INFECTION AND IMMUNITY, June 1987, p. 1381–1386
0019-9567/87/061381-06$02.00/0

Vol. 55, No. 6

Antigenic Specificity of Convalescent Serum from Cattle with *Haemophilus somnus*-Induced Experimental Abortion

LYNETTE B. CORBEIL,†* JILL E. ARTHUR, PHILLIP R. WIDDERS, JOE W. SMITH, AND ANTHONY F. BARBET‡

Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040

Received 12 December 1986/Accepted 26 February 1987

The antigens of *Haemophilus somnus* recognized by convalescent bovine serum were studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting with a protein A-peroxidase conjugate. The same two 76K and 40K antigens were predominant in whole-bacterium preparations and in outer-membrane-enriched, Triton X-100-insoluble fractions. The surface location of these two antigens was confirmed by absorbing antiserum with whole, live bacteria. Absorption with *H. somnus* removed antibody reactivity for the 76K antigen and reduced reactivity for the 40K antigen. Absorption with *Pasteurella multocida*, *Actinobacillus equuli*, or *Escherichia coli* did not reduce reactivity, and results with *Pasteurella haemolytica* were equivocal. The two immunodominant antigens detected in this study were conserved in isolates of *H. somnus* from thromboembolic meningoencephalitis, pneumonia, reproductive failure, or asymptomatic carriers. Convalescent sera from nearly all 17 cattle studied recognized these two antigens. Other antigens were recognized less consistently. Although other antigens may also be involved, the 76K and 40K surface antigens of *H. somnus* appear to be important candidates for a subunit vaccine or an immunodiagnostic assay.

Haemophilus somnus is a gram-negative pleomorphic bacillus that causes thromboembolic meningoencephalitis (TEME) in cattle (2, 11, 14, 15), as well as septicemia, arthritis, pneumonia, and reproductive failure (14, 16). Not only is this organism an important pathogen, but also many apparently healthy animals carry it on their nasal, vaginal, or preputial mucosae (12, 13, 14, 34). Little is known about the antigenic specificity of the immune response after infection or about antigenic differences between isolates from asymptomatic carriers and diseased animals. We have shown previously that abortion can be produced experimentally in cattle with an isolate from a bovine abortion (35) and that pneumonia can be produced in calves with an isolate from bovine pneumonia (R. P. Gogolewski, C. W. Leathers, H. D. Liggitt, and L. B. Corbeil, Vet. Pathol., in press). Convalescent serum from calves with experimental pneumonia was protective (R. P. Gogolewski, H. D. Liggitt, and L. B. Corbeil, Abstr. 1st Int. Vet. Immunol. Symp., 1986, p. 121). Since *H. somnus* appears not to have a capsule or pili (31) and crude outer-membrane antigens protect against TEME (32), we hypothesized that the outer-membrane antigens recognized by convalescent serum would include protective antigens on the surface of the bacterium.

Diagnosis is also a problem in *H. somnus* infection. Cultural diagnosis is used, but the organism is fastidious and is often overgrown by normal flora. Furthermore, isolation of *H. somnus* after antibiotic treatment of the host is often not possible, and the presence of the mucosal carrier state of *H. somnus* often complicates bacteriological diagnosis. Serological diagnosis of *H. somnus* disease is made by a microagglutination test in some laboratories. Although this test is very sensitive, many cattle without *H. somnus* disease have positive titers (9). Thus, a more specific serological test would be very useful. Serological assays with whole bacteria as the antigen may detect cross-reactive antibody, thereby producing high background titers in the microagglutination test or in the enzyme-linked immunoabsorbent assay. Therefore, a subunit antigen recognized by convalescent but not by preinfection sera should be the basis for a more specific diagnostic assay.

We now report an analysis of the antigens recognized by convalescent serum and show that these antigens are at least partially exposed on the surface of the outer membrane of *H. somnus*.

MATERIALS AND METHODS

Bacterial cultures. Bovine isolates of *H. somnus* were obtained from clinical cases of TEME, pneumonia, or reproductive failure which were submitted to the Washington Animal Disease Diagnostic Laboratory or from cultures of the vaginal or preputial flora of clinically normal animals. Isolates were frozen at −70°C in phosphate-buffered saline–glycerol (40:60) within two subcultures from initial isolation. Isolate 8025 was a laboratory strain originally obtained from a case of TEME, and isolates 91-1, 43826, and B83-145 were received from P. Little, Ontario Veterinary College, Guelph, Ontario, Canada. The isolate (649-4) used for experimental infection, was obtained from an aborted bovine fetus. These isolates were grown on Columbia blood-agar plates containing 10% bovine blood at 37°C in 10% CO_2 overnight and then were used to seed brain heart infusion broth containing Tris base (0.1%) and thiamine monophosphate (0.001%) (BHI-TT). For electrophoresis, broth cultures were grown to late log phase (5 to 6 h) yielding approximately 10^9 organisms per ml. Cells were centrifuged, washed, and suspended in 5 ml of 0.5 M Tris (pH 6.8). Clinical isolates of other bacteria (*Escherichia coli* [isolate JL9 from calf septicemia], *Pasteurella multocida* [isolate 251 from bovine lung], *Pasteurella haemolytica* [isolate 0587 from bovine lung], and *Actinobacillus equuli* [isolate 1723-6

