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Immunoglobulin-Binding Activity among Pathogenic and Carrier Isolates of *Haemophilus somnus*

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Nonimmune binding of immunoglobulin to whole bacteria was quantitated for North American isolates of *Haemophilus somnus* recovered from cattle with pneumonia, reproductive failure (abortion), or thromboembolic meningoencephalitis or from the vagina or prepuce of carrier cattle. Quantitative binding activity covered a wide range, with most pathogenic and carrier isolates demonstrating significant immunoglobulin-Fc binding. Isolates for which Fc binding was not detectable were recovered only from the prepuces of asymptomatic bulls. Expression of Fc-binding activity correlated with the presence of the 41,000-molecular-weight protein (41K protein) and 270K protein. Isolates that lacked Fc-binding activity did not possess 41K or 270K protein. A 33K protein was detected in isolates that lacked Fc-binding activity but not in isolates that bound Fc.

Haemophilus somnus is the etiological agent in a range of systemic and mucosal disease syndromes of cattle (9). A feature of infections with this organism is its persistence at mucosal sites in both asymptomatic and diseased animals. It can be recovered from the nasal and genital mucosae of clinically normal cattle (9) and, following experimental pulmonary infection in calves, has been isolated from bronchoalveolar washings up to 5 weeks after challenge (8).

The basis for this persistence in cattle has not been defined. Studies of the bacterial surface have failed to identify relevant structures, such as long O-side chains to bacterial lipopolysaccharide (7, 9a; T. J. Inzana, R. Gogolewski, and L. B. Corbeil, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, B-183, p. 54) or a capsule (15), commonly associated with evasion of host defenses by gram-negative pathogens (11, 16). Bacterial outer membrane proteins can contribute to pathogenicity (1), as do the outer membrane proteins of *Neisseria gonorrhoeae* (10). Proteins in the outer membrane of *H. somnus* that bind bovine immunoglobulin by a nonimmune mechanism have been identified (20, 21). Bacterial proteins of 41,000 and 270,000 molecular weight (41K and 270K proteins) reacted with the Fc region of immunoglobulin G. Similar microbial binding of host molecules has been associated with evasion of host defenses by pathogenic streptococci (14, 17) and staphylococci (6, 13, 14).

The aim of this study was to monitor quantitative Fc-binding activity among disease and carrier isolates of *H. somnus* in order to investigate the association between Fc receptor activity and pathogenicity. Binding of Fc to disease and carrier isolates of *H. somnus* was measured by enzyme-linked immunosorbent assay (ELISA). Possession of the 41K and 270K receptor proteins was investigated by Western blot (immunoblot); probing was done with antisera specific for these bacterial antigens.

Bovine isolates of *H. somnus* were recovered from North American clinical cases of animals with thromboembolic meningoencephalitis, pneumonia, and reproductive failure (abortion) and from the asymptomatic carrier state in bovine vagina and prepuce (4). Isolates were preserved in 60% glycerol in phosphate-buffered saline and stored at −70°C.

Bacteria were cultured in brain-heart infusion supplemented with 0.1% Tris base and thiamine monophosphate (1 μg/ml) (19). Cultures were incubated with shaking in air at 37°C for 5 to 6 h, harvested by centrifugation, and suspended in 1% Formalin in saline.

Bovine immunoglobulin G was purified from normal serum by ion-exchange chromatography (20). Bovine immunoglobulin G-Fc fragments were prepared by papain digestion and isolated by ion-exchange chromatography (20). The purity of the immunoglobulin preparations was monitored by immunoelectrophoresis (20).

Binding of the bovine Fc fragment to whole bacteria was titrated in ELISA (20). Endpoints were calculated as the concentration of immunoglobulin-Fc resulting in an optical density at the approximate midpoint of the titration curve (optical density, 0.30). Bacterial isolate 8025, a laboratory standard recovered from an animal with thromboembolic meningoencephalitis, was included in each microdilution plate as a reference standard, and results for each isolate were expressed as an Fc-binding index, calculated as the ratio of the isolate endpoint to the reference standard endpoint. Results were calculated on the basis of at least two separate experiments and are presented as means of those observations.

