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Effect of Specific Binding of Human Albumin, Fibrinogen, and Immunoglobulin G on Surface Characteristics of Bacterial Strains as Revealed by Partition Experiments in Polymer Phase Systems

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Four strains of gram-positive cocci with different combinations of positive binding of human proteins were investigated with respect to changes in physicochemical surface properties after specific protein binding. *Staphylococcus aureus* Cowan I, two group A β-hemolytic streptococci, and one group G streptococcal strain were studied; they represented three different combinations of reactivity for human serum albumin, human immunoglobulin G, and fibrinogen. Using single-tube partition of bacterial cells in a dextran-polyethylene glycol system of constant polymer concentration but varying ionic compositions, it was possible to detect changes in the partition of bacteria after specific protein binding. There was a correlation between the binding of radiolabeled human proteins to the bacterial strains and the effect of human proteins on the partition of the bacteria in the phase systems. Thus, the specific binding of proteins to the bacteria changes their physicochemical surface properties. These types of bacteria-protein interactions may play an important role in modulating host-parasite relationships.

The surface characteristics of bacteria play an important role in pathogenicity (32). Several factors seem to modulate cell surface charge and degree of hydrophobicity. Recently, an increasing number of specific interactions between surface structures of streptococci and human proteins have been discovered. Fibrinogen-binding properties were found first in some group A streptococci but are now known to reside in all group A, C, and G strains (9, 13, 19, 39). Immunoglobulin G (IgG) and IgA binding seems to depend on separate surface structures (7, 16, 17, 25). A binding of aggregated β_2-microglobulin to group A, C, and G streptococci has been demonstrated (17, 18). Haptoglobin shows affinity for some streptococci (15). Albumin binds to a specific receptor on group C and G streptococci (20, 26). *Staphylococcus aureus* organisms are also known to bind various immunoglobulins as well as fibrinogen (11, 24). The high density of receptors for some of these proteins suggests that a positive protein binding might influence the physicochemical properties of the bacterial cell surface.

Partition between two imiscible liquid phases composed of different aqueous polymer solutions has been used to investigate surface properties of *Salmonella typhimurium* and *Escherichia coli* (21, 22, 34-37). Particles, such as bacteria, distribute between the two phases and the interface in such aqueous phase systems (1-3). Factors determining the partition include several more or less independent variables such as surface charge, hydrophobic-hydrophilic composition of the particle, and spatial conformation (3).

To study the effects of protein-bacteria interactions on surface characteristics, we used single-tube partition of bacterial cells in a dextran-polyethylene glycol (PEG) system of constant polymer concentrations with varying ionic compositions. The results obtained indicate that the presence of receptors for the proteins studied influences the partition of the bacteria. These types of bacteria-protein interactions may play an important role in modulating the host-parasite relationship.

MATERIALS AND METHODS

Bacterial strains. Strains with different combinations of positive binding of human proteins were selected for partition studies and included one *S. aureus* strain (Cowan I), two group A streptococcal strains (AM1 and AW43), and one group G strain (G148). As shown in Table 1, these strains represented three different combinations of reactivity for the proteins studied. One strain, G148, showed specific binding of human serum albumin (20, 26). All strains showed specific binding of human IgG (16, 20, 25), whereas only the group A streptococci and *S. aureus* showed specific binding of fibrinogen (19). All strains

were kept at 4°C on blood agar plates before use. Tryptone broth or Todd-Hewitt broth was inoculated and incubated overnight at 37°C. Bacteria were harvested by centrifugation at 3,000 rpm for 15 min and washed twice in phosphate-buffered saline (0.03 M phosphate, 0.12 M NaCl, pH 7.2). The optical density at 540 nm was measured, and the bacterial concentration was calculated from a standard curve and adjusted to 10^9 organisms per ml.

Radiolabeling of bacteria. Bacteria were labeled with ^{51}Cr (The Radiochemical Centre, Amersham, England). Fifty microCuries of ^{51}Cr was added to 10^9 bacteria in phosphate-buffered saline containing 0.05% Tween 20 and incubated at 37°C for 4 h. The bacteria were then deposited by centrifugation, washed four times in phosphate-buffered saline–Tween, and suspended in sterile water to contain 2.5×10^8 bacteria per ml. Radioactivity in the last supernatant never exceeded 2% of that in the pellet. Radioactivity per bacterial cell was in the range of 10^{-3} cpm.

Human protein preparations. Human serum albumin and human fibrinogen were purchased from Kabi AB (Stockholm, Sweden). IgG1 myeloma protein was obtained as previously described (16). Human, mouse, and fetal bovine sera used were pools of normal serum samples. Bovine serum albumin was obtained from Sigma Chemical Co., St. Louis, Mo. Proteins and sera were diluted in sterile water.

Radiolabeling of human albumin, IgG, and fibrinogen. Human serum albumin and IgG were labeled with ^{125}I (The Radiochemical Centre) by using the chloramine-T method (23). Fibrinogen was radiolabeled by using the electrolytic method of Rosa et al. (28) as described by Harboe and Fölling (12).

Polymer two-phase systems. PEG 6000 (Union Carbide, New York) and dextran T500 (Pharmacia Fine Chemicals, Uppsala, Sweden), separating into an upper and lower phase, respectively, were used for the liquid polymer phase system (2). The phase systems were prepared from a 20% (wt/wt) stock solution of dextran and a 40% (wt/wt) stock solution of PEG. Salt and buffer stock solutions were made in concentrations 10 times higher than that of the final concentration in the phase system. Single-tube partitions at constant polymer compositions were performed in plastic tubes (70 by 12 mm; AB CERBO, Trollhättan, Sweden). Total capacity of each system was 2 g. The polymer solutions were weighed in each tube, and appropriate buffer and salt solutions were then added with a carefully calibrated pipette (Oxford Laboratories, Foster City, Calif.). Partition of the bacteria more in favor of the lower phase was obtained through addition of NaCl (2).

To select a phase system suitable for each strain, a series of single-tube experiments were set up (Table 2). The phase composition selected for investigating the group G *Streptococcus* (G148) and the group A *Streptococcus* (AM1) was: 5% (wt/wt) dextran T500; 4% (wt/wt) PEG 6000; 0.01 M sodium phosphate; pH 7.8. For *S. aureus* and group A *Streptococcus* (AW43), the following composition was used: 5% (wt/wt) dextran T500; 4% (wt/wt) PEG 6000; 0.01 M lithium phosphate; pH 6.8.

Thus in a typical experiment 0.5 g of 20% dextran T500 and 0.2 g of 40% PEG 6000 were weighed in, and then 200 μl of 0.1 M buffer and 600 μl of sterile water were added. Adding 400 μl of bacteria and 100 μl of protein solution in sterile water to the phase system gave the desired final concentration of all components. All phase systems were prepared and experiments were carried out at room temperature.

Phase partition experiments. Initially, incubation of protein with bacteria was performed before adding the cells to the phase systems. The proteins were mixed with bacteria in phosphate-buffered saline, and the mixtures were allowed to stand at room temperature for 45 min. The bacteria were then washed twice, suspended in sterile water, and added to the phase system. Similar results were obtained when proteins and bacteria were added separately to the

TABLE 1. *Binding of radiolabeled human proteins to bacterial strains*

Bacterial strains	% Binding[a]		
	Human albumin	IgG	Fibrinogen
S. aureus	2	77	16
Group A *Streptococcus*, AW43	5	26	53
Group A *Streptococcus*, AM1	8	67	57
Group G *Streptococcus*, G148	70	75	7

[a] Figures denote percent uptake of 0.4 μg of human serum albumin, 0.5 μg of human IgG, and 5.0 μg of fibrinogen to 2×10^8 bacteria. The uptake is expressed as percentage of added radioactivity (16, 19, 20, 25, 26).

TABLE 2. *Polymer two-phase systems with partition behavior of bacterial strains*

Bacterial strains	Two-phase systems[a]											
	1			2			3			4		
	T	I	B	T	I	B	T	I	B	T	I	B
S. aureus	**70.7**	24.4	4.9	40.0	**55.5**	4.5	**88.2**	9.2	2.6	**80.7**	16.3	3.0
Group A *Streptococcus*; AW43	3.7	**55.4**	40.9	2.7	35.6	**61.7**	5.8	**88.8**	4.4	29.9	**66.2**	3.9
Group A *Streptococcus*; AM1	27.6	**68.8**	3.6	7.8	**84.8**	7.4	33.0	**60.2**	6.8	**71.2**	24.5	4.3
Group G *Streptococcus*; G148	12.8	**84.2**	3.0	9.4	**87.7**	2.9	16.2	**81.6**	2.2	28.7	**67.9**	3.4