* Corresponding author.
† Present address: Department of Pathology H811F, University of California at San Diego Medical Center, San Diego, CA 92103.
‡ Present address: University of Florida, College of Veterinary Medicine, Gainesville, FL 36201.

from equine trachea]) were also frozen in phosphate-buffered saline–glycerol within two subcultures from initial isolation and were similarly grown in BHI-TT.

Sera. Bovine sera were collected before and after challenge with *H. somnus* (isolate 649-4). The clinical and isotypic antibody responses to this challenge have been described in an earlier report (35). Briefly, 19 pregnant cows were challenged by intravenous (11 animals) or intrabronchial (8 animals) inoculation of 4×10^{10} *H. somnus*. Abortion or fetal resorption was recorded for five cows challenged intravenously and for one cow challenged intrabronchially. Isolation of large numbers of *H. somnus* from aborted tissues and placental lesions consistent with *H. somnus* infection confirmed the role of this organism in the etiology of the abortions. Sera were collected at weeks 0, 1, 2, 5, and 30 and stored in aliquots at −20°C. Seventeen serum samples collected preinfection and at 5 weeks postinfection were used in this study because one of the aborting cows died shortly after abortion because of a uterine rupture, and one animal was not bled at 5 weeks. For production of hyperimmune serum, one of the convalescent animals was immunized with two biweekly subcutaneous injections of 10^{10} formalinized *H. somnus* cells from isolate 649-4 beginning at 6 weeks postinfection. The animal was bled 14 weeks after challenge.

SDS-PAGE. Bacterial suspensions (containing approximately 5×10^8 cells per ml) were boiled for 3 min in sample buffer containing 2-mercaptoethanol as described by Laemmli (19). Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 1.5-mm-thick slab gels used stacking and gradient gels of 5% and 7.5 to 15% polyacrylamide, respectively. Electrophoresis was carried out at a constant current of 30 mA for 4 h with a slab cell apparatus (Bio-Rad Laboratories, Richmond, Calif.). The gels were either stained with 0.075% Coomassie brilliant blue or electrotransferred to nitrocellulose.

Outer-membrane Triton X-100 extraction. Late-phase bacteria from 100 ml of BHI-TT broth culture were washed, suspended in 25 ml of 0.05 M Tris (pH 7.8) containing 2 mM $MgCl_2$, and sonicated for 3 min in 15-s bursts at a probe intensity of 80 W with a Braunsonic 1510 sonicator. After being centrifuged at 12,000 × *g* for 14 min to remove whole bacteria, the supernatant was centrifuged at 255,000 × *g* for 1 h to sediment outer membranes. The pellet was suspended in 0.5 ml of 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4) and extracted twice with 2% Triton X-100 as described by Schnaitman (29).

Immunoblotting procedure. Upon completion of electrophoresis, the SDS-PAGE gel was equilibrated in transfer buffer (25 mM Tris–192 mM glycine [pH 8.3] with 20% methanol) for 30 min and then transferred electrophoretically to a nitrocellulose membrane by using a Trans-Blot Cell (Bio-Rad) (33). These blots were then incubated in bovine sera diluted with Tris-buffered saline (pH 7.5) containing 0.05% Tween 20 (TBS-Tween) for 2 h at room temperature. After two 10-min washes in TBS-Tween, bound antibody was detected by incubation in a 1:2,000 dilution of protein A-horseradish peroxidase conjugate (Zymed Laboratory, South San Francisco, Calif.) in TBS-Tween for 2 h at room temperature. After the membrane was washed, color was developed in TBS with 0.05% 4-chloro-1-naphthol, 5% methanol, and 0.015% hydrogen peroxide. Immunoblots or portions thereof were overstained with 0.1% amido black to detect molecular weight standards or to identify nonimmunoreactive protein bands.

Antibody absorption. Bovine convalescent serum (from cow 14) was incubated with live bacteria at 4°C to remove antibody to bacterial surface components. Cells from 50 ml of a log-phase broth culture of bacteria (2×10^9 to 4×10^9 cells per ml) were washed twice in phosphate-buffered saline by centrifugation at 10,000 × *g* for 10 min and suspended in 1 ml of a 1:100 dilution of heat-inactivated (30 min at 56°C) convalescent serum in phosphate-buffered saline. The ratio of pellet to serum volume was about 1:2. After 3 h of incubation at 4°C, the absorbed serum was recovered by centrifugation. Depletion of antibody activity to bacterial antigens was monitored by Western blotting with a final concentration of 1:2,000 of unabsorbed convalescent serum or of convalescent serum absorbed with homologous *H. somnus* (isolate 649-4), *E. coli* (isolate JL9), *A. equuli* (isolate 1723-6), *P. multocida* (isolate 251), or *P. haemolytica* (isolate 0587).

RESULTS

The protein profile of *H. somnus* after SDS-PAGE revealed many proteins. Preliminary studies showed that when nitrocellulose electroblots were reacted with bovine serum and developed with anti-bovine immunoglobulin G (IgG)-peroxidase conjugate, background reactivity with preimmune sera was high. This was not so with a protein A-peroxidase conjugate which detects primarily IgG2 (35). Because of this and because our earlier studies had shown that IgG2 antibodies predominate in the bovine immune response to *H. somnus* infection (35), all studies reported here were done with a protein A-peroxidase conjugate. With this conjugate, preimmune serum was negative at a 1:1,000 dilution, whereas convalescent serum (from cow 14) at the

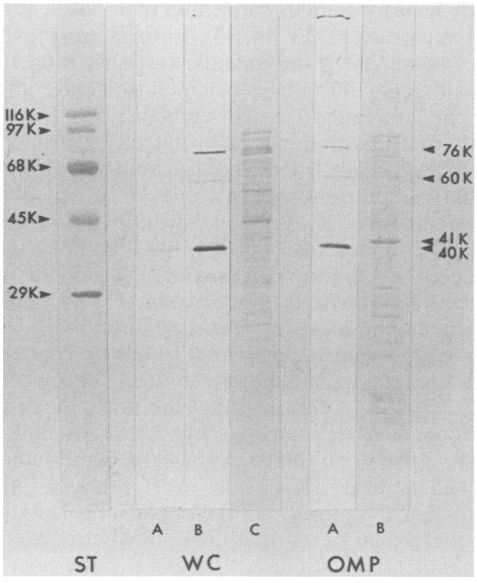

FIG. 1. Protein and antigen profiles of whole *H. somnus* cells (WC) and outer-membrane-enriched preparations (OMP) of *H. somnus* isolate 649-4. ST, Molecular size standards stained with amido black. WC: lane A, reacted with preimmune serum (1:1,000); lane B, convalescent serum from cow 14 (1:1,000); lane C, stained with amido black. OMP: lane A, reacted with convalescent serum from cow 14 (1:1,000); lane B, stained with amido black. Note that the same 76K, 60K, and 40K antigens were detected in whole-cell *H. somnus* preparations and outer-membrane preparations. Also, note that the 41K major outer membrane protein was not recognized by convalescent serum. A faint reaction with the 40K antigens was detected by preimmune serum.

same dilution showed two very intense bands with apparent molecular weights of 76,000 and 40,000 (Fig. 1). Both of these antigens appeared to be present in the outer membrane since outer-membrane-enriched fractions prepared by Triton X-100 extraction (Fig. 1) did not show decreased intensity of the 76K and 40K bands in Western blots with the same convalescent serum. The major outer-envelope protein of *H. somnus* (with an apparent molecular weight of 41,000) was not recognized at all by convalescent serum (Fig. 1) as shown by staining of the 41K protein with amido black but not by staining with protein A-peroxidase. In other studies, when an individual strip from an immunoblot was overstained with amido black, it was clear that there was a 40K immunoreactive band and a nonimmunoreactive 41K protein. This was also true in preliminary studies with an anti-bovine IgG conjugate. The data obtained from immunoblots overstained for protein is not shown because it is difficult to differentiate between antigen bands stained by the peroxidase conjugate and protein bands stained only with amido black without color photographs. The surface location of the 76K and 40K antigens was further demonstrated by absorption of convalescent serum with whole, live *H. somnus* bacteria (isolate 649-4) (Fig. 2). Antibody reactivity with the 76K antigen was essentially removed by absorption, while reactivity with 40K antigen was greatly reduced. Absorption of this serum with other closely related gram-negative bacteria (*P. multocida*, *A. equuli*, or *E. coli*) did not significantly reduce reactivity for the 76K or 40K antigen. In Fig. 2, the serum absorbed with *P. haemolytica* appeared to give slightly lighter bands at both the 76K and the 40K regions, perhaps because of dilution of serum during absorption or because some antigen remained at the top in this lane. Other absorptions with *P. haemolytica* showed no decrease in either 76K or 40K protein reactivity (data not shown).

