JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1989, p. 1767–1774 Vol. 27, No. 8
0095-1137/89/081767-08$02.00/0
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Pulmonary Persistence of *Haemophilus somnus* in the Presence of Specific Antibody

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Received 28 December 1988/Accepted 17 April 1989

Chronic experimental *Haemophilus somnus* pneumonia was produced in five 8- to 12-week-old calves to investigate host-parasite relationships in the respiratory tract. Calves were depressed and pyrexic and coughed intermittently for 3 days and then recovered except for sporadic coughing. Bacteria persisted in the lung for 6 to 10 weeks or more. Immunoglobulin G1 (IgG1), IgG2, and IgM but no IgA antibodies specific for *H. somnus* were detected in serum. Bronchoalveolar lavage samples contained detectable IgG1, IgG2, IgM, and IgA antibodies specific for *H. somnus* throughout most of the experiment. The kinetics of the isotypic antibody response against *H. somnus* in serum and bronchoalveolar lavage fluids differed, suggesting that both local and systemic antibody responses had occurred. Persistence of pulmonary infection for 10 weeks or more in the presence of antibody may be due to an inappropriate distribution of isotypes, toxicity of *H. somnus* for bovine macrophages, and perhaps other factors. Three of the calves were challenged with a 10-fold-higher dose of *H. somnus* at 10 weeks after the original inoculation. Immunity against *H. somnus* was indicated by the rapid clearance of bacteria from the lungs and the presence of minimal pneumonia at necropsy 3 days after bacterial challenge.

Haemophilus somnus is an important cause of bovine pneumonia (1, 14, 18; S. C. Groom and P. B. Little, Conf. Res. Workers Anim. Dis. 1985, abstr. 240, p. 44). Additionally, *H. somnus* causes thromboembolic meningoencephalitis (18, 37), septicemia (18), arthritis (18), and abortion (2, 18, 42) and is part of the genital and nasal flora of normal cattle (5, 16, 17), apparently unassociated with disease. This diverse pathogenic potential of *H. somnus* and the economic importance of the above diseases indicate that this organism is a significant bovine bacterial pathogen. The host-parasite relationship may also have some similarities to that of the human pathogen *Haemophilus influenzae* since this organism also causes pneumonia (20, 24), meningitis (20, 24, 27, 38), bacteremia or septicemia (27, 32, 38), arthritis (24), occasional genital infections (38), and a carrier state (13, 24).

The few reported studies of experimental *H. somnus* pneumonia have concentrated on acute disease (12) or have studied acute to chronic infection but have been limited to evaluation of pathologic changes (7, 19, 31). No reported studies have focused on the pathogenesis and host response in *H. somnus* pneumonia by sequential monitoring of bacterial infection as well as systemic and local antibody responses. The systemic antibody response to *H. somnus* infection has been studied in thromboembolic meningoencephalitis (36) and *H. somnus*-induced abortion (42), and preliminary data have been reported on *H. somnus* pneumonia (11). Except for the latter, these reports focus on the antibody response following systemic infection even though essentially all *H. somnus* infections are likely to originate on mucosal surfaces. Therefore, it is important to understand the progression of host-parasite interactions following mucosal infection. To address this question, we characterized the host-parasite relationship throughout the course of experimental chronic *H. somnus* pneumonia by quantitating bacteria, inflammatory cells, and the isotypic antibody responses in bronchoalveolar lavage fluid and serum. Additionally, resistance of convalescent calves to subsequent intrabronchial challenge with a 10-fold-higher concentration of *H. somnus* was also studied. Since little is understood about persistence of bacteria in the respiratory tract, the study could have broader biologic significance than that restricted to this experimental model.

MATERIALS AND METHODS

Bacteria. *H. somnus* 2336 was passaged once in a calf before use in the study. This strain was originally isolated in pure culture at the Washington Animal Disease Diagnostic Laboratory from the lungs of a vealer calf during an outbreak of *H. somnus* pneumonia. Isolates from the primary culture were stored at −70°C in 60% glycerol in phosphate-buffered saline.

Animals. Holstein bull calves were purchased from dairies which had no evidence of *H. somnus* disease. During experiments, calves were housed indoors on rubber mats and fed grain and alfalfa pellets.

Chronic experimental *H. somnus* pneumonia. Five 8- to 12-week-old calves were inoculated with approximately 10^7 CFU of *H. somnus* essentially as described previously (11). Calves were inoculated on two different occasions for ease of handling. Calves E5 and E7 were inoculated on one day, and calves 93, 94, and 95 were inoculated on a different day. Bacteria from the second or third 18-h subculture on 10% bovine blood-Columbia blood agar plates (Difco Laborato-

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ries, Detroit, Mich.) were used for inoculation. A standard suspension of bacteria (10^7 CFU) was prepared in 1 ml of sterile saline and added to 1 ml (calves 93, 94, 95) or 4 ml (calves E5, E7) of sterile RPMI 1640 tissue culture medium (M. A. Bioproducts, Walkersville, Md.). Intrabronchial inoculation of RPMI 1640 medium alone induces minimal neutrophilic inflammation that is not detectable grossly 24-h postinoculation (H. D. Liggitt and R. P. Gogolewski, unpublished data). The bacteria were inoculated into the right caudal lung lobe with a flexible fiberoptic bronchoscope (calves 93, 94, and 95) (diameter, 6 mm; Machida, Norwood, N.J.) or a polyethylene nasotracheal tube (calves E5 and E7) (diameter, 6.5 mm; Bev-a-line tubing; Cole Palmer Instrument Co., Chicago, Ill.). Animals were examined clinically, and cardinal signs were monitored daily. Bronchoalveolar lavage was performed before inoculation and at weekly intervals thereafter for 10 weeks (calves 93, 94, and 95) or 6 weeks (calves E5 and E7). Lungs were instilled with 60 ml of sterile, lactated Ringer solution (Travenol Laboratories, Deerfield, Ill.) which was retrieved immediately. Lavage samples were well mixed, and 250 μl was removed for total and differential cell counts. The remaining lavage fluid was centrifuged at 10,000 × *g* for 20 min, and all but 2 ml of the supernatant was decanted and frozen for subsequent antibody determinations. The pellet was suspended in 2 ml of supernatant, and bacterial counts were done in duplicate by the drop method (3). Nasal swabs were taken immediately before bronchial lavage. Each week both nasal swabs and lavage fluids were cultured on Columbia blood agar plates and on plates containing media selective for *H. somnus* (35, 39). Serum was collected before inoculation and at weekly intervals after inoculation and was stored in small aliquots at −20°C. Two calves (E5 and E7) were culturally positive for 5 weeks and then negative 6 weeks after inoculation; so they were necropsied at that time. The other three calves carried *H. somnus* for 9 (93) or 10 (94, 95) weeks. These three calves were challenged at 10 weeks postinoculation with approximately 10^8 CFU of *H. somnus*, and bronchoalveolar lavage fluid was cultured daily for *H. somnus* for 3 days prior to necropsy.

ELISA. Antibody titers in serum were determined as described previously (11, 42). Briefly, Formalin-fixed *H. somnus* organisms (approximately 10^7 CFU per well) were coated onto polystyrene microdilution plates (Costar, Cambridge, Mass.). Antibodies were detected with monoclonal antibodies to bovine immunoglobulin G1 (IgG1), IgG2, IgA, and IgM (provided by A. Guidry, U.S. Department of Agriculture, Beltsville, Md., and W. Davis, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman) and peroxidase-conjugated, affinity-purified goat anti-mouse IgG (gamma) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.). Reactions were developed with hydrogen peroxide in 5-aminosalicylic acid, and plates were read in the dual-wavelength mode (630 and 490 nm) of an enzyme-linked immunosorbent assay (ELISA) reader (Dynatech Laboratories, Inc., Alexandria, Va.). For each serum sample, regression lines of optical density against serum dilution were determined. Titers were calculated as the reciprocal of the serum dilution at an optical density of 0.2. Variation between plates was controlled for by including the same high-titered positive control serum on each plate and correcting the serum endpoint as follows: corrected sample endpoint = (sample endpoint for plate X × mean high-titered serum endpoint of all samples)/high-titered serum endpoint for plate X. For IgA determinations, a standard bovine vaginal secretion was used as a positive control. The methods for detection of antibodies against *H. somnus* in bronchoalveolar lavage samples were identical, but activity was expressed as optical density at 490 nm in undiluted lavage fluid after subtracting mean background optical density at 490 nm with antigen but without bovine lavage fluid.

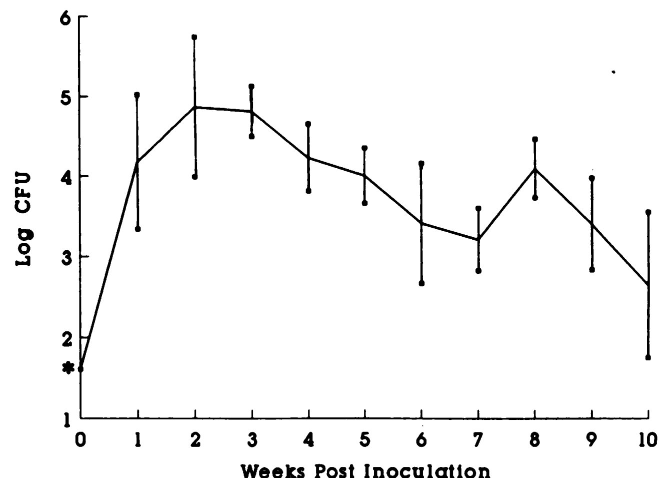

FIG. 1. Bacterial persistence in calves with experimental *H. somnus* pneumonia. Colony counts of *H. somnus* in total volume of bronchoalveolar lavage fluid collected each week from experimentally infected calves. Data points represent mean CFU per milliliter of lavage fluid ± standard error of the mean. At zero time, no *H. somnus* was detected with undiluted lavage fluid in plate counts (*, <1.6 log CFU).

Statistics. The simple correlation coefficients between bacterial numbers and isotypic antibody responses throughout infection were calculated (45).

RESULTS

Clinical findings. All calves were depressed and febrile (mean temperature, 39.9°C; range, 39.6 to 41°C) for 2 to 3 days following inoculation. Inappetence and coughing occurred irregularly over the same period. After 3 days, fever and inappetence were not generally observed, but sporadic coughing continued until the time of necropsy.

Bacterial cultures. Although *H. somnus* was not recovered from the lungs of any calves prior to inoculation, *H. somnus* persisted in lungs for long periods or was not cleared by the termination of the experiment (Fig. 1). Two calves (E5 and E7) had cleared infection six weeks after inoculation, but only one of the other three calves (93) had cleared *H. somnus* 10 weeks after inoculation. In all five calves, *Pasteurella multocida* was also isolated occasionally from lavage fluid, but always in lesser numbers than *H. somnus*. *Mycoplasma* spp. were isolated occasionally from calves 94 and 95. On one occasion in calf 95, two colonies of *Pasteurella haemolytica* were isolated from lavage fluid.

Similarly, *H. somnus* was not recovered from nasal swabs of any calf before inoculation. After inoculation, nasal cultures were positive for *H. somnus* periodically through week 4 in calves E7 and 90, week 6 in calf 93, and week 8 in calf 94. Nasal cultures from calf E5 were always negative for *H. somnus*.

Bronchoalveolar lavage cells. Macrophage counts per milliliter of lavage fluid remained relatively constant throughout the infection, but neutrophil counts increased dramatically

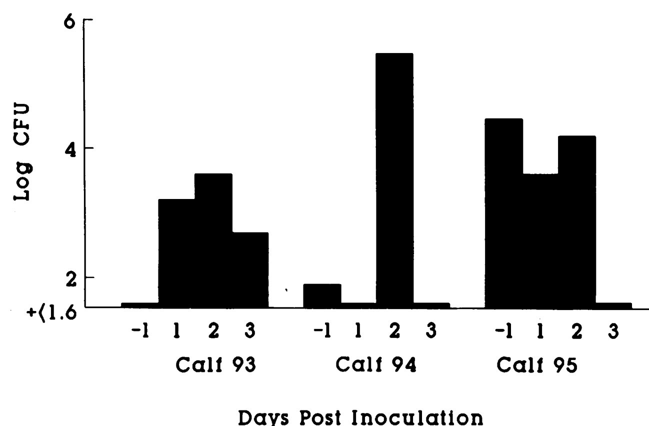

FIG. 2. Bacterial recovery following challenge of convalescent calves. Colony counts of *H. somnus* in total volume of lavage fluid collected 1 day prior to challenge with 10^8 *H. somnus* at week 10 (day −1) and daily for 3 days postchallenge. Day 3 lavage fluids were obtained at necropsy. The lower limit of detection was 1.6 log CFU; therefore, negative cultures are reported as <1.6 log CFU (+).

after inoculation and remained high. Lymphocyte counts were always 2% or less of total cells lavaged.

Necropsy and histopathologic examination of calves which cleared infection. Calves E5 and E7 were necropsied 6 weeks after inoculation when no *H. somnus* was isolated from bronchoalveolar lavage fluid. These two calves had minimal pulmonary lesions. Gross changes were limited to subpleural and interlobular fibrosis in the caudal portion of the right caudal lung lobe, correlating with the site of inoculation. Histologically, scattered foci of plasma cells, lymphocytes, and macrophages were present within areas of fibrosis; however, the macrophages were not degenerate and vasculitis was not seen. Bronchus-associated lymphoid tissue was markedly hyperplastic. Calves 93, 94, and 95 were not examined until after challenge.

Challenge experiment. Calves 93, 94, and 95 were challenged at 10 weeks with 10^8 CFU of *H. somnus* (10 times the original dose). This was done to determine whether calves convalescing from chronic *H. somnus* pneumonia were resistant to challenge with *H. somnus*. Coughing, fever, and depression did not occur in these calves during the 3 days after challenge. In calves 94 and 95, bacteria were cleared from the lung by 3 days after challenge (Fig. 2), whereas calf 93 had moderate numbers of bacteria in lavage fluid (2.4×10^2 CFU ml^{-1}). We could be certain that the inoculated lobe was lavaged each time because fiberoptic bronchoscopy was used to identify the appropriate bronchus. On day 3, we could be certain not only that the inoculated lobe was lavaged but also that the precise area of the lesion was lavaged because the procedure was done at necropsy, making the site visible. Necropsy examination of all three calves revealed minimal gross lesions at the site of inoculum deposition which were only observed following serial slicing of the right caudal lung lobe. Although the extent of the lesions was minimal, histologically there was a lobular distribution of focally severe pneumonia, characterized by various degrees of suppurative bronchopneumonia, necrotic bronchiolitis, lobular necrosis, neutrophilia, vasculitis, degeneration of alveolar macrophages, and dilation plus plugging of lymphatics with fibrin thrombi. These changes were most prominent in calf 93 and to a lesser extent in calf 95, while calf 94 had very mild histologic changes. Hyperplasma of bronchus-associated lymphoid tissue was pronounced in all three calves.

Antibody responses. Serum IgG1 antibody titers against *H. somnus* increased during the first 2 weeks and then stabilized at relatively high levels, remaining there for the duration of the experiment (Fig. 3). Serum IgG2 titers followed essentially the same pattern as IgG1 titers but were always lower than IgG1 titers and took about 1 week longer to peak. Although IgG2 titers were not as high as IgG1 titers, the relative increase in IgG2 during infection was greater than that observed for IgG1 (Fig. 3). Minimal changes occurred in serum IgM, and serum IgA was not detected (Fig. 3).

Bronchoalveolar lavage samples were tested undiluted and were not corrected on the basis of total protein or albumin concentration. This was done because our intent was to evaluate total antibody present at the site of inflammation rather than to discriminate between local and systemic production of antibody, since antibody from any source might be important in protection. In bronchoalveolar lavage fluid, marked variation between animals was observed in antibody levels against *H. somnus*, as indicated by the extent of the standard errors of the mean (Fig. 4). Lavage fluid IgM peaked at week 2 and began to drop after week 3; IgG1 peaked at 2 to 3 weeks and subsequently dropped off slowly; IgG2 antibody did not peak until week 6, decreased until week 8, and then rose slightly. The kinetics of IgA responses was intermediate between IgG1 and IgG2 responses. There was no parallel between serum and bronchoalveolar lavage antibody kinetics; however, there appeared to be an inverse relationship between the concentration of IgG2 and *H. somnus* counts in bronchoalveolar lavage fluids (Fig. 1 and 4). When IgA antibody activity decreased, bacterial counts decreased also. Statistical analysis was done to determine whether there was a correlation between bacterial numbers and isotype of antibody throughout infection (Table 1). No negative correlations were detected between any of the isotypes of antibody and bacterial numbers, probably owing to the small number of calves and variable data. However, a direct correlation was demonstrated between IgA ($P < 0.001$), IgG1 ($P < 0.002$), and IgM ($P < 0.005$) activity and bacterial numbers but not between IgG2 and bacterial numbers ($P > 0.50$).

DISCUSSION

The results of this study demonstrated the ability of *H. somnus* to persist for long periods within the lungs of calves. The differences in times of clearance are not surprising considering that the calves were outbred animals from two different farms with different interacting normal flora (6) and immunologic background. The variation in time of clearance was not thought to be mainly due to variations in technique because the volume of the inocula in both groups (E5 plus E7 and 93, 94, and 95) and the diameters of the nasotracheal tube and fiberoptic bronchoscope were similar. In all five calves, clinical signs of infection were of short duration, but pulmonary infection persisted for 6 weeks to more than 10 weeks in the absence of clinical abnormalities other than sporadic coughing. This finding may have important implications for the spread of infection. Previous studies indicate a low prevalence of *H. somnus* in the nasal cavity (18, 33) and a relatively high carriage rate in the urogenital tract (5, 17, 18), causing some investigators (18) to conclude that the urogenital tract is the most significant infective nidus for transmission of *H. somnus*. Our results indicate that even when nasal cultures are continually negative (calf E5) or

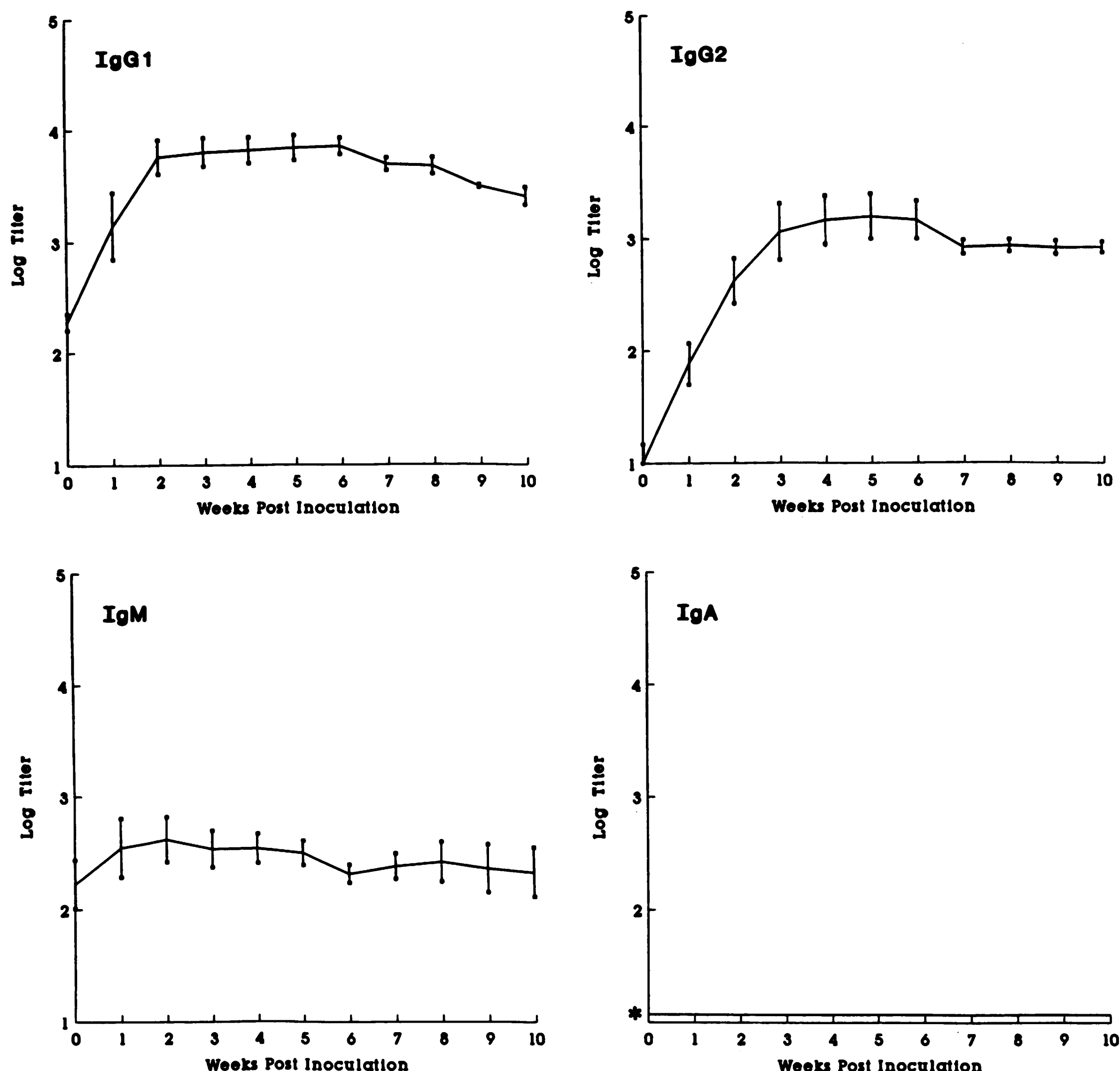

FIG. 3. Serum antibody against *H. somnus* as detected by ELISA with whole formalinized *H. somnus* cell antigen. Isotypic antibody titers were determined with monoclonal mouse antibodies to bovine IgG1, IgG2, IgM, and IgA. Note that serum IgA antibodies were not detected (*).

sporadically positive, bronchoalveolar lavage fluids may continue to contain high numbers of *H. somnus*. Bronchoalveolar lavage fluid is considered to represent washings from the level of the bronchi down to the alveoli. The lobar pattern of the bovine lung guards against excessive dispersion of the washings; therefore, we are confident that the lavage fluid isolates represented bronchoalveolar colonization. The fact that selective media (39) were used in addition to Columbia blood agar plates makes it likely that the detection rate for *H. somnus* in our study was as high as current methods allow. Since *H. somnus* was often detected from bronchial lavage samples when nasal cultures were negative, we conclude that the organisms had colonized the bronchi rather than being picked up from the upper respiratory tract at the time of lavage. At necropsy, culture results of lung samples and the existence of small foci of resolving pneumonia supported the contention that *H. somnus* was obtained from the lung. Thus, it appears that, once established, *H. somnus* survives more readily in the bronchoalveolar area than on the nasal mucosa. Furthermore, coughing may transmit infection even when nasal cultures are negative.

The systemic humoral immune response to *H. somnus* during experimental pneumonia was similar to the response we reported earlier for two calves (11). Since IgG1 and IgG2 titers remained high throughout the course of infection, systemic IgG responses may be useful diagnostically. IgG2 titers were lower than IgG1 titers, but owing to very low preinoculation titers, IgG2 concentrations increased approximately 100-fold during infection, whereas IgG1 titers increased much less. The lack of detectable IgA in serum during infection is not surprising since cattle have very low serum IgA concentrations (10) and serum IgA titers are not detected in bovine venereal vibriosis, another bacterial mucosal infection of cattle (4). In the present study, both IgG1 and IgM titers were relatively high prior to infection, as has been noted in our previous *H. somnus* experimental infection studies (42). It is likely that this is due to cross-reacting natural antibody since these calves came from herds with no history of *H. somnus* disease, the calves were not asymptomatic carriers of *H. somnus*, and most cattle have natural antibodies to many different gram-negative bacteria. The role of systemic antibody responses in defense against *H. somnus* infection is not absolutely clear. However,

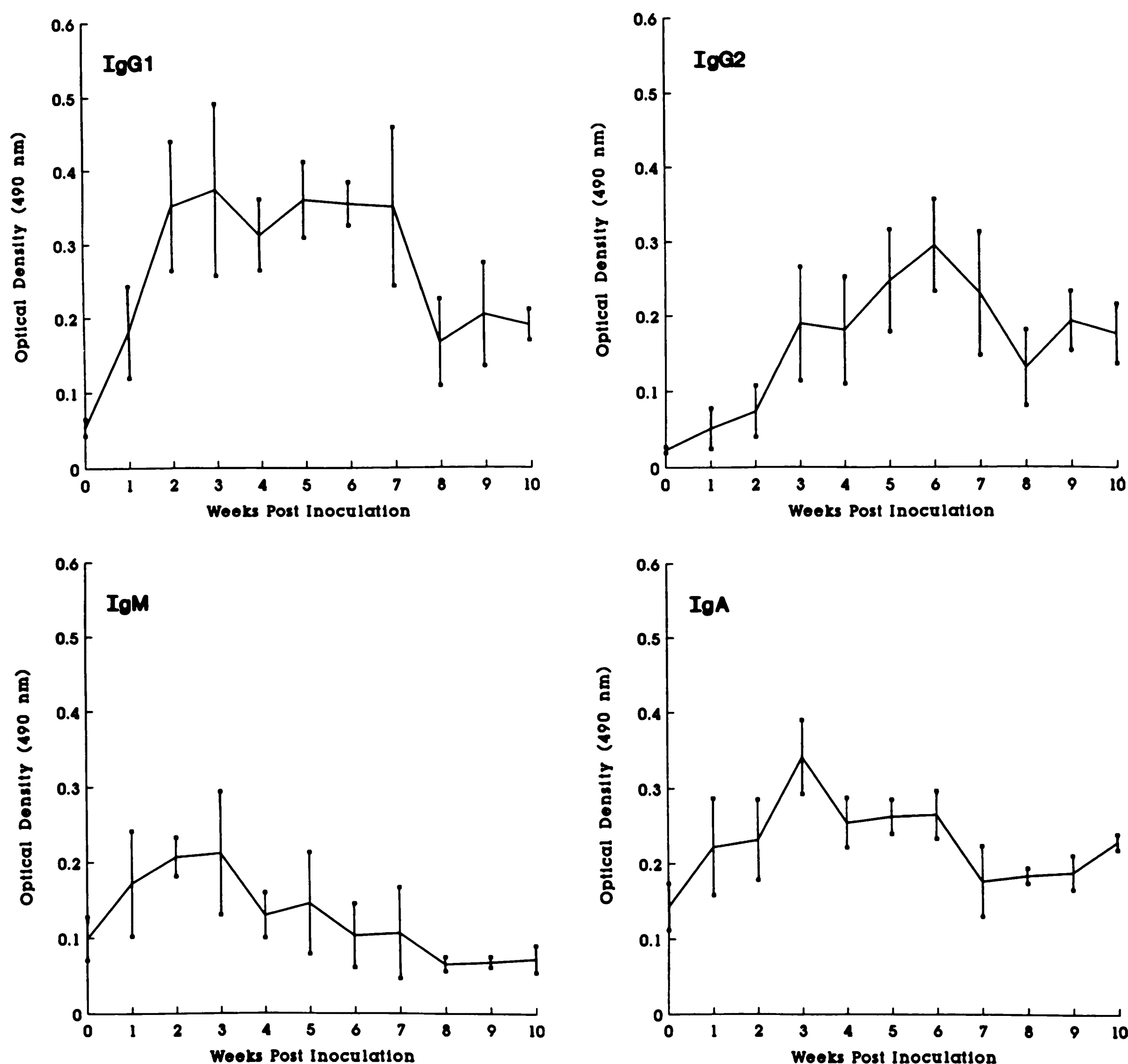

FIG. 4. Bronchial lavage antibody against *H. somnus* as detected by ELISA with whole formalinized *H. somnus* as antigen. Antibody activity of each isotype was determined with mouse monoclonal antibodies to bovine IgG1, IgG2, IgM, and IgA.

prevention of bacterial invasion with resultant septicemia and thromboembolic meningoencephalitis would appear to be an obvious role for the systemic response since these are both common systemic manifestations of *H. somnus* disease. Furthermore, the marked serum exudation of *H. somnus* pneumonia (12) indicates that serum antibody would be present in infected pulmonary tissue so that defense against *H. somnus* in pulmonary tissue should result from a systemic antibody response.

TABLE 1. Correlation coefficients between bacterial counts and immunoglobulin isotype of antibodies to *H. somnus* in bronchial lavage fluids throughout infection

Isotype	r^a	P^b
IgG1	0.46	<0.002
IgG2	0.08	>0.5
IgM	0.43	<0.005
IgA	0.62	<0.001

[a] r, Correlation coefficient.
[b] P, Probability under the hypothesis that $r = 0$.

Local immune responses must be involved also since the kinetics of isotypic responses in serum differs from responses in bronchoalveolar lavage fluids. Although titers in serum and bronchoalveolar lavage fluids cannot be compared directly because of differences in methods of determination, the kinetics of these responses can be compared. The most striking example is the lack of a detectable IgA response in serum versus a striking increase in IgA in lavage fluids by 3 weeks. The lavage and serum IgG1 and IgM responses are similar except for the greater magnitude of the increase in lavage fluid during the first 2 weeks and the greater decrease in antibody in lavage fluid in the last 2 weeks. However, serum and lavage IgG2 antibody responses differed considerably, as was found by Pringle et al. (30) in studies of total immunoglobulin concentrations in bovine serum and lavage fluids. Serum IgG2 antibody concentrations had peaked by 3 weeks, whereas lavage IgG2 titers did not peak until 6 weeks. Furthermore, serum IgG2 titers stabilized after 3 weeks, but lavage IgG2 antibody activity dropped after 6 weeks. This suggests that at least some of the IgG2 and IgA anti-*H. somnus* antibodies were

locally synthesized or selectively transferred. Further evidence for a local contribution to the immune response was the prominent hyperplasia of bronchus-associated lymphoid tissue seen at necropsy.

The recovery of *H. somnus* from bronchoalveolar lavage for prolonged periods in all calves is consistent with the failure of this humoral response to clear infection. Since bacterial persistence in this study occurred in the lungs of experimental animals receiving a relatively low inoculum dose (in comparison with other studies), this is probably representative of the field situation. The mechanism of bacterial persistence in the lung is intriguing. Specific *H. somnus* antibody is probably involved in keeping bacterial numbers low since we have already demonstrated that convalescent-phase serum from calves E5 and E7 protected other calves against intrabronchial challenge with *H. somnus* in passive transfer experiments (11). Persistence of low numbers of *H. somnus* over a prolonged period in calves 93, 94, 95, E5, and E7 may be attributed to (i) an inappropriate mucosal antibody response; (ii) impairment of host defense; (iii) *H. somnus*-mediated evasion of host defenses.

An antibody response of inappropriate isotype distribution may impair effective clearance of *H. somnus* from the lung. A precedent for this would be blocking of bactericidal and opsonizing effects of IgG antibody against nontypeable *H. influenzae* by specific antibody in bronchopulmonary lavage fluids (26). These investigators (26) proposed that the blocking effect of IgA may permit pulmonary colonization and development of pneumonia in people who already have seemingly adequate bactericidal and opsonizing antibody. In studies of *H. influenzae* type b (34), anticapsular IgG was bactericidal and opsonic, but IgA was not. In normal calves, mean ratios of total IgG/IgA in bronchoalveolar washings are approximately 1, but the majority of the calves studied had more IgA than IgG in bronchoalveolar washings (44). In our study, IgA specific for *H. somnus* was detectable in bronchoalveolar lavage fluid throughout the study. Since isotype-specific antibody activity was detected by ELISA with different monoclonal antibodies for each isotype, it is not appropriate to make quantitative comparisons between isotypes. However, kinetics of responses can be compared. When IgG2 activity increased and IgA activity decreased at 4 to 6 weeks, *H. somnus* counts decreased also, indicating that the predominance of IgG2 may have contributed to clearing. When IgA was high, *H. somnus* numbers were also high. There was a significant positive correlation between IgA and IgG1 and IgM antibody activity and bacterial numbers throughout infection but not between IgG2 and bacterial numbers. IgG2 is known to be the most opsonic bovine isotype (25), and Watson (40) proposed that the other isotypes may interfere with opsonization by blocking antigenic epitopes for IgG2. Thus, the relative amount of potentially blocking and protective isotypes may determine persistence or clearing.

Pulmonary persistence of *H. somnus* in chronic *H. somnus* pneumonia may also be related to *H. somnus*-induced compromise of phagocytic defenses. Suppression of bovine neutrophil (8, 15) and bovine alveolar macrophage (23) function by *H. somnus* has been reported. Additionally, we found that *H. somnus* is toxic for bovine alveolar macrophages in vitro (H. D. Liggitt, L. Huston, and L. B. Corbeil, Conf. Res. Workers Anim. Dis. 1984, abstr. 174, p. 31) and that there is extensive degeneration of alveolar macrophages in vivo in acute experimental *H. somnus* pneumonia (12). Although *H. somnus* is phagocytosed when antibody is present (23), it has been shown to survive in the macrophage for a few hours (23) before degeneration of the macrophage. In our study, *H. somnus* numbers were partially controlled by host defenses, but it appears likely that the ability of this organism to evade phagocytic defenses by suppression of phagocytes contributes to its persistence in the lung for weeks after the acute phase of the infection. The degenerate macrophages in the small lesions of the three rechallenged calves provide additional evidence supporting this hypothesis. Another means of evading the host phagocytic defense may involve the recent demonstration that *H. somnus* binds immunoglobulin nonspecifically by an Fc-mediated mechanism (41) similar to that which occurs in staphylococci (22), streptococci (21), and other gram-negative bacteria including *Brucella abortus* (28) and *Taylorella equigenitalis* (43). In our earlier histopathologic studies of *H. somnus* pneumonia (12), *H. somnus* was usually seen extracellullary in modified Gram stains of plastic-embedded sections (R. P. Gogolewski and L. B. Corbeil, unpublished data). Thus, it may be that immunoglobulin Fc binding to *H. somnus* promotes evasion of phagocytosis, as has been shown for the staphylococcal immunoglobulin Fc receptor (protein A) (9, 29), or it may mask antigens involved in other protective mechanisms.

The ability of calves convalescing from experimental *H. somnus* pneumonia to develop active resistance to *H. somnus* challenge at 10 times the initial dose was shown by clearance of *H. somnus* infection in 2 days in two of three calves and decreasing numbers of *H. somnus* in lavage fluid from the other calf. Also, the presence of minimal gross lesions at necropsy 3 days after challenge indicates protection. Although increased resistance with age could be responsible for part of this defense, this is an unlikely explanation because the incidence of naturally occurring *H. somnus* pneumonia peaks at 7 to 9 months of age (S. C. Groom and P. B. Little, Conf. Res. Workers Anim. Dis. 1985, abstr. 240, p. 44) and our calves were 2 to 3 months old at the initiation of infection. That immune defense is involved in the observed protection of the three convalescent animals is further supported by our findings that convalescent-phase serum from two of these five animals was passively protective against experimental pneumonia in other calves (11).

In summary, *H. somnus* persisted for long periods in the lungs of calves in the absence of clinical disease. Serum antibody responses were characterized by early IgG1 and IgM increases and a later but sustained increase in IgG2. Antibodies against *H. somnus* in bronchoalveolar lavage fluid were also reflected by early IgG1, IgA, and IgM increases and later IgG2 increases. Increases in levels of IgA and IgG2 in bronchoalveolar lavage fluid did not parallel serum responses of homologous isotypes. Numbers of viable *H. somnus* from lungs were directly correlated with IgG1, IgM, and IgA antibodies against *H. somnus* in lavage fluid but not with IgG2 antibodies, suggesting a possible protective effect of IgG2 and a blocking effect for the other isotypes. Destruction of alveolar macrophages by *H. somnus* may also be a factor in persistence in the lung. The development of protection against *H. somnus* pneumonia in calves rechallenged with *H. somnus* 10 weeks after initial challenge was demonstrated by the rapid clearance of bacteria from the lungs and the presence of minimal pneumonia 3 days after bacterial challenge.

ACKNOWLEDGMENTS

The technical assistance of Ruth Cutright and Ronald M. Silflow is gratefully acknowledged.

This work was supported in part by U.S. Department of Agriculture grants 83-CRSR-2-2282, 84-CRSR-2-2528, and 87-CRSR-2-3077. R.P.G. is the recipient of a National Research Service award (1 F32 AI 07848).

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