Bacterial antigens for electrophoresis and immunoblotting were prepared from filtered bacterial-culture supernatant (21). Antigens were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (21) and transferred electrophoretically to nitrocellulose. Separated antigens on nitrocellulose were reacted with rabbit antiserum to the bacterial Fc receptors (21), consisting of a mixture of equal proportions of the anti-41K serum (1/1,500 dilution) and the anti-270K serum (1/1,500 dilution). Immunoreactive bands were visualized by incubation with goat anti-rabbit immunoglobulin G conjugated to peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) followed by substrate (21). The significance in this study of immune binding of Fab or whole immunoglobulin G contaminants in the Fc preparation is minimized by the fact that the 41K Fc receptor, a major outer membrane protein of *H. somnus*, is nonimmunogenic (3, 7). Antibody specific for this protein was not detectable in normal or immune bovine serum (2, 5). Antibody binding to

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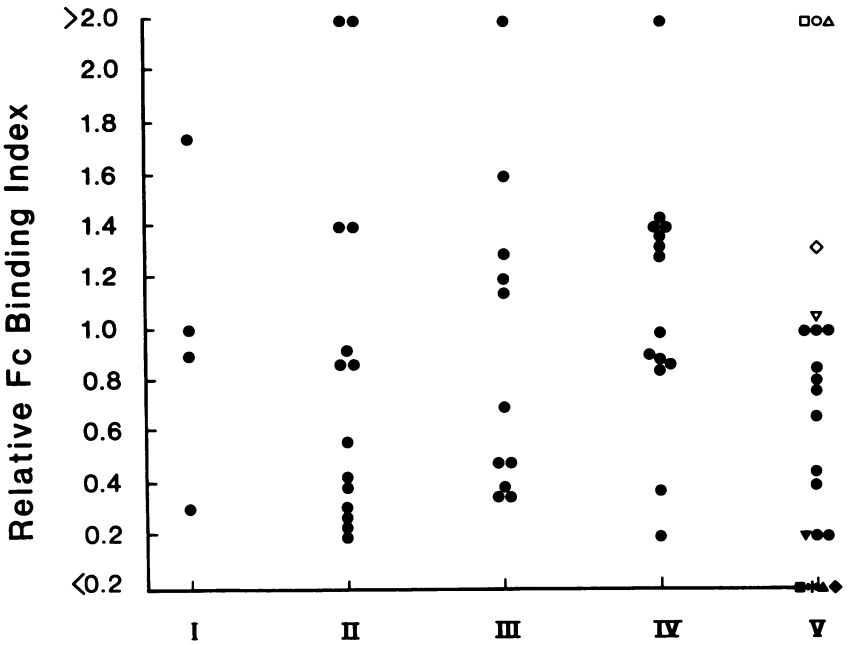

FIG. 1. Fc-binding activity among bovine isolates of *H. somnus* recovered from cattle with thromboembolic meningoencephalitis (I), pneumonia (II), or reproductive failure (III) or with an asymptomatic carrier state in vagina (IV) or prepuce (V). Results are expressed as a binding index, calculated as the ratio of the endpoint for each isolate relative to that for the standard isolate, 8025. Average endpoint to binding by 8025 was 75 μg of Fc per ml. Ten preputial isolates were selected for further characterization as high-binding isolates (18P [□], 19P [○], 02973 [△], 1565 [◇], and 129Ph [▽]) or low-binding isolates (1P [*], 129Pt [▼], 130P [▲], 133P [◆], and 0834 [■]).

bacterial antigens was analyzed by densitometry with a Hoeffer scanning densitometer (model 1650) with GS360 software.

Nonimmune immunoglobulin binding was detected among isolates of *H. somnus* recovered from both diseased and carrier cattle (Fig. 1). A wide range in relative binding indices was found, but this range was comparable among isolates recovered from cattle with pneumonia, thromboembolic meningoencephalitis, or reproductive failure (abortion) and from the vaginas and prepuces of carrier animals. All pathogenic and vaginal carrier isolates displayed detectable binding of bovine immunoglobulin-Fc, but four preputial isolates that showed no binding in this assay were identified (Fig. 1).

The capacity of individual isolates to bind bovine Fc fragment in ELISA correlated with the presence of the 41K and 270K bacterial proteins (Fig. 2). All pathogenic and vaginal carrier isolates possessed antigens of approximately 41,000 and 270,000 molecular weights that were recognized by immune serum in blots (data not shown). The electrophoretic and antigenic profiles in an outer membrane preparation from 10 preputial isolates, selected on the basis of high or low binding activity in ELISA (Fig. 1), are presented in Fig. 2 and Table 1. Isolates 18P, 19P, 1565, 129Ph, and 02973, which bound Fc in ELISA (Fig. 1), possessed protein bands that reacted with antisera to the 41K and 270K antigens (Table 1). In contrast, isolates 1P, 129Pt, 130P, 133P, and 0834, which showed no or minimal binding of Fc in ELISA (Fig. 1), did not contain the 41K or 270K protein (Fig. 2). An additional 33K major outer membrane protein was detected in these five isolates lacking Fc-binding activity (Fig. 2). This protein was not observed in other isolates and might be a variant form of the 41K protein, having lost structural components necessary for Fc-binding activity.

All isolates recovered from cattle with disease syndromes (thromboembolic meningoencephalitis, pneumonia, and abortion) displayed detectable Fc binding and possessed the Fc-binding proteins (data not shown). While quantitative Fc receptor activity was variable, the consistent expression of activity and the presence of the relevant bacterial proteins among pathogenic isolates of *H. somnus* could characterize the Fc receptor as a useful marker of bacterial virulence. On this basis, the carrier isolates expressing Fc receptor activity should also be considered potential pathogens.

Surface acquisition of bovine immunoglobulin might also enhance bacterial virulence. *H. somnus* apparently lacks factors that, in other gram-negative bacteria, are associated with evasion of phagocytosis and complement-mediated bactericidal activity (11, 16). The organism does not possess a bacterial capsule (15), and the bacterial lipopolysaccharide has oligosaccharide moieties of molecular sizes similar to those of *Escherichia coli* J5 (7, 9a; Inzana et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986). Yet this pathogen is capable of surviving systemically for production of thromboembolic meningoencephalitis and abortion (9, 19) and mucosally for up to 5 weeks in the lungs of experimentally infected calves (8). Immunohistological examination of pneumonic lesions from calves infected with *H. somnus* demonstrated a predominance of extracellular bacteria in the presence of neutrophils and degenerate macrophages (8). Thus, *H. somnus* is capable of extracellular survival in vivo, implying evasion of phagocytosis and complement-dependent killing. A bacterial surface mechanism for acquiring host constituents might enhance evasion of defenses by masking bacterial targets for deposition of complement or specific antibody (18). Similarly, persistence at an infection site could be favored by bacterial suppression of phagocyte function. In this respect, the inability of bovine phagocytes—both polymorphonuclear leukocytes (5) and macrophages (12)—to kill *H. somnus* in vitro is probably important to the persistence of this organism in vivo.

The resistance of *H. somnus* to phagocytic killing has been

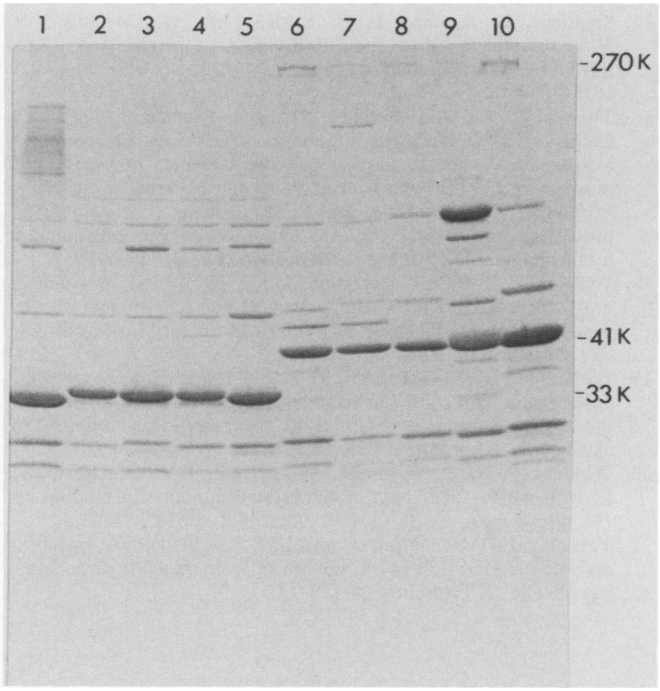

FIG. 2. Protein profiles of preputial isolates of *H. somnus* identified as low-binding isolates (0834, 1P, 133P, 130P, and 129Pt [lanes 1 through 5, respectively]) or high-binding isolates (02973, 129Ph, 18P, 1565, and 19P [lanes 6 through 10, respectively]). Bacterial antigens released into the culture supernatant and sedimented by ultracentrifugation were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Separated antigens were visualized by staining with Coomassie blue. High-binding isolates revealed 270K and 41K protein antigens that reacted with antisera specific for the bacterial Fc receptors (Table 1). The low-binding isolates did not contain the 270K or 41K protein.

attributed to bacterial release of soluble inhibitors, including guanine, adenine, and GMP (2). These soluble factors inhibit phagocytosis and bactericidal activity (2) and are likely responsible, at least in part, for the presence of *H. somnus* outside phagocytes at infectious foci in vivo (8). Bacterial pathogenicity, however, is unlikely to be dependent on a single bacterial virulence mechanism. The leukotoxin produced by *H. somnus* (H. D. Liggitt, L. Huston, and L. B. Corbeil, Abstr. Conf. Res. Workers Anim. Dis., abstr. 174, 1984) must favor extracellular survival, and cell-associated and soluble Fc receptors might also limit phagocytosis and complement-dependent killing.

Differences in resistance to host defenses between pathogenic and preputial isolates of *H. somnus* have been described elsewhere. In a study of susceptibility to the bactericidal effects of fresh, normal bovine serum, pathogenic isolates were relatively resistant to serum killing, while a proportion of preputial isolates were susceptible (4). These differences in serum susceptibility are most likely related to variations in bacterial surface structure. Resistance of pathogenic *N. gonorrhoeae* to serum killing has been associated with the possession of particular variants of outer membrane proteins (10). The bacterial basis for increased serum sensitivity among preputial isolates of *H. somnus* has not been defined.

This study has identified differences between pathogenic and preputial isolates in the protein composition of the bacterial outer membrane. However, the association between expression of bacterial Fc receptors and increased resistance to serum killing remains unclear. Some preputial isolates that were characterized for serum susceptibility (4) were also used in the present study. Isolates 11P, 26-16S, 20P, 22P, and 129Ph, which were positive for Fc receptor activity in this study, were classified as serum resistant (7). Isolates 133P, 1P, 124P, and 24P were classified as serum susceptible (4), and of these, 133P and 1P lacked Fc-binding activity (Fig. 1). Both 124P and 24P bound immunoglobulin, although binding by 24P was minimal, i.e., at the lower limit of assay sensitivity. Although interpretation of these results could be confounded by the presence of different clones in the original bacterial isolate (4), resistance to serum killing is consistently associated with expression of Fc-binding activity. However, the role of nonimmune binding by *H. somnus* in protecting against complement killing and killing by polymorphonuclear leukocytes requires further investigation. The isolates of *H. somnus* lacking the Fc receptor proteins can serve as the basis for further studies to define the role of nonimmune immunoglobulin binding in bacterial pathogenicity.

TABLE 1. Integrated densitometric analysis of separated bacterial antigens (Fig. 2) reacted with antisera specific for the 270K and 41K Fc receptor proteins

Isolate[a]	Relative peak area	
	270K protein	41K protein
02973	1,884	8,073
129Ph	311	7,575
18P	664	4,787
1565	534	643
19P	1,743	5,040

[a] Low-binding isolates 0834, 1P, 133P, 130P, and 129Pt showed no detectable bands on immunoblot.

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