of colonial morphology: opaque, semitransparent, and transparent. Pneumococcal phase variation has been implicated recently in the ability of pneumococci to colonize the nasopharynx in an infant rat model. Opaque variants were unable to colonize the nasopharynx, whereas the transparent variants were stable and effective colonizers (17). In contrast, organisms of both phenotypes produced sepsis with equal efficiency when introduced intraperitoneally. These results suggest that organisms with the transparent phenotype possess a selective advantage in the colonization of the nasopharynx. We sought to determine the mechanism of the difference in colonization at the level of cellular attachment.

Pneumonia is believed to result from aerosolized spread of the bacteria from the nasopharynx down into the lung, where they attach to epithelial cells lining the alveoli (18). Pneumococci readily gain access to the blood circulation from the alveolar space, suggesting an aggressive interaction with the vascular endothelial cells (EC) of the alveolar capillaries (12). Adherence in the nasopharynx has been determined to involve the recognition of *N*-acetyl-D-glucosamine β1-3 galactose (Glc Nacβ1-3Gal) glycoconjugate receptors on buccal epithelial cells (1). In contrast, adherence to lung and EC involves two classes of receptors on resting pneumocytes (LC) and vascular EC containing *N*-acetyl-D-galactosamine linked either β1,3 or β1,4 to galactose (GalNAcβ1-3Gal and GalNAcβ1-4Gal) (6). In the presence of inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), the presentation of receptors on eukaryotic cells changes dramatically (5). Although resting receptor populations persist, cytokine stimulation of LC and vascular EC in vitro results in a >50% increase in pneumococcal adherence associated with the appearance of a new specificity for GlcNAc and for platelet-activating factor (PAF) receptors on LC and vascular EC. Pneumococci are able to adhere to these de novo PAF receptors apparently via the phosphorylcholine determinant of the cell wall teichoic acid (5), a determinant which is shared between the pneumococcal cell wall and the natural ligand PAF (3). PAF receptors have been identified in a number of tissues and cells including lung, brain, and leukocytes (4).

To begin to dissect the effect of phase variation on pneumococcal adherence to target eukaryotic cells, we compared the ability of opacity variants to adhere to cells representative of three in vivo niches: (i) buccal epithelial cells, which may correlate with nasopharyngeal colonization, (ii) human type II LC representative of the alveolar site of infection, and (iii) human vascular EC representative of the endovascular site of infection. Further, the ability of opacity variants to adhere during the evolution of pneumococcal disease was assessed using cytokine-activated LC and EC and cell lines bearing transfected PAF receptors.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The strains examined in this study were isolates from blood cultures (17) (Table 1). A pair of unencapsulated pneumococcal opacity variants, P109 (very opaque) and P110 (very transparent), derived from R6 were also studied (13). Bacteria were grown on tryptic soy agar (Difco, Detroit Mich.) containing 3% sheep blood (Micropure Medical Inc., Stillwater, Mich.) (sheep blood plates) for 18 h at 37°C and harvested from the plate into 1 ml of Dulbecco's phosphate-buffered saline (DPBS; Whittaker Bioproducts, Walkersville, Md.). Pneumococci were labelled with fluorescein iso-

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TABLE 1. Strain characteristics for pneumococcal opacity variants

Strain	Serotype or characteristic	Phenotype	Source or reference
P68	18C	Transparent	17
		Semitransparent	17
		Opaque	17
P62	9V	Opaque	17
		Semitransparent	17
		Transparent	17
P13[a]	Unencapsulated	Transparent	13
		Opaque	13
R6[b]	Unencapsulated	Transparent	13
		Opaque	13

[a] Unencapsulated variant of a type 9V strain.

[b] R6 displays four phenotypes. The most opaque (strain P109) and most transparent (P110) variants of R6 were compared in the cited study (13).

thiocyanate (FITC; 1 mg/ml; Sigma Chemical Company, St. Louis, Mo.) as previously described (6). The bacteria were washed twice by centrifugation (13,000 × *g*, 3 min), resuspended in 1 ml of albumin buffer (6), and diluted to between 10^5 and 10^7 CFU/ml.

Hemagglutination of neuraminidase-treated bovine erythrocytes. Neuraminidase-treated bovine erythrocytes were prepared according to Andersson et al. (1). Stabilized bovine erythrocytes (Sigma) were suspended in 10 ml of DPBS according to the manufacturer's instructions, incubated with an equal volume of neuraminidase (1 U/ml; Sigma) for 30 min at 37°C, washed twice by centrifugation (13,000 × *g*, 3 min), and resuspended in albumin buffer, and 100-fold dilutions were prepared from a 5% stock concentration. Hemagglutination was assessed by light microscopy following coincubation (5 min, room temperature) of equal volumes of the erythrocyte dilutions with the pneumococcal strains (10^9 CFU/ml, 10 μl each). Hemagglutinating activity of each opacity variant was expressed as the erythrocyte dilution at which agglutination was lost, i.e. at which <5% of the erythrocytes were seen to be agglutinated. Agglutinating activity for each opacity variant was determined on at least six separate occasions. In order to test for competitive inhibition of binding by monosaccharides, glucose, GlcNAc, or GalNAc (50 mM in albumin buffer) was added to the bacteria (2 × 10^7 CFU/ml) for 15 min and the mixture was centrifuged to remove the sugar prior to the hemagglutination assay.

Human type II LC and vascular EC. The human type II LC line A549 (American Type Culture Collection) was cultured in nutrient mixture F12, Ham medium (Sigma), supplemented with 10% fetal calf serum (Sigma) (6). Primary cultures of human umbilical vein EC (passage 1; Clonetics Corp., San Diego, Calif.) were grown in medium 199 (Sigma) supplemented as previously described (complete medium) (7). At confluence the cells were prepared for subculture with trypsin–0.05% EDTA (Sigma). For adherence assays, cells were transferred to Terasaki 60-well culture dishes (Robbins Scientific, Sunnyvale, Calif.) coated with fibronectin (50 μg/ml) and cultured for another 24 to 48 h for confluent monolayers.

Cells transfected with human PAF receptors. Human TSA cells ATCC CRL1593) are a derivative of human embryonic kidney 293 cells stably transfected with the simian virus 40 large T antigen. TSA cells transfected with the pCDM8 human Flag-PAF receptor plasmid by the addition of cationic liposomes were obtained from N. Gerard (Boston, Mass.) (5, 9). Control cells were transfected with the vector alone. The presence of PAF receptors was confirmed by immunohistochemical staining of the Flag-PAF receptor with monoclonal antibody (data not shown) (5, 9).

Adherence of pneumococcal opacity variants to eukaryotic cells. Adherence of pneumococci to LC and EC was investigated using a protocol previously described (3). Human buccal epithelial cells were obtained from scraping of the buccal mucosa, washed, and suspended at a final concentration of 10^6 cells per ml as described previously (1). Initially, binding of bacteria was detected in parallel by FITC labelling and by Gram stain. No differences were observed, and therefore further experiments were conducted using FITC labelling alone. FITC-labelled bacteria (10^5 to 10^7 CFU/ml; 10 μl per well) were incubated with cell monolayers for 30 min at 37°C. Adherence of FITC-labelled pneumococci (10^7 CFU/ml) was also assessed following coincubation (30 min at 37°C) with monolayers of control TSA cells or those bearing transfected human PAF receptors. Pneumococcal adherence to LC and EC was also assessed following cytokine activation. LC were incubated with 5 ng of IL-1α (Sigma) per ml for 4 h at 37°C, and EC were incubated with 5 ng of TNF-α (Boehringer) per ml for 3 h at 37°C (5). Nonadherent bacteria were removed by washing the LC and EC monolayers five times and the TSA monolayers three times with medium 199. Monolayers were then fixed in 2.5% gluteraldehyde, and adherent pneumococci were counted visually with an inverted microscope (Diaphot-TMD; Nikon Inc., Melville, N.Y.) equipped for fluorescence with an IF DM-510 filter. Adherence was expressed as the number of attached bacteria per 100 eukaryotic cells.

TABLE 2. Hemagglutinating capacity of pneumococcal opacity variants

Strain	Endpoint dilution for hemagglutinating activity[a]			
	Opaque		Transparent	
	No sugar	+GlcNAc[b]	No sugar	+GlcNAc[b]
P68	1:10^5	1:10^3	1:10^7	1:10^3*
P62	1:10^5	1:10^5	1:10^7	1:10^3*
P13	1:10^3	1:10^3	1:10^7	1:10*
R6	1:10^3	1:10^3	1:10^9	1:10^3*

[a] Hemagglutinating activity was assessed following 5 min of coincubation of 100-fold dilutions of neuraminidase-treated bovine erythrocytes with pneumococcal strains (10^9 CFU/ml). Values represent the lowest fold dilutions of erythrocytes from a 5% stock solution at which hemagglutination was lost, i.e., <5% of erythrocytes agglutinated. Variation between assays was ±1 dilution. Experiments were performed on six separate occasions.

[b] Pneumococci were incubated with GlcNAc (50 mM), centrifuged, resuspended to 10^9 CFU/ml, and added to the hemagglutination assay mixture. *, significant ($P < 0.05$) loss in hemagglutinating capacity in the presence of GlcNAc compared with the control.

Values for two wells were averaged, and each experiment was performed on at least six separate occasions.

Adherence of pneumococcal opacity variants to immobilized carbohydrates. To identify the glycoconjugate receptor specificities recognized by pneumococcal opacity variants, pneumococci were allowed to adhere to a number of carbohydrates which have been identified as effective analogs of receptors present on resting (1, 8) and cytokine-activated (5, 6) LC and EC. Solid-phase binding assays were carried out as previously described (6, 10). Four monosaccharides (glucose, Gal, GalNAc, and GlcNAc) and three complex sugars (lactosylceramide, asialo-GM2, and globoside) were tested. Stock solutions of the monosaccharides (100 mM) and complex carbohydrates (2 mM) were prepared in 10% chloroform in methanol (vol/vol). Dilutions of the monosaccharides (50 mM) and complex carbohydrates (0.016 mM) were made in methanol. Terasaki 60-well culture dishes (Robbins Scientific) were coated with 10 μl of monosaccharides or glycoconjugates in methanol per well or methanol as a control and allowed to evaporate to dryness at 4°C. The wells of the microtiter plate were then blocked by overnight incubation at 37°C with 10% (wt/vol) bovine serum albumin (BSA; Sigma) in PBS, pH 7.4. Excess BSA was decanted, and the wells were washed once with 1% BSA in PBS. FITC-labelled pneumococci (10^7 CFU/ml) were allowed to adhere to the plates for 30 min at 37°C, and adherence was quantitated visually with an inverted fluorescence microscope. Adherence was expressed as the number of attached bacteria counted in a 40× field. Values for two wells were averaged, and each experiment was performed on six separate occasions.

Statistics. Differences between groups were tested by the Wilcoxon signed ranks test. All results are expressed as means and standard deviations of at least six experiments.

RESULTS

Adherence to buccal epithelial cells and hemagglutination by pneumococcal opacity variants. The ability of opacity variants to adhere to buccal epithelial cells was assessed directly. Transparent variants demonstrated approximately double the adherence capability of their opaque counterparts. Adherence values for unencapsulated R6 were 28 ± 9 and 60 ± 10 bacteria per buccal epithelial cell for opaque and transparent phenotypes, respectively. Similarly, for encapsulated P62, transparent variants adhered more avidly than their opaque counterparts (64 ± 12 and 27 ± 6, respectively). This difference was more thoroughly documented by an assay of hemagglutination of neuraminidase-treated bovine erythrocytes which correlates with binding to the glycoconjugate GlcNAcβ1-3Gal, a receptor suggested to be important on buccal epithelial cells (1). The ability of opacity variants to agglutinate treated bovine erythrocytes was assessed. Hemagglutination by encapsulated and unencapsulated transparent pneumococcal variants was found to be 10^2- to 10^6-fold greater than that by their opaque counterparts (Table 2). Neither glucose nor GalNAc had any effect on hemagglutination. In contrast, GlcNAc

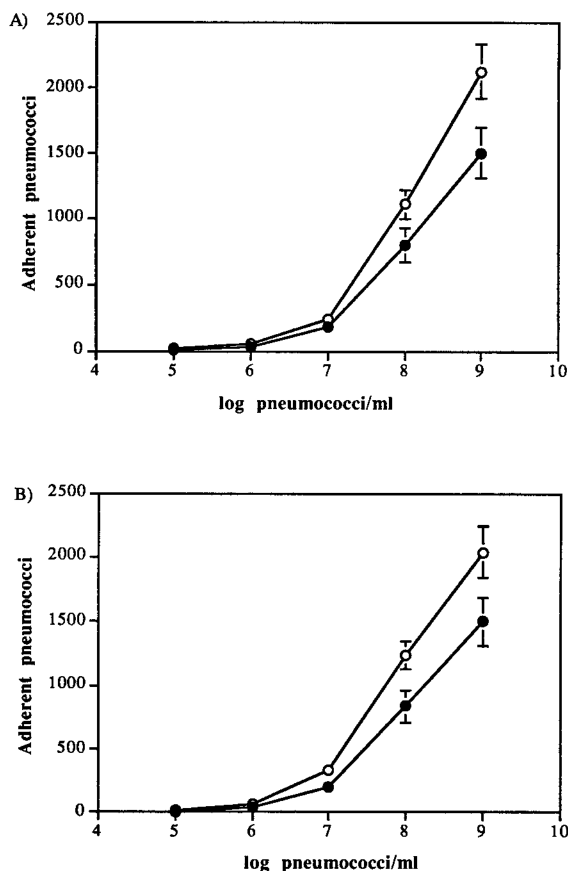

FIG. 1. Adherence of unencapsulated R6 opaque (●) and transparent (○) pneumococcal variants to human cultured vascular EC (A) and type II LC (B). Adherent bacteria were determined as the number of attached bacteria per 100 type II LC or EC. Results shown are the means ± standard deviations for duplicate wells in at least six independent experiments.

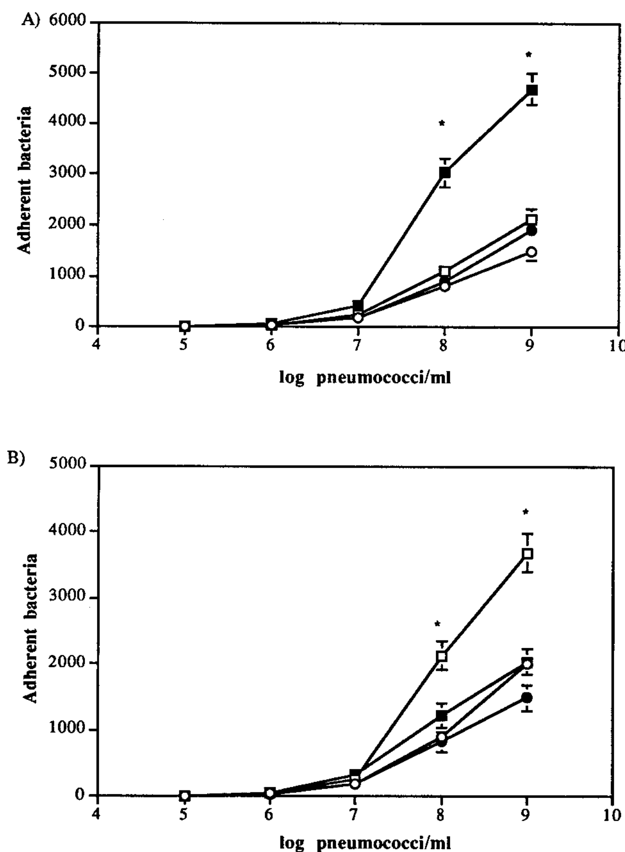

FIG. 2. Adherence of pneumococcal opacity variants to cytokine-stimulated and resting EC (A) and LC (B). EC monolayers were stimulated with TNF-α (10 ng/ml, 3 h at 37°C), and LC were stimulated with IL-1α (5 ng/ml, 4 h at 37°C). Similar results were obtained for encapsulated and unencapsulated pneumococcal opacity variants, and data shown are for unencapsulated R6 opaque (circles) and transparent (squares) variants. Cytokine-stimulated monolayers were coincubated (30 min, 37°C) with 10^5 to 10^9 CFU of pneumococci per ml, and bacteria adherent to resting (open symbols) and cytokine-stimulated (closed symbols) EC and type II LC were determined as the number of attached bacteria per 100 eukaryotic cells. Results are the means ± standard deviations for duplicate wells in at least six independent experiments. *, $P < 0.05$ compared with resting cells.

produced a 10^4- to 10^6-fold decrease in the ability of transparent pneumococcal opacity variants to cause hemagglutination. No effect on the hemagglutinating capacity of any opaque pneumococcal strains was observed.

Adherence of pneumococcal opacity variants to cultured human type II LC and vascular EC. Adherence of opacity variants to both LC and EC was dose dependent, with a threshold dose of 10^5 CFU/ml being required to detect attachment (Fig. 1). At higher concentrations adherence increased, and at concentrations of more than 10^9 CFU/ml adherent pneumococci were too numerous to count. All transparent variants, with and without a capsule, adhered consistently ~33 to 46% more than the corresponding opaque variants to both cell types (shown for R6 opacity variants in Fig. 1). Semitransparent variants P62 and P68 displayed intermediate binding capacity between their transparent and opaque counterparts. For example, at an input concentration of 10^7 CFU/ml, adherence of transparent, semitransparent, and opaque variants of strain P62 was 271 ± 10, 219 ± 8, and 190 ± 10/100 LC and 245 ± 18, 210 ± 9, and 181 ± 16/100 EC, respectively.

Effect of cytokine stimulation on adherence of pneumococcal opacity variants to cultured human type II LC and vascular EC. Adherence of all transparent variants of pneumococci increased by ~90% following stimulation of LC with IL-1 and by ~130% following treatment of EC with TNF-α (Fig. 2). In contrast, no increase in adherence to cytokine-stimulated cells was observed for opaque variants of any strain investigated even at very high inocula (Fig. 2). An intermediate increase in adherence to TNF-stimulated monolayers was observed for semitransparent phase variants of P62 and P68.

Effects of carbohydrates on adherence of pneumococcal opacity variants. Pneumococci have been demonstrated to adhere to five different carbohydrate determinants, Gal, GalNAc, GlcNAc, asialo-GM2 (GalNAcβ1-4Gal), and globoside (GalNAcβ1-3Gal), each reflective of a different eukaryotic cell type (1, 5, 6, 8). Transparent variants were able to adhere directly to immobilized Gal, GalNAc, GlcNAc, asialo-GM2, and globoside and were unable to adhere to glucose or lactosylceramide (Table 3). Opaque variants shared this binding ability with one notable exception: opaque variants demonstrated significantly less binding to GlcNAc (Table 3). This is consistent with the finding reported above that opaque strains adhered poorly to activated cells, since GlcNAc has been shown to inhibit pneumococcal binding to activated cells but not to resting cells (5). Also consistent with this observation, GlcNAc inhibited the adherence of transparent pneumococci to activated EC by 56% while decreasing the adherence of opaque strains by only 20% (Table 4). Asialo GM2 and globoside, which are equally active

TABLE 3. Direct adherence of pneumococcal opacity variants to immobilized glycoconjugates

Serotype or characteristic	Adherent bacteria[a]						
	Glc	Gal	GalNAc	GlcNAc	Asialo GM2	Globoside	Lactosyl ceramide
Encapsulated							
P62 opaque	53 ± 9	78 ± 8	115 ± 10	80 ± 11*	485 ± 5	190 ± 11	112 ± 10
P62 transparent	57 ± 11	84 ± 12	121 ± 7	119 ± 12	463 ± 20	202 ± 10	108 ± 8
P68 opaque	49 ± 7	83 ± 10	118 ± 9	78 ± 4*	476 ± 13	213 ± 12	123 ± 11
P68 transparent	52 ± 8	90 ± 9	124 ± 10	112 ± 5	454 ± 16	199 ± 11	134 ± 10
Unencapsulated							
P13 opaque	56 ± 8	78 ± 7	125 ± 11	85 ± 9*	478 ± 18	222 ± 10	121 ± 5
P13 transparent	61 ± 7	85 ± 8	116 ± 9	137 ± 11	482 ± 15	197 ± 9	114 ± 8
R6 opaque	60 ± 8	93 ± 10	114 ± 11	79 ± 7*	474 ± 17	192 ± 12	119 ± 7
R6 transparent	59 ± 3	100 ± 9	117 ± 8	124 ± 8	485 ± 12	200 ± 9	120 ± 12

[a] Pneumococci (10^7 CFU/ml) were coincubated with immobilized monosaccharides (50 mM) or glycoconjugates (0.016 mM) for 30 min at 37°C. Adherent bacteria in a 40× microscope field were enumerated using an inverted fluorescence microscope. Values are means ± standard deviations of six experiments, with each experiment being the mean of two replicate wells. *, significantly less ($P < 0.05$) than adherence by organisms with the corresponding transparent phenotype.

in inhibiting adherence of opaque and transparent pneumococci to resting cells (5), did not show an increased ability to inhibit adherence to activated cells (Table 4).

Adherence of pneumococcal opacity variants to transfected human PAF receptors. Pneumococci bind to the human PAF receptor (5). This property appeared to be significantly compromised in opaque variants. At an input concentration of 10^7 CFU/ml adherence of transparent and opaque variants of encapsulated P62 was 332 ± 20 and 92 ± 11 and that of unencapsulated R6 was 286 ± 15 and 169 ± 10/100 transfected TSA cells, respectively. Adherence of opaque variants, although significantly ($P < 0.05$) reduced was still greater than adherence of either opaque or transparent variants to untransfected cells (17 ± 8/100 TSA cells). The impaired ability of opaque variants to adhere to the PAF receptor transfectants was observed over a wide concentration range, remaining ~50% of the corresponding value for transparent variants (Fig. 3).

DISCUSSION

During the course of pneumococcal colonization and infection, pneumococci are presumed to attach to nasopharyngeal cells, LC, and vascular EC. Establishment of pneumococci at each different site in the host involves recognition of host cell surface carbohydrates such as GlcNAcβ1-3Gal on nasopharyngeal cells (1) and GalNAcβ1-3Gal and GalNAcβ1-4Gal on lung and vascular endothelial cells (6). We have shown that, when compared with opaque variants, transparent pneumococci exhibit significantly enhanced ability to attach to some of these eukaryotic cell ligands. Transparent variants bound more avidly (~100%) to buccal epithelial cells. This was reflected at the mechanistic level in a strongly enhanced ability to recognize GlcNAcβ1-3Gal as measured by agglutination of GlcNAcβ1-3Gal-bearing erythrocytes (10^2- to 10^6-fold more efficiently than opaque variants). Thus, in the initial encounter of pneumococci with the nasopharynx, transparent variants appear to have a great advantage. This observation is consistent with and suggests a mechanism for the documented ability of transparent variants to colonize this site effectively in vivo while opaque variants are eliminated (17).

The aerosolization of pneumococci into the lung is a first step toward pneumonia. Presumably the first encounter with pulmonary cells would involve resting epithelium. Transparent pneumococci adhered only modestly better (33 to 46%) to resting, cultured type II LC and vascular EC than did their opaque counterparts. In accord with these results, opaque and transparent pneumococcal variants adhered to a similar degree to analogs for the two receptors on resting LC and EC, asialo-GM2 (GalNAcβ1-4Gal) and globoside (GalNAcβ1-3Gal), and to their substituents GalNAc and Gal. These results indicate that during the initial encounter between naive lung cells and pneumococci, the advantage of transparent strains may be small, an observation that supports the roughly equivalent ability of organisms of the two phenotypes to establish bacteremia when injected intraperitoneally, a route circumventing the restrictions imposed by the nasopharynx (17).

In the presence of inflammatory stimuli, LC and EC rapidly become activated and change the presentation of receptors on their surfaces. Such activation of both LC and EC occurs in the presence of cytokines and has been shown to be associated with the de novo appearance of PAF receptors (5). The PAF receptor strongly supports adherence of pneumococci to acti-

TABLE 4. Inhibition of adherence of pneumococci to TNF-activated human vascular EC by carbohydrates

Strain	% Inhibition of adherence by [a]:									
	Control	Asialo GM1 (μg/ml)			Globoside (μg/ml)			GlcNAc (mM)		
		500	100	20	500	100	20	50	25	12.5
Transparent	100 (389 ± 16)	59	46	40	67	58	54	56	49	34
Opaque	100 (233 ± 11)	57	49	39	67	56	51	20	11	2

[a] Pneumococci (10^7 CFU/ml) were incubated with the indicated concentration of carbohydrate for 10 min prior to exposure to TNF-activated EC. Values are means ± standard deviations of four experiments expressed as percent inhibition of the number of adherent bacteria per 100 TNF-activated human vascular EC as compared with the control in the absence of sugar. Values in parentheses are numbers of bacteria per 100 activated cells.

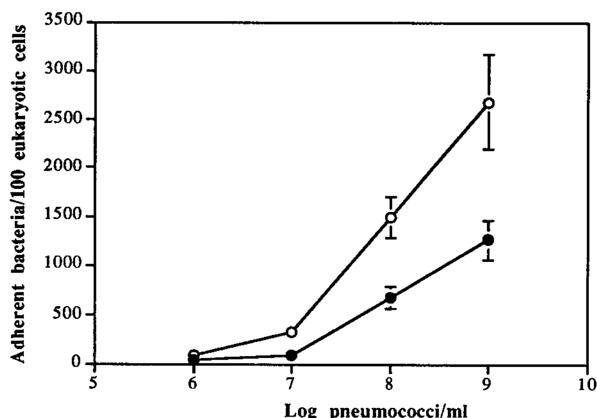

FIG. 3. Adherence of pneumococcal opacity variants to PAF receptor transfectants. Opaque (●) and transparent (○) variants of unencapsulated R6 were coincubated (30 min, 37°C) at the indicated concentrations with monolayers of COS cells bearing the PAF receptor. Values are the numbers of attached bacteria per 100 eukaryotic cells. Results are the means ± standard deviations for duplicate wells in three independent experiments.

vated cells, increasing the number of bacteria per cell from ~11 for resting cells to >30 for activated, PAF-receptor-bearing cells (5). The association of pneumococci with the PAF receptor enhances internalization of the bacteria, a step perhaps permissive to invasion from the lung into the bloodstream. This interaction is inhibitable by GlcNAc, a saccharide that shows no effects on adherence to resting cells. One possible mechanism by which virulent pneumococci could adapt their cognate, surface adhesins to match this change in host cell targets would be phase variation. Adherence of transparent organisms, but not of opaque variants, to LC nearly doubled and increased ~130% to EC following cytokine stimulation. This additional adherence was inhibitable by GlcNAc, and the transparent variants also demonstrated a greater direct adherence to GlcNAc immobilized on a microtiter plate. Further, transparent bacteria were significantly more able to adhere to human PAF receptors transfected into TSA cells. These results suggest that organisms with the transparent phenotype differs strongly from those with the opaque phenotype by the ability to recognize novel receptors expressed on activated LC and EC. Translated to the in vivo situation, this would be expected to confer an advantage to organisms with the transparent pneumococcal phenotype in producing more invasive disease in the face of the inflammatory response during pneumonia, meningitis, and bacteremia.

For other human respiratory pathogens such as *Haemophilus* and *Neisseria* spp., spontaneous phase variation of key surface structures allows adaptation to the various challenges encountered in the nasopharynx, lung, and bloodstream (14–16). In these pathogens, phase variation in colonial morphology has allowed the identification of several cell surface proteins of importance at key stages in the host-parasite interaction and thus has provided insight into adaptive strategies utilized by the bacterium in vivo. The biochemical basis of opacity variation in pneumococci is unknown. A genetic locus conferring opacity has been identified, although its function has not been clarified (13). The ability of phase variation to affect adherence to two carbohydrates with independent specificities on different cells (buccal epithelial GlcNAcβ1-3Gal and activated LC and EC GlcNAc) as well as adherence to the PAF receptor suggests that phase variation may involve coordinate regulation of a number of adhesive ligands. Pneumococcal adherence appears to involve both proteins and cell wall-related ligands (7), the latter most probably being the phosphorylcholine of the teichoic acid interacting with the PAF receptor (5). The ability of a locus to modulate cell wall as well as protein adhesins suggests an unusual level of complexity underlying the regulation of pneumococcal adherence.

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