Preinfection serum and serum collected 5 weeks after experimental infection were used to determine the range of immune responses in an experimental abortion study described previously (35). All postinfection sera from the 17 cattle studied reacted more strongly with the 76K protein than did preinfection sera, but the 76K antigen bands were not very intense with postinfection sera from animals 1, 17, and P1 (Fig. 3). The 40K antigen reaction was also much more intense with postinfection sera than with preinfection sera from all 17 animals, but postinfection sera from animals 15 and P1 gave very faint reactions (Fig. 3). Several other antigens (including the 270K and 60K antigens) were recognized by some sera but not by others at the dilutions used in this study. The major outer-envelope protein (41K) was not recognized by these sera either. Sera collected from many of the 17 cows at 30 weeks postinfection also recognized the two major antigens (data not shown).

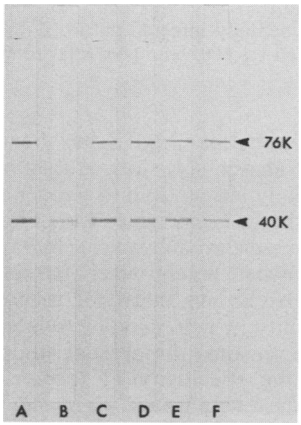

FIG. 2. Reactivity of absorbed convalescent serum from cow 14 with *H. somnus* isolate 649-4. Lanes A, unabsorbed convalescent serum; same serum absorbed with: B, *H. somnus* isolate 649-4; C, *E. coli* isolate JL9; D, *A. equuli* isolate 1723-6; E, *P. multocida* isolate 251; and F, *P. haemolytica* isolate 0587. All absorptions were done with live log-phase bacteria for 3 h at 4°C to ensure that only surface antigens would absorb antibodies.

Conservation of the surface antigens among *H. somnus* isolates was studied with the 20 isolates from TEME, pneumonia, reproductive failure, or asymptomatic carriers (Fig. 4) that were used in our previous studies (7). Bovine convalescent serum (from cow 14) recognized the 60K and 40K antigens in all isolates and the 76K antigen in at least 14 of 23 isolates. The position of the 40K antigen, and perhaps of the 76K antigen, varied slightly from isolate to isolate.

DISCUSSION

Although many proteins were demonstrated in the protein profile of *H. somnus*, only a few appeared to be important immunogens as determined by immunoblotting. The two major antigens detected in this study appeared to be part of the outer membrane, and antibodies to these antigens were absorbed out with homologous whole, live *H. somnus*, indicating that the 76K and 40K antigens were surface exposed or partially exposed. This is significant since outer membrane proteins of *Neisseria gonorrhoeae* (4, 26), *Yersinia* species (27), *Bordetella bronchiseptica* (25), and *E. coli* (1) have been shown to be important in virulence. Also, antibodies to outer-membrane-protein antigens have been shown to protect against several bacterial infections including *Haemophilus influenzae* (3, 17, 23), *Salmonella typhimurium* (18), and *Neisseria meningitidis* (10). Although not all outer membrane proteins stimulate the production of protective antibodies (23), the surface exposure of *H. somnus* 76K and 40K outer membrane proteins suggests that antibodies to them may be protective. In support of this hypothesis are the observations that no pili or capsules were demonstrated in ultrastructural studies of the bacterium (31) and that the crude outer membranes of *H. somnus* protect against TEME (32). Since convalescent serum was protective in our own studies (Gogolewski et al., Abstr. 1st Int. Vet. Immunol. Symp., 1986), it is likely that an antibody is important in protection. Of course, the outer membrane of *H. somnus* contains not only protein but also lipopolysaccharide which may be involved in pathogenesis and protective host responses. Furthermore, surface-exposed protein antigens which were not detected by our methods may be important in protection also. Nevertheless, detection of two or more surface-exposed immunogens recognized by protective convalescent serum shows that they are likely to be useful diagnostic antigens and may indicate that these are protective antigens.

Studies of other gram-negative bacteria have demonstrated that the major outer-envelope protein is protective (10, 18). In SDS-PAGE, the outer membrane protein which stains most intensely (the major protein) is often the porin (4). However, the major protein in outer-membrane-enriched fractions of *H. somnus* found by SDS-PAGE was not recognized by convalescent serum. The reason for this lack of response to the major envelope protein is not known but may be due to a lack of immunogenicity, lack of surface exposure, or loss of antigenicity during SDS-PAGE blotting.

Not only is the specificity of the antibody response important in protection, but also the isotype is of functional

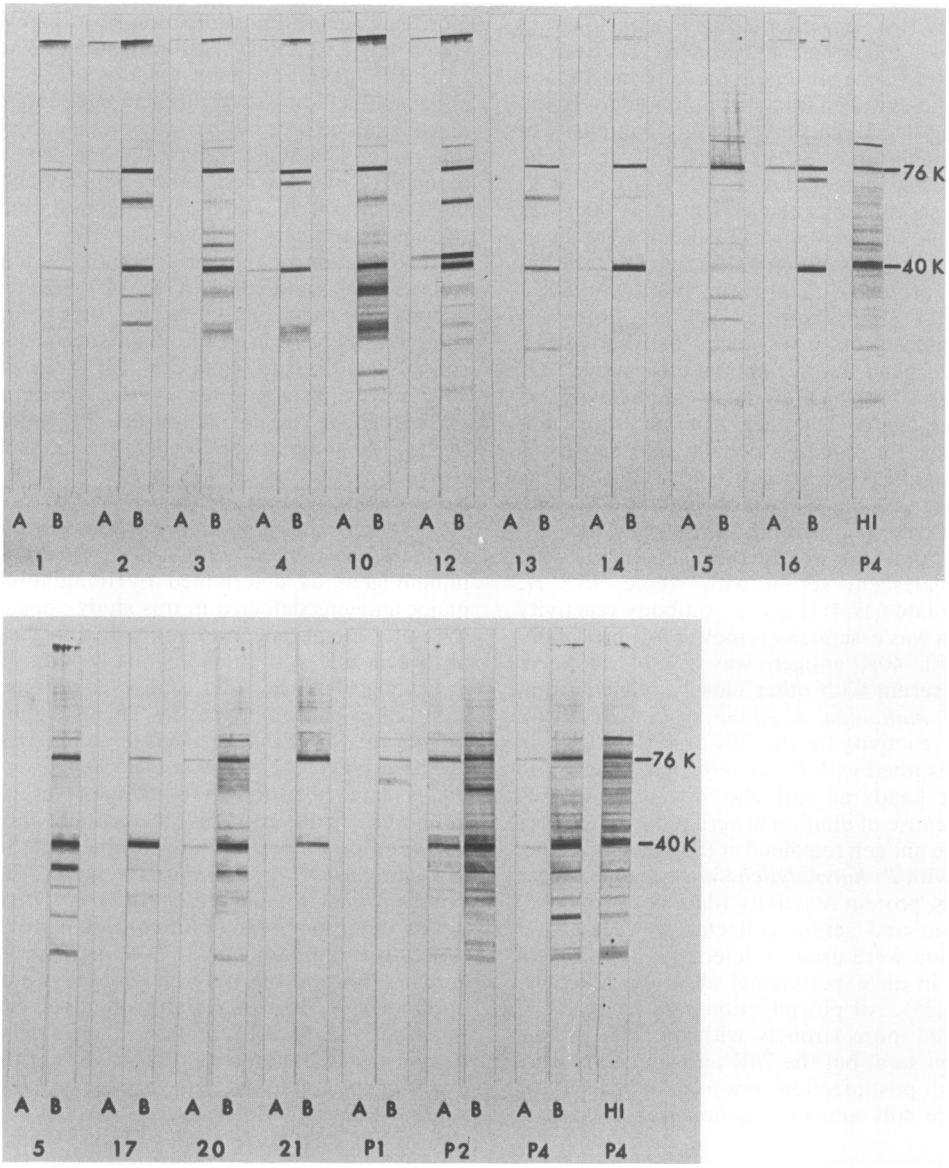

FIG. 3. *H. somnus* whole-cell antigens recognized by convalescent serum from experimentally infected cattle. Lanes: A, preimmune serum; B, 5-wk-postinfection serum; HI, hyperimmune serum. All serum samples were diluted 1:1,000. Numbers refer to individual animals.

significance in defense. In this study, we used protein A-peroxidase to detect antibody reactivity in Western blots. Although bovine IgG1 will bind to protein A under some conditions of high pH and molarity (28), this reagent is generally considered to bind preferentially to bovine IgG2 (20), which is consistent with results in our laboratory with purified bovine IgG2 (P. R. Widders, unpublished data). Also, IgG2 responses are likely to be relevant to bovine disease. We have already reported that cattle have natural antibodies to *H. somnus*, but that most of this cross-reactivity is caused by the IgM and IgG1 isotypes (35). Furthermore, in the sera from experimental abortion used in the present study, IgG2 antibodies detected by enzyme-linked immunosorbent assay were of the lowest titer prechallenge, increased the most after challenge, and persisted longest when compared with the IgG1 and IgM responses (35). Cows with the lowest prechallenge IgG2 anti-*H. somnus* titers were the animals which aborted (35). In other bovine bacterial infections, IgG2 has been implicated in protection also. Nanssen (24) showed that cattle deficient in IgG2 were especially susceptible to pyogenic infection, and Corbeil et al. (8) showed that calves were most susceptible to pneumonia when serum and nasal IgG2 concentrations reached their lowest levels after passive transfer via colostrum. It is also known that IgG2 is more opsonic than IgG1 (22), a quality which may be related to protective ability. For these reasons, the present study was limited to investigation of the specificity of responses detected by protein A-peroxidase conjugate.

The variation in the specificity of the response of individual animals was determined by Western blot analysis of the preinfection and postinfection sera of 17 cows. Since the postinfection sera recognized the 76K and 40K proteins with greater intensity than the preinfection sera, it can be concluded that these two antigens are among the dominant immunogens for cattle. This underscores their potential

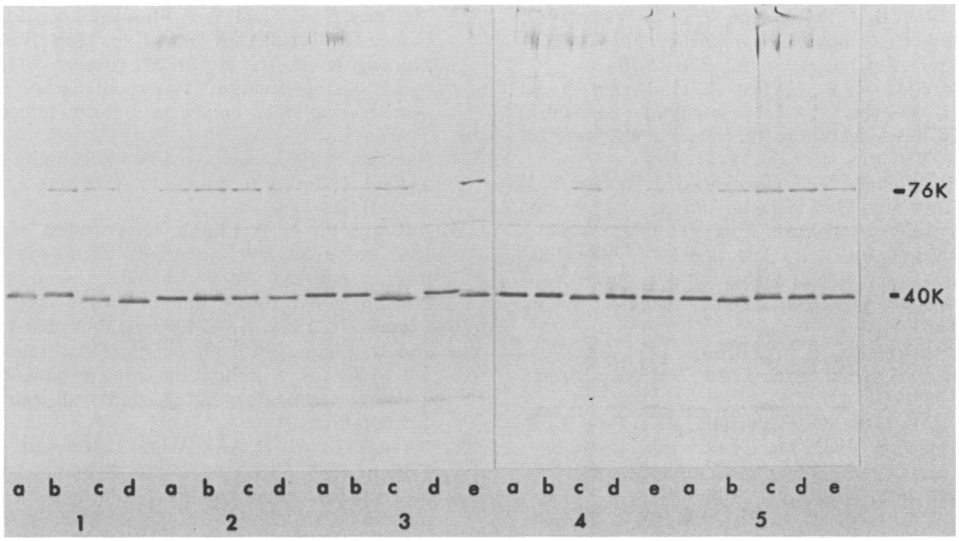

FIG. 4. Conservation of *H. somnus* antigens among whole-cell isolates from various syndromes. Preputial isolates (group 1): a, 1P; b, 24P; c, 127P; and d, 129 Pt. Vaginal isolates (group 2): a, 41Vc; b, 202V; c, 208V; and d, 221V. Reproductive-failure isolates (group 3): a, 1030; b, 2069; c, 649-4; d, 0570; and e, B83-145. TEME isolates (group 4): a, 43826; b, 0289; c, 8025; d, 109B, and e, 91-1. Pneumonia isolates (group 5): a, 1542; b, 2336; c, 3581; d, 3415-2; and e, 1297. Serum used to detect antigen was convalescent serum from cow 14. Note that the 40K and 60K antigens were present in all isolates and the 76K antigen was detected in most isolates.

usefulness both as vaccines and as immunodiagnostic antigens. A few individual animals had considerable antibody to these two antigens before challenge. This is not surprising since the cattle came from varied backgrounds. Although the animals were healthy at the time of the study, it is not known whether they had had *H. somnus* disease or had been carriers in the past.

Western blot studies of conservation of surface antigens among *H. somnus* isolates showed that the immunodominant 76K and 40K antigens, as well as the fainter 60K antigen, were the most conserved among the outer-membrane antigens detected. Using agglutination or enzyme-linked immunosorbent assay, Canto et al. (5, 6) have demonstrated antigenic diversity among *H. somnus* isolates, as well as a common antigen(s). Our results agree with theirs in that some antigens were seen in some isolates but not in others, whereas the 76K, 60K, and 40K antigens appeared to be common. Since the same antigens were recognized in isolates from TEME, pneumonia, and reproductive failure, it may be that a subunit vaccine containing one or more of these antigens would protect against all three syndromes. Similarly, from the results of this study, we conclude that serodiagnostic tests with these antigens would be likely to detect all three syndromes.

To detect cross-reactions with proteins of other related bacteria, absorptions were done with clinical isolates of *P. multocida*, *P. haemolytica*, *A. equuli*, and *E. coli*. The first three organisms were chosen because they are both taxonomically (21) and antigenically (6, 30) related to *H. somnus*. *E. coli* was included because of its ubiquity. Since the intensity of the bands was not reduced after absorption with the live cells of three of these species, but absorption with the live homologous isolate removed reactivity for 76K *H. somnus* antigen and reduced activity for the 40K antigen, we concluded that the 76K and 40K surface antigens was not shared by these three closely related bacteria. Further work needs to be done with *P. haemolytica*, but since repeated absorption appeared not to decrease reactivity to the 76K and 40K antigens, major cross-reactivity is not likely. If other studies also show lack of cross-reactivity with other bacteria, these two antigens may be useful as specific diagnostic reagents.

In this study, we have demonstrated that two immunodominant antigens in outer-membrane-enriched fractions of *H. somnus* are recognized by antibodies in convalescent serum which bind to protein A. These antigens were present in most *H. somnus* isolates and were recognized by most convalescent sera. The conservation of these antigens and the ubiquity of the bovine antibody response to them in experimentally infected cattle indicates that they are promising candidate antigens for subunit vaccines and diagnostic reagents.

ACKNOWLEDGMENTS

This work was supported in part by the Washington State Beef Commission, by Formula Funding under Section 1433, Public Law 95-113, and by U.S. Department of Agriculture grants 83-CRSR-2-2282 and 84-CRSR-2-2423.

LITERATURE CITED

1. **Aguero, M. E., A. Lieselotte, A. G. DeLuca, K. N. Timmis, and F. C. Cabello.** 1984. A plasmid-encoded outer membrane protein, TraT, enhances resistance of *Escherichia coli* to phagocytosis. Infect. Immun. **46**:740–746.
2. **Bailie, W. E., H. D. Anthony, and K. D. Weide.** 1966. Infectious thromboembolic meningoencephalitis (sleeper syndrome) in feedlot cattle. J. Am. Vet. Med. Assoc. **148**:162–166.
3. **Barenkamp, S. J.** 1986. Protection by serum antibodies in experimental nontypable *Haemophilus influenzae* otitis media. Infect. Immun. **52**:572–578.
4. **Blake, M. S.** 1985. Implications of the active role of gonococcal porins in disease, p. 251–258. *In* G. K. Schoolnik (ed.), The pathogenic neisseriae. American Society for Microbiology, Washington, D.C.
5. **Canto, G. J., and E. L. Biberstein.** 1982. Serological diversity in *Haemophilus somnus*. J. Clin. Microbiol. **15**:1009–1015.
6. **Canto, J., E. L. Biberstein, T. A. Schulte, and D. Behymer.** 1983. Cross-reactivity of *Haemophilus somnus* antibody in agglutination and complement fixation tests and in the enzyme-linked immunosorbent assay. J. Clin. Microbiol. **17**:500–506.

7. **Corbeil, L. B., K. Blau, D. J. Prieur, and A. C. S. Ward.** 1985. Serum susceptibility of *Haemophilus somnus* from bovine clinical cases and carriers. J. Clin. Microbiol. **22**:192–198.
8. **Corbeil, L. B., B. Watt, R. R. Corbeil, T. G. Betzen, R. K. Brownson, and J. L. Morrill.** 1984. Immunoglobulin concentrations in serum and nasal secretions of calves at the onset of pneumonia. Am. J. Vet. Res. **45**:773–778.
9. **Corbeil, L. B., P. R. Widders, R. P. Gogolewski, J. Arthur, T. J. Inzana, and A. C. S. Ward.** 1986. *Haemophilus somnus*: bovine reproductive and respiratory disease. Can. Vet. J. **27**:90–94.
10. **Frasch, C. E., M. S. Peppler, T. R. Cate, and J. M. Zahradnik.** 1982. Immunogenicity and clinical evaluation of group B *Neisseriae meningitidis* outer membrane protein vaccines. Semin. Infect. Dis. **4**:263–267.
11. **Griner, L. A., R. Jensen, and W. W. Brown.** 1956. Infectious embolic meningoencephalitis in cattle. J. Am. Vet. Med. Assoc. **129**:417–421.
12. **Humphrey, J. D., P. B. Little, D. A. Barnum, P. A. Doig, L. R. Stephens, and J. Thorsen.** 1982. Occurrence of *Haemophilus somnus* in bovine semen and in the prepuce of bulls and steers. Can. J. Comp. Med. **46**:215–217.
13. **Humphrey, J. D., P. B. Little, L. R. Stephens, D. A. Barnum, P. A. Doig, and J. Thorsen.** 1982. Prevalence and distribution of *Haemophilus somnus* in the male bovine reproductive tract. Am. J. Vet. Res. **43**:791–794.
14. **Humphrey, J. D., and L. R. Stephens.** 1983. *Haemophilus somnus*: a review. Vet. Bull. **53**:987–1004.
15. **Kennedy, P. C., E. L. Biberstein, J. A. Howarth, M. Frazier, and D. L. Dungworth.** 1960. Infectious meningoencephalitis in cattle, caused by a *Haemophilus*-like organism. Am. J. Vet. Res. **21**:403–409.
16. **Kesiter, D. M.** 1981. *Haemophilus somnus* infections in cattle. Compend. Cont. Ed. **3**:S260–S264.
17. **Kimura A., P. A. Gulig, G. H. McCracken, Jr., T. A. Loftus, and E. J. Hansen.** 1985. A minor high-molecular-weight outer membrane protein of *Haemophilus influenzae* type b is a protective antigen. Infect. Immun. **47**:253–259.
18. **Kuusi, N., M. Nurminen, H. Saxen, M. Valtonen, and P. H. Mäkelä.** 1979. Immunization with major outer membrane proteins in experimental salmonellosis of mice. Infect. Immun. **25**:857–862.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Lawman, G. J. P., D. R. Ball, E. M. Hoffmann, L. E. DesJardin, and M. D. P. Boyle.** 1986. Production of *Brucella abortus*-specific protein A-reactive antibodies (IgG_2) in infected and vaccinated cattle. Vet. Microbiol. **12**:42–53.
21. **Mannheim, W.** 1981. Taxonomic implications of DNA relatedness and quinone patterns in *Actinobacillus*, *Haemophilus*, and *Pasteurella*, p. 265–280. *In* M. Kilian, W. Frederiksen, and E. L. Biberstein (ed.), *Haemophilus*, *Pasteurella*, and *Actinobacillus*. Academic Press, Inc. (London), Ltd., London.
22. **McGuire, T. C., and A. J. Musoke.** 1981. Biologic activities of bovine IgG subclasses. Adv. Exp. Med. Biol. **137**:359–366.
23. **Munson, R. S., Jr., and D. M. Granoff.** 1985. Purification and partial characterization of outer membrane proteins P5 and P6 from *Haemophilus influenzae* type b. Infect. Immun. **49**:544–549.
24. **Nanssen, P.** 1972. Selective immunoglobulin deficiency in cattle and susceptibility to infection. Acta Pathol. Microbiol. Scand. Sect. B **80**:49–54.
25. **Novotny, P., A. P. Chubb, K. Cownley, and J. A. Montaraz.** 1985. Adenylate cyclase activity of a 68,000-molecular-weight protein isolated from the outer membrane of *Bordetella bronchiseptica*. Infect. Immun. **50**:199–206.
26. **Parsons, N. J., A. A. A. Kwaasi, P. V. Patel, P. M. V. Martin, and H. Smith.** 1985. Association of resistance of *Neisseriae gonorrhoeae* to killing by human phagocytes with outer-membrane proteins of about 20 kilodaltons. J. Gen. Microbiol. **131**:601–610.
27. **Portnoy, D. A., H. Wolf-Watz, I. Bolin, A. B. Beeder, and S. Falkow.** 1984. Characterization of common virulence plasmids in *Yersinia* species and their role in the expression of outer membrane proteins. Infect. Immun. **43**:108–114.
28. **Schmerr, M. J. F., J. M. Patterson, M. J. Van Der Moaten, and J. M. Miller.** 1985. Conditions for binding bovine IgG_1 to protein A-Sepharose. Mol. Immunol. **22**:613–616.
29. **Schnaitman, C. A.** 1971. Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. J. Bacteriol. **108**:545–552.
30. **Stephens, L. R., J. D. Humphrey, P. B. Little, and D. A. Barnum.** 1983. Morphological, biochemical, antigenic, and cytochemical relationships among *Haemophilus somnus*, *Haemophilus agni*, *Haemophilus haemoglobinophilus*, *Histophilus ovis*, and *Actinobacillus seminis*. J. Clin. Microbiol. **17**:728–737.
31. **Stephens, L. R., and P. B. Little.** 1981. Ultrastructure of *Haemophilus somnus*, causative agent of bovine infectious thromboembolic meningoencephalitis. Am. J. Vet. Res. **42**:1638–1640.
32. **Stephens, L. R., P. B. Little, B. N. Wilkie, and D. A. Barnum.** 1984. Isolation of *Haemophilus somnus* antigens and their use as vaccines for prevention of bovine thromboembolic meningoencephalitis. Am. J. Vet. Res. **45**:234–239.
33. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose. Proc. Natl. Acad. Sci. **76**:4350–4354.
34. **Ward, A. C. S., L. B. Corbeil, W. D. Mickelsen, and V. F. Sweet.** 1983. A selective medium for gram-negative pathogens from bovine respiratory and reproductive tracts. Proc. Am. Assoc. Vet. Lab. Diagn. **26**:103–112.
35. **Widders, P. R., L. G. Paisley, R. P. Gogolewski, J. F. Evermann, J. W. Smith, and L. B. Corbeil.** 1986. Experimental abortion and the systemic immune response to "*Haemophilus somnus*" in cattle. Infect. Immun. **54**:555–560.