[a] System 1, 5% (wt/wt) dextran, 4% (wt/wt) PEG 6000, 0.01 M sodium phosphate (pH 7.8); system 2, same except pH 6.8; system 3, 5% dextran, 4% PEG 6000, 0.01 M Lithium phosphate (pH 6.8); system 4, 5% dextran, 3.6% PEG 6000, 0.01 M lithium phosphate (pH 6.8). Data indicate percentage of bacterial cells in PEG-rich top phase (T), interphase (I), and dextran-rich bottom phase (B). The bacteria were labeled with ^{51}Cr. Boldface numbers indicate the phase into which the majority of the cells partition.

phase systems and the bindings were allowed to take place within the systems. Proteins, in amounts as indicated, were added in 100-μl volumes, and 10^8 bacteria were added in 400-μl volumes. The systems were mixed thoroughly by inverting the tubes about 50 times. After 45 min of settling time, sufficient for phase separation and maximal protein binding, 200 μl of the top or bottom phase, or both, was withdrawn from each tube, and the radioactivity in each sample was measured in a gamma scintillation counter (LKB-Wallace 1280 Ultro Gamma; Biotec, Stockholm, Sweden). Percent bacterial cells in the top and the bottom phase was calculated. The distribution of bacteria is presented as percentage of cells in the dextran-rich bottom phase. In experiments determining the partition coefficient of proteins alone, 500 μl of ^{125}I-labeled protein in sterile water was added to the phase systems. The partition coefficient (K) is defined as concentration in top phase divided by concentration in bottom phase.

RESULTS

Effect of serum on bacterial partition. One hundred microliters of normal human serum, diluted 1:50 in sterile water, was added together with 10^8 bacterial organisms to the phase systems. The partition of all four strains studied was strongly influenced by the addition of serum (Fig. 1). The group G *Streptococcus* (G148) showed the largest change in partition behavior. At 40 mM NaCl, the addition of human serum resulted in a change in partition of the group G *Streptococcus* from 22 to 84% in the bottom phase (Fig. 1C). In a second series of experiments, normal human serum diluted from 1:50 to 1:640 was added to the group G *Streptococcus* and resulted in a dose-dependent transfer of the bacterial cells from the upper phase and the interphase into the bottom phase (Fig. 1D).

The effect of normal serum from other species was also studied. Fetal bovine serum had no influence on the partition of the two group A streptococci or the *S. aureus* strain. A slight change in the partition of the group G *Streptococcus* was obtained when tested with bovine serum (less than 10% as compared to more than 60% with the same amount of human serum). The addition of mouse serum to the group G *Streptococcus*, which is capable of binding murine IgG, resulted in a marked change in partition, in contrast to the lack of any influence on the partition of a group A streptococcal strain.

Effect of albumin on bacterial partition. Human serum albumin (10 μg per 10^8 bacteria) was added together with the bacterial cells to the phase systems. No effect was seen on the partition of the *S. aureus*, whereas a slight effect on the partition of the group A streptococci was seen at high NaCl concentrations (Fig. 2A and B). In contrast, human serum albumin had a strong effect on the partition of the group G streptococcus (Fig. 2C).

Experiments using varying concentrations of human serum albumin were also performed. As seen in Fig. 2D, low concentrations of albumin had an early marked effect on the partition of the group G *Streptococcus*. At 40 mM NaCl, the addition of 1.5 μg of human serum albumin per 10^8 bacteria resulted in a change in partition from 38 to 67% in the bottom phase. The addition of different concentrations showed that the influence is a dose-dependent and saturable process.

Bovine albumin does not bind to the receptor for human albumin in strain G148. Control experiments were therefore performed to measure the effect of bovine serum albumin on the dis-

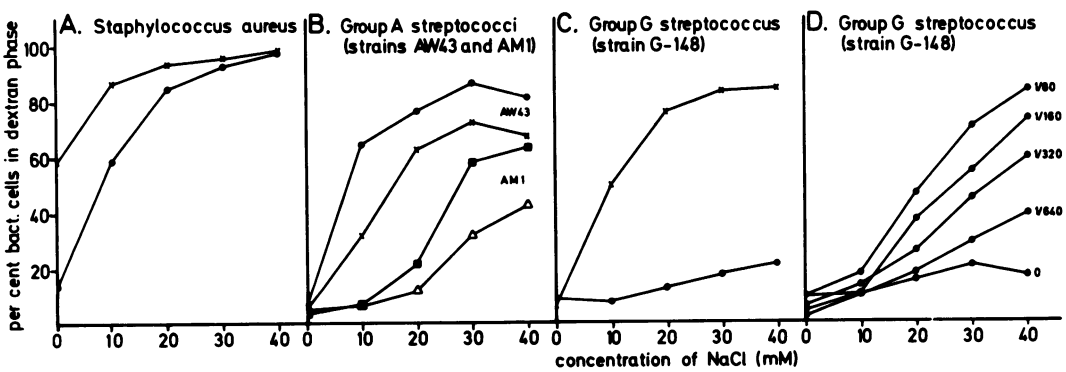

FIG. 1. *Effect of normal human serum on partition of bacterial cells in the dextran-PEG phase system at constant polymer concentrations but different ionic compositions. Symbols:* (●, ■) *bacterial cells only;* (×, △) *bacterial cells with human serum added at a 1:50 dilution (A, B, and C). The effect of varying dilutions of serum on strain G148 is shown in (D). In Fig. 1A, 2A, 3A, 3B, and 4A the distribution is presented as percent bacterial cells in dextran phase plus interface, as calculated by the subtraction of percent in the PEG phase from the total 100%.*

tribution of the bacterial cells in the phase systems. Bovine albumin had no effect on the partition of the group A streptococci or *S. aureus*, but it had a slight effect on the partition of the group G *Streptococcus*, equivalent to the effect of fetal bovine serum.

To study the partition of protein alone, ^{125}I-labeled human serum albumin was added to the systems. An increase in NaCl concentration resulted in a slight decrease of the partition coefficient in the systems used (a decrease in K value from 1.7 to 0.68).

Effect of IgG on bacterial partition. Ten micrograms of human IgG1 myeloma protein was added together with 10^8 bacterial cells to the phase systems. As shown in Fig. 3, the addition of IgG influenced the partition of all four strains studied. *S. aureus* Cowan I showed a marked change in partition (from 14 to 59%), whereas the other strains showed a lower but definite change. The addition of increasing amounts of IgG (2.5 to 20 μg) to *S. aureus* revealed that the effect was dose dependent (Fig. 3B). The partition coefficient of radiolabeled soluble IgG was decreased through the addition of NaCl, a change opposite the effect of IgG when bound to the group A streptococci (a decrease in K value from 7.2 to 1.4).

Effect of fibrinogen on bacterial partition. The effect of 5 μg of human fibrinogen on the partition of 10^8 bacterial cells was studied. Fibrinogen had a low but definite influence on the distribution of *S. aureus* and the group A streptococci in the phase systems used (Fig. 4). There was no effect of fibrinogen on partition of the group G *Streptococcus* lacking a receptor for this protein (Table 1). A decrease in partition coefficient of radiolabeled fibrinogen was observed when the NaCl concentration was increased (a decrease in K value from 6.2 to 1.8).

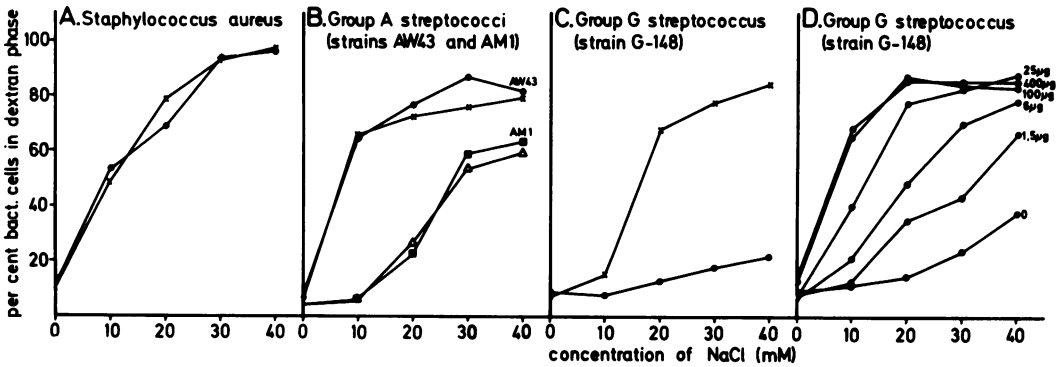

FIG. 2. *Effect of human serum albumin on partition of bacterial cells in the dextran-PEG system at constant polymer concentrations but different ionic compositions. Symbols: (●, ■) bacterial cells only; (×, △) bacterial cells and 10 μg of human serum albumin added (A, B, and C). The effect of varying concentrations of human serum albumin on strain G148 is shown in (D).*

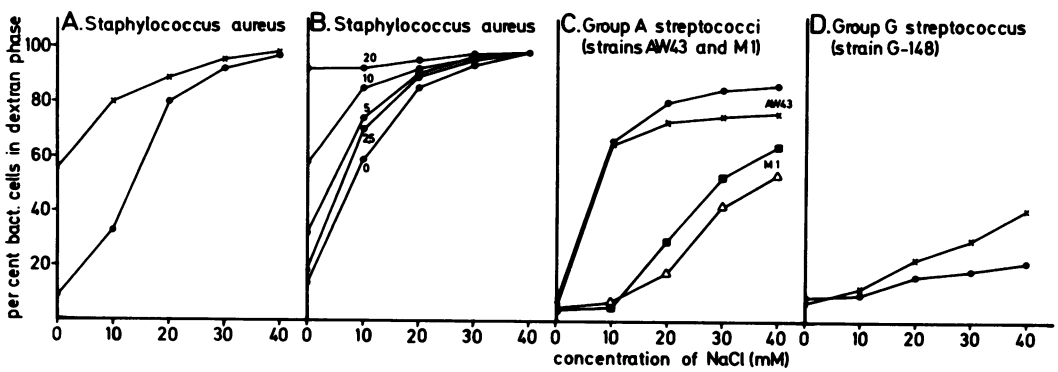

FIG. 3. *Effect of human IgG1 myeloma protein on partition of bacterial cells in the dextran-PEG system at constant polymer concentrations but different ionic compositions. Symbols: (●, ■) bacterial cells only; (×, △) bacterial cells and 10 μg of IgG1 added (A, C, and D). (B) Effect of varying concentrations of human IgG1 on S. aureus.*

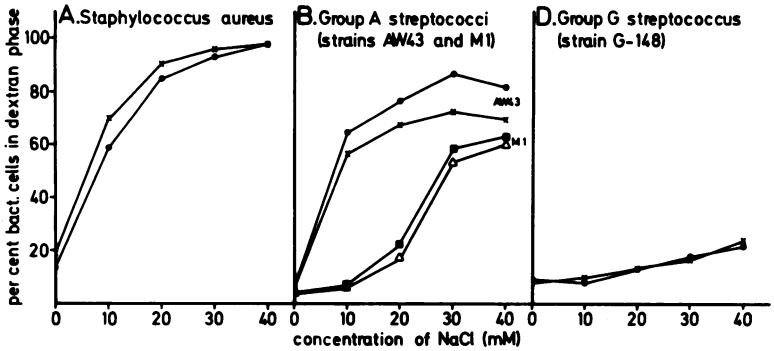

FIG. 4. *Effect of human fibrinogen on partition of bacterial cells in the dextran-PEG system at constant polymer concentrations but different ionic compositions. Symbols: (●, ■) bacterial cells only; (×, △) bacterial cells and 5 μg of fibrinogen added.*

DISCUSSION

The present studies describe marked changes in surface characteristics of four bacterial strains after specific binding of human proteins as revealed by partition in polymer phase systems. The bacteria used carry surface receptors for human serum albumin, IgG, and fibrinogen in different combinations (9, 11, 13, 16, 19, 20, 24–26, 39). As seen in Tables 1 and 3, there is a correlation between the binding of radiolabeled human proteins to the bacterial strains (Table 1) and the effect of human proteins on the partition of the bacterial strains in the dextran-PEG system (Table 3). The biological significance of the bacterial protein binding and subsequent changes of physicochemical surface properties of the bacteria is not yet clear. Such interactions might, however, contribute to interference with the entry of bacteria into the host and host cells and also to interference with host defense mechanisms.

In aqueous polymer phase systems it is possible to separate particles according to their surface properties by partition between the two imiscible phases and the interface (2). The polymers are gentle to the cell particles and to proteins (1). Phase partition has the further advantage that bacteria and protein can be partitioned together and therefore allow the specific protein-protein interactions to take place within the system. Only if there is an interaction between protein and the bacteria will the presence of protein change their distribution. Experiments performed in our laboratory have also documented the possible use of the polymer phase system for discovering unrecognized specific protein binding to bacterial cells.

The effect of bovine and murine proteins on the strains studied was completely in agreement with previous results on direct binding of radiolabeled protein, with one exception. The slight but definite change in partition of the group G *Streptococcus* with bovine serum albumin and of the group A streptococci with human serum albumin has not been confirmed by direct binding studies and needs to be further investigated.

TABLE 3. *Effect of binding of human proteins to bacterial strains on partition in the dextran-PEG system*[a]

Bacterial strains	Serum	Human serum albumin	IgG	Fibrinogen
Staphylococcus aureus	↓↓	—	↓↓↓	↓
Group A *Streptococcus*, AW43	↑↑	↑	↑↑	↑↑
Group A *Streptococcus*, AM1	↑↑	—	↑↑	↑
Group G *Streptococcus*, G148	↓↓↓	↓↓↓	↓↓	—

[a] One, two, and three arrows, respectively, denote low, medium, and high effect of protein on partition of bacterial cells in the phase systems selected for each individual bacterial strain. A dash represents no effect. The direction of the arrow denotes a change in partition in favor of the top phase (↑) or the bottom phase (↓) in the presence of protein as compared to the partition of bacteria alone.

The change in distribution of bacteria after protein binding might be ascribed to the partition properties of the bound protein itself. However, the partition properties of bound protein could not be the sole factor influencing the distribution of bacteria, since the binding of one protein results in a change in partition in favor of the top phase of some strains (AW43 and AM1), but a change in partition in favor of the bottom phase of other strains (*S. aureus* and G148; Table 3). In spite of the lack of correlation between the partition behavior of protein alone and the observed change in partition of bacteria after specific protein binding, it is possible that bound protein expresses different physicochem-

ical properties as compared to protein in solution. As for immunoglobulin, there is an orientation of the bacteria-bound immunoglobulin molecules with their more hydrophilic Fab parts outward (16). A third mechanism mediating the observed surface changes might be that the binding of protein leads to a masking of bacterial cell surface properties. Receptor areas will be covered through the binding of protein to the bacterial cell surface and are no longer available to the polymers. In support of this, it is of particular interest that each individual bacterial strain shows the same change in partition after positive protein binding, whereas individual proteins have opposite effects on the partition of the different bacterial strains (Table 3).

Determination of surface characteristics of *S. typhimurium* has revealed that rough, nonvirulent bacteria are subject to hydrophobic and ionic interactions, whereas hydrophilicity and absence of charge are characteristic of smooth bacteria (22, 34). Therefore, it might be possible to predict the outcome of the interaction between protein-coated bacteria and host cells on the basis of results obtained in polymer two-phase systems. Experiments have been initiated to further characterize the change in surface properties of gram-positive cocci after specific binding of protein, with emphasis on susceptibility to ionic and hydrophobic interactions.

Extensive studies have been performed on the correlation between the physicochemical properties of bacteria and proneness to phagocytic engulfment (33, 36–38, 41, 42) as well as attachment to intestinal mucosal cells (27). From these investigations it is evident that the physicochemical cell surface properties of the bacteria play a crucial role in the interaction of bacteria with human cells. Many in vitro studies on adherence of streptococci to epithelial surfaces have been performed in physiological saline (4, 5, 10). Other authors have shown that there are surface differences between microorganisms grown in vivo and in vitro (31–33). The marked effects of specific protein binding to the bacteria on their surface properties as shown in our studies indicate that these types of interactions have to be taken into account in studies of virulence. Some low avid interactions between serum proteins and bacterial surfaces of more nonspecific type might also influence host-parasite interactions. However, in the present studies such low avid interactions did not influence the polymer phase partition of the investigated bacteria to any detectable extent.

Interference with host defense mechanisms after surface acquisition of host proteins has been demonstrated in experimental *Schistosoma mansoni* infection (6). This mammalian parasite is capable of specific binding of blood group substances and histocompatibility antigens to its surface (8, 29). Receptors for IgG Fc and human β_2-microglobulin have also been discovered on the surface of *S. mansoni* (14, 40). The existence of receptors for some types of human host proteins in such widely separated parasite species as schistosomes and streptococci seems to indicate that binding of host proteins might be a more widespread phenomenon in nature. Such binding might influence the outcome of host-parasite relationships as emphasized by the marked changes in surface properties of coated bacteria noted in our present studies. Their systematic elucidation will therefore provide a more solid basis for future virulence studies.

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