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Capsulelike Surface Material of *Mycoplasma dispar* Induced by In Vitro Growth in Culture with Bovine Cells Is Antigenically Related to Similar Structures Expressed In Vivo

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Electron microscopy has been used to show that *Mycoplasma dispar* produces an external capsulelike material in vivo that has an affinity for both ruthenium red and polycationic ferritin. This extracellular material is lost upon passage in culture medium but can be regained with a single passage on bovine lung fibroblast (BLF) cells. To confirm that the extracellular material associated with cell-grown mycoplasmas was the same as that observed in infected calves, rabbit antibodies were produced to purified capsulelike material isolated by protease digestion of cell-grown organisms. These antibodies bound to capsulelike material on the surface of *M. dispar* cells colonizing the bronchial epithelium of infected calves and to capsulelike material from cell-grown mycoplasmas. Calves infected with *M. dispar* produced antibodies in lung secretions that were capable of binding to the purified capsulelike material. The Fab fragments of rabbit antibodies to in vitro-produced capsulelike material could block this binding, indicating that the capsulelike material was similar in both in vivo-grown and cell-grown organisms. The carbohydrate nature of the capsular material suggested by the ruthenium red and polycationic staining characteristics was confirmed by its binding to *Ricinus communis* agglutinin, a galactose-specific lectin. These studies confirm that capsule material produced during infections with *M. dispar* share antigenic determinants with the material produced under in vitro conditions and that association with mammalian cells induces production of this material.

A classic tenet in microbiology is that capsular structures are virulence determinants of pathogenic microorganisms (11). Participation of capsules in the avoidance of host defense mechanisms, and therefore in the pathogenic process, was demonstrated with bacterial species such as *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* (6). It was also shown that the induction of an extracellular surface polysaccharide layer resulted in increased adherence of *Candida albicans* to inert surfaces (20). With *Cryptococcus neoformans*, capsule expression was also correlated with antiphagocytic and adherence properties (15). The capsular galactan produced by *Mycoplasma mycoides* subsp. *mycoides* has long been known to promote lesions and to prolong mycoplasmosis in infected cattle (17). With other capsulated pathogenic mycoplasmas such as *M. hyopneumoniae* (25) and *M. gallisepticum* (26), a correlation between increased virulence, capsule presence, and attachment to host cells has been shown.

Mycoplasma dispar is commonly associated with bovine pneumonia in calves and has been considered to produce capsules during natural infections (4, 7, 8). It has been proposed that the capsule of this mycoplasma plays a role in the pathogenesis of the disease by participating in the attachment process or by inducing immunosuppresive effects on the host (4, 7). A precise definition of the nature of this capsule or its role in disease, however, has not been obtained. Characterization of the *M. dispar* capsule is hampered by the inability of the organism to produce capsules when grown in vitro. In this paper we report a biological system that allows the in vitro production of *M. dispar* capsulelike material and its preliminary identification with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). We then compare the in vitro product with that produced under in vivo conditions.

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MATERIALS AND METHODS

Cell cultures and mycoplasma. Mycoplasma-free bovine lung fibroblasts (BLF cells) derived by trypsin digestion from bovine calf lung tissues (0.5% trypsin for 30 min at 37°C) were grown in Eagles minimal essential medium (MEM) supplemented with 10% fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.) and used below the 15th passage level. The absence of mycoplasma contamination was ascertained by culturing the cells without antibiotics and then staining with the fluorescent dye 4′,6′-diamidino-2-phenylindole dihydrochloride (Calbiochem, San Diego, Calif.). The SD-O strain of *M. dispar*, isolated from a pneumonic calf (28), was cloned twice and used at passage levels 7 and 50. *M. dispar* was grown in MEM and in Friis modified broth medium as previously described (13). Logarithmic-phase cultures were centrifuged for 10 min at 500 × *g* to remove debris, and the resulting supernatant was centrifuged at 34,000 × *g* for 30 min. The pellet was washed three times with phosphate-buffered saline solution (PBS) at pH 7.2. Protein concentrations were determined by the method of Lowry et al. (18), with alkali treatment before the assay to solubilize membranes (1). Mycoplasma suspensions were stored after quick freezing at −70°C in 1 ml aliquots (30 mg of protein per ml).

M. dispar was cocultured with BLF cells by infecting confluent monolayers in 450-cm^2 roller bottles with 4 × 10^6 mycoplasma CFU. Before the infection, the mycoplasmas were washed once in PBS (pH 7.2) and resuspended in the same volume of cell culture medium containing 10% fetal bovine serum. A modification of this procedure consisted of growing *M. dispar* inside sterile dialysis bags (molecular

weight cutoff, 12,000 to 14,000) in BLF cultures. Mycoplasmas were harvested from cell culture supernatants or from the dialysis bags after 22 h of growth at 37°C by differential centrifugation, washed with PBS, and stored as described above.

Lectin agglutination. Lectin-mediated agglutination and inhibition of the agglutination of Friis medium-grown and cell- grown *M. dispar* was assayed as described by Schiefer et al. (23). Serial twofold dilutions of concanavalin A (Vector, Burlingame, Calif.), *Dolichus biflorus* agglutinin, *Ulex europeus* agglutinin I, peanut agglutinin, *Ricinus communis* agglutinin I, and *R. communis* agglutinin II in 25 μl of 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered saline solution (pH 7.8) were performed in U-bottom microtitration plates. The starting concentration in all lectin solutions was 1 mg/ml. Mycoplasma suspension (25 μl) at 1:10 dilution in the same HEPES-buffered solution were added to each well; after mixing, plates were incubated at 37°C for 2 h. Agglutination was detected visually, and the titer was determined as the highest lectin dilution causing agglutination. One agglutination unit was considered to be the amount of lectin present in the highest lectin dilution causing agglutination. To confirm the specificity of the reaction, inhibition of the agglutination was assayed by using specific carbohydrates. Briefly, 25 μl of a twofold dilution of the carbohydrate stock was mixed with an equal volume of each lectin solution (containing 2 agglutinating units) and incubated for 10 min at 37°C. Then 25 μl of this mixture was mixed with an equal volume of mycoplasma suspension, and the reaction was incubated and read as before.

Preparation of capsulelike material. Cell-grown *M. dispar* was used as a source of capsulelike material. Mycoplasma suspensions were digested with proteinase K (PK; E. Merck, Darmstadt, Germany) as described by Hitchcock and Brown (5). Briefly, 0.1 mg of PK was added to 1 ml of mycoplasma suspension and incubated at 60°C for 1 h. In some instances after digestion, PK activity was inhibited by the addition of phenylmethylsulfonyl fluoride (10 mM). For electrophoretic analysis, samples were treated immediately by adding an equal volume of sample buffer (2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.063 M Tris [pH 8.8]) and boiling for 90 s.

Electrophoresis and staining procedures. Discontinuous SDS-PAGE was performed with 4% acrylamide stacking gels and 12.5% acrylamide separating gels and the two-buffer system of Laemmli (16). Protein bands were visualized by Coomassie blue staining (22). Carbohydrate bands were visualized by staining with a modified silver staining method (5, 14), with periodic acid-Schiff reagent, or with Alcian blue (12). Apparent molecular weights were determined by using low-molecular-weight standards from Sigma Chemical Co. (St. Louis, Mo.).

Experimental calves. Four colostrum-deprived calves caught at birth in sterile bags were raised in individual isolation rooms. Two nasal and ocular samples obtained before experimental infection were cultured for mycoplasmas to exclude the possibility of an upper respiratory mycoplasma infection. No clinical evidence of disease was recorded in any of the four calves before inoculation. Two calves were inoculated twice by intratracheal cannulation with 10^{10} CFU of *M. dispar* suspended in 10 ml of sterile PBS at 15-day intervals. Two control calves were inoculated twice with 10 ml of sterile PBS. All calves were killed 20 days after the last inoculation, and lung lavage fluid was obtained by instillation of 500 ml of PBS with 1 mM EDTA. The lung lavage fluid was treated by adding a 1:100 dilution of Sputolysin (Calbiochem, San Diego, Calif.) and centrifuged to remove cells before storage at −20°C. Lung immunoglobulins were precipitated by the addition of 33% (vol/vol) saturated ammonium sulfate solution (pH 7.8). The precipitated immunoglobulins were dissolved in 10 mM Tris Tricine–0.85% NaCl–0.02% sodium azide–1 mM EDTA (pH 8.4) and dialyzed against the same buffer at 4°C for 72 h.

Rabbit antisera. Antiserum to the capsulelike material was prepared in the following way. An SDS-PAGE gel slice containing the low-molecular-weight band obtained after PK digestion of 3×10^{11} CFU of cell-grown *M. dispar* was passed through a 20-gauge needle several times, suspended in 3 ml of sterile PBS, and stored at −70°C in 1-ml aliquots. An aliquot was mixed with 0.1 ml of 10-mg/ml hemocyanin (Sigma) and emulsified with an equal volume of incomplete Freund adjuvant (Miles Scientific, Naperville, Ill.). Bovine lung fibroblast monolayers were subjected to three cycles of freezing and thawing followed by sonication. The suspension was centrifuged for 30 min at 100,000 × *g* and supernatants emulsified with an equal volume of incomplete Freund adjuvant were used as the antigen. New Zealand White rabbits were inoculated subcutaneously with 2 ml of the antigen preparation, and two booster inoculations were given at 10-day intervals. Ten days after the last injection, the rabbits were bled out. Serum immunoglobulins were precipitated by the addition of 50% (vol/vol) saturated ammonium sulfate solution (pH 7.8), dialyzed as described above, and stored at 4°C.

Preparations of Fab antibody fractions. Fab fragments were prepared by using a kit from Pierce Chemical Co. (Rockford, Ill.). Briefly, rabbit antibodies were treated with immobilized papain for 5 h at 37°C and centrifuged, and the supernatant was passed through a protein A affinity column. The excluded Fab fragments were collected by washing the column with 10 mM Tris buffer (pH 7.5). The Fab fragment containing fraction was stored at 4°C.

Microscopy. The presence of capsulelike material was determined in medium- and cell-grown mycoplasmas by transmission electron microscopy (TEM). Cell-grown mycoplasmas from cocultures were divided into two fractions, cell associated and non-cell associated. Non-cell-associated mycoplasmas were obtained from cell culture supernatants; cell-associated organisms were obtained by scraping lung fibroblast cultures to remove the cell monolayer and pelleting them.

Mycoplasmas were sedimented by centrifugation at 14,000 × *g* for 3 min, and the pellets were fixed in 3% glutaraldehyde–cacodylate buffer with and without 1% ruthenium red (Sigma) for 3 h at 4°C. The pellets were then washed three times by incubating them with 0.1 M cacodylate buffer (pH 7.2) for 30 min between centrifugation steps. Postfixation was done in 1% osmium tetroxide (Sigma) in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h at room temperature. Pellets were then dehydrated through an acetone series and embedded in Epon 812 resin.

Mycoplasmas were labeled with polycationic ferritin (Sigma) as described by Jacques and Foiry (10). Briefly, mycoplasmas were harvested by centrifugation, washed once in PBS, and fixed in 3% glutaraldehyde–cacodylate buffer for 2 h at 22°C. Fixed mycoplasmas were suspended in 0.1 M sodium cacodylate buffer for 30 min and allowed to react with polycationic ferritin (1 mg/ml). The reaction was stopped by 10-fold dilution in buffer. Organisms were then washed three times (0.1 M sodium cacodylate buffer), immobilized in 4% agar, washed five times, postfixed in 2% osmium tetroxide for 2 h, and then washed again with

cacodylate buffer. Samples were dehydrated as described above and embedded in Epon 812 resin.

Estimates of the amounts of encapsulated to nonencapsulated mycoplasmas were obtaining by direct counting of 100 mycoplasma cells in each of 25 fields at a magnification of ×27,000 under TEM. The significance of treatment mean differences was evaluated by analysis of variance.

For immunogold labeling, Friis medium-grown or cell-grown mycoplasmas were harvested as described above, incubated with different dilutions (1:2, 1:5, 1:10) of lung lavage or rabbit anti-capsulelike material antibodies for 1 h at 22°C, and washed twice with 1% bovine serum albumin in PBS. The mycoplasma suspension was then reacted with protein A-gold (Sigma) (1.5 mg/ml) by incubating at 37°C for 1 h, centrifuged at 14,000 × *g* for 5 min, washed twice with 1% bovine serum albumin in PBS, fixed with 3% glutaraldehyde-cacodylate buffer, and processed for TEM. No osmium tetroxide postfixation step was done on these preparations. Controls consisted of samples without the primary antibody to determine the nonspecific attachment of protein A-gold.

Paraffin-embedded lung sections (5 μm thick) of calves infected with *M. dispar* were also examined by using immunogold labeling. Deparaffinized, rehydrated sections were reacted with rabbit anti-capsulelike material diluted 1:10 in PBS with 0.5% bovine serum albumin for 30 min at 37°C in a moist chamber. After the sections were washed twice with PBS and once with H_2O, they were air dried and then reacted with gold-labeled anti-rabbit antibodies (Sigma) diluted 1:50 in PBS with 0.5% bovine serum albumin. After 30 min of incubation at 37°C in a moist chamber, samples were washed and dried as described above. Sections were counterstained with hematoxylin, dehydrated, and mounted.

Quantitation and specificity of antibodies against capsulelike material. *M. dispar* capsular material was bound to agarose beads coated with *R. communis* agglutinin I (Vector, Burlingame, Calif.). Purified capsular material was allowed to react with the beads for 2 h at 37°C. The beads were washed five times with 10 mM HEPES-buffered saline (pH 8.5) and then reacted with dilutions of lung lavage or rabbit antibodies for 90 min at 4°C. The agarose-bound lectin, capsulelike material, and antibody complexes were washed five times in the above buffer and then reacted with 0.1 μCi of ^{125}I-labeled protein G (Amersham) for 1 h at 25°C with shaking. The radioactive beads were washed five times with the above buffer, and bound radioactivity was quantitated by using a gamma counter (Beckmann Instruments, Inc., Palo Alto, Calif.). Controls consisted of immunoglobulins from normal rabbit serum or from noninfected calf lung lavage fluid in standard reaction mixtures. Nonspecific reactions of globulins, Fab fractions, and ^{125}I-labeled protein G with agarose-bound lectin were also tested.

To determine the specificity of the rabbit antibodies against capsulelike material, two series of experiments were performed. In the first set of experiments, Fab fragments from immunized or control rabbit antibodies were used to block lung lavage-derived antibody binding to agarose-bound lectin. Fab fragments were incubated with capsular material attached to agarose-bound lectin for 1 h at room temperature before the addition of lung lavage-derived antibodies. In the second set of experiments, lung fibroblasts (infected and noninfected) were radiolabeled with [^{14}C]glucose (5 μCi/ml) for 2 h at 37°C in a glucose-free labeling medium. Cells were washed and treated consecutively with RNase (0.1 mg/ml, 15 min at 37°C), DNase (0.1 mg/ml, 1 h at 37°C), and PK (0.1 mg/ml, 1 h, 60°C). The resulting material was reacted with a 1:10 dilution of rabbit antibodies against capsulelike material or against BLF cells for 1 h at 4°C, and the antigen-antibody complexes were precipitated with 6% agarose–protein A (Sigma, 100 μg/ml, 1 h at room temperature). The beads were washed three times, and the radioactivity was quantitated by using liquid scintillation. Controls consisted of reaction mixtures with preimmune rabbit sera or without primary antibodies.

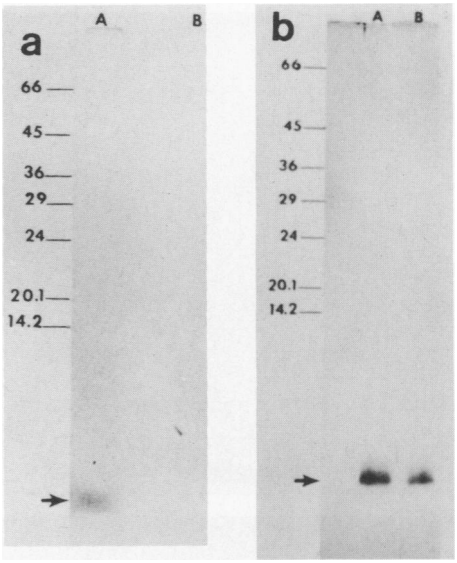

FIG. 1. (a) SDS-PAGE separations of PK-digested *M. dispar* SD-O stained with silver and Coomassie blue. *M. dispar* was cultured with bovine lung fibroblasts (lane A) or on Friis medium (lane B). (b) SDS-PAGE separations of PK-digested cell-grown *M. dispar* SD-O stained with Alcian blue (lane A) and Schiff reagent (lane B). The arrows indicate low-molecular-weight, PK-resistant bands. The numbers on the left indicate apparent molecular masses in kilodaltons.

RESULTS

Identification of a capsulelike, proteinase-resistant band by SDS-PAGE. After PK digestion, the capsulelike material was analyzed by SDS-PAGE. The electrophoretic profiles of Friis medium-grown or cell-grown *M. dispar* are presented in Fig. 1a. A low-molecular-weight PK-resistant band was detected in silver-stained gels with cell-grown *M. dispar* (lane A) but not with Friis medium-grown *M. dispar* (lane B). This material was also sensitive to staining with Alcian blue and periodic acid-Schiff reagent (Fig. 1b).

Detection of capsulelike material by TEM and light microscopy. Capsulelike material was detected by TEM with ruthenium red, polycationic ferritin, and immunogold labeling procedures (Fig. 2 and 3). When mycoplasmas were examined for capsulelike material by TEM, significant differences were observed between medium-grown mycoplasmas and those cocultured with lung fibroblasts (Table 1). When *M. dispar* was passaged in Friis medium, no differences in the percentage of organisms with capsulelike material were observed between the 7th and 50th passages (data not shown). Of the mycoplasmas grown in direct contact with lung fibroblasts, 66% ± 12% were surrounded with capsulelike material, whereas only 29% ± 5% of mycoplasmas present in the cell culture supernatant showed capsulelike material. This difference in percentage is significant.

A significant proportion of *M. dispar* grown separated

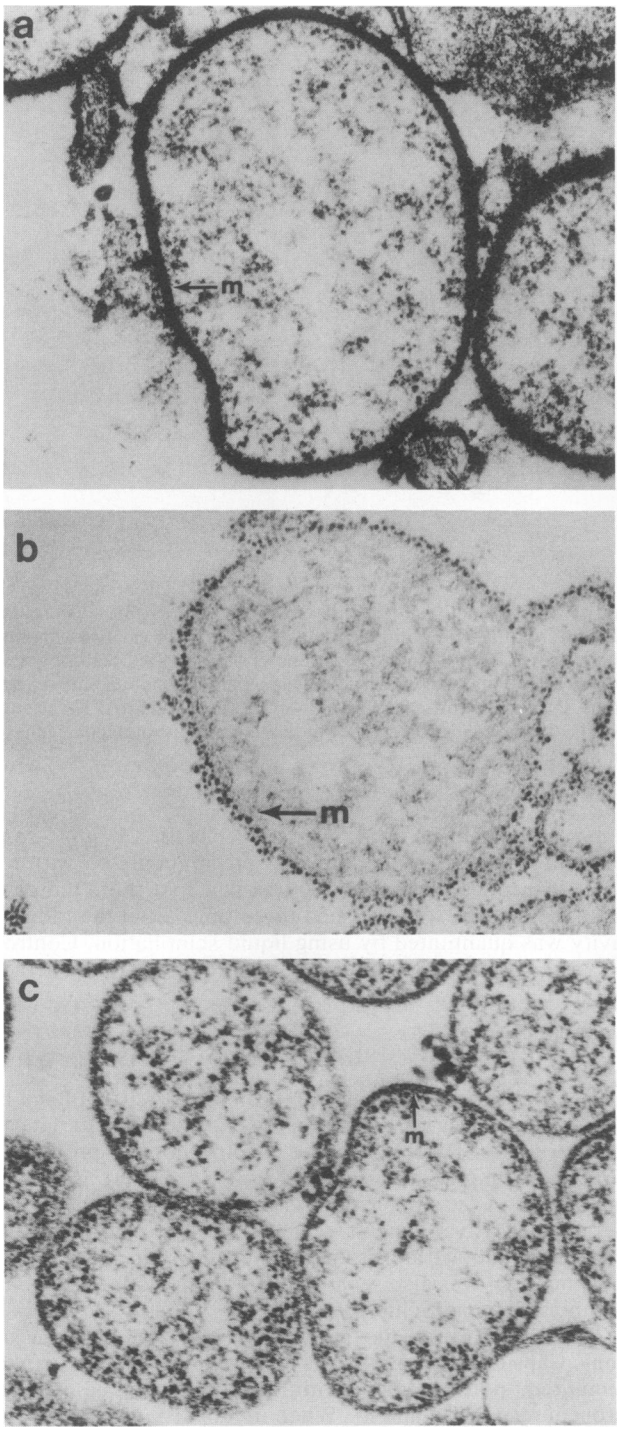

FIG. 2. Electron micrographs of cell-grown *M. dispar* stained with ruthenium red (a) or labeled with polycationic ferritin (b). Friis medium-grown *M. dispar* was stained with ruthenium red (c) as described in the text. Magnification, ×57,510. m, membrane.

from cell surfaces by a dialysis membrane were surrounded by capsulelike material (Table 1). This was in contrast to mycoplasmas grown in Friis medium or MEM (Table 1). Both lung lavage and rabbit antibodies against capsulelike material reacted with cell-grown mycoplasmas, as shown by immunogold labeling (Fig. 3). A positive immunogold reaction was detected in paraffin-embedded lung sections of calves infected with *M. dispar* when reacted with rabbit antibodies (Fig. 4). Gold labeling was observed on the surface of airway epithelia, indicating the presence of capsular antigens of *M. dispar* (Fig. 4a). This type of labeling was not observed with noninfected lung sections (Fig. 4b).

Agglutination of cell-grown and medium-grown mycoplasmas by lectins. Cell-grown and Friis medium-grown *M. dispar* were agglutinated by lectins that react with galactose residues (Table 2). The highest titers were observed when cell-grown mycoplasmas were reacted with *R. communis* I and II agglutinins. Inhibition of the agglutination was observed when D-galactose or lactose was added to the reaction. A lesser degree of agglutination was observed with Friis medium-grown mycoplasmas and with lectins that react with glucose residues.

Quantitation and specificity of anti-capsular antibodies. Capsulelike material obtained from cell-grown *M. dispar* could bind to *R. communis* agglutinin, as shown by the ability of rabbit antibodies against capsulelike material to recognize agarose-bound lectin after incubation with PK digests. Antibodies from rabbits inoculated with capsulelike material precipitated 1,520 ± 85 cpm. In contrast, antibodies from noninoculated rabbits precipitated 562 ± 57 cpm.

Lung lavage-derived antibodies from *M. dispar*-infected calves, but not those from noninfected calves, also recognized the capsulelike material. Antibodies derived from the lung lavage of infected calves precipitated 3,462 ± 356 cpm, whereas those derived from noninfected calves precipitated 681 ± 142 cpm, suggesting that the capsulelike material and the antibodies against it are produced during infection. In both cases, precipitation of the capsulelike material by antibodies from inoculated animals was significantly higher than that by antibodies from noninoculated animals.

The specificity of the calf antibodies was shown by the ability of Fab fragments from rabbit antibodies to block the positive response given by lung lavage antibodies. Fab fragments from normal rabbit globulins failed to inhibit the binding of lung lavage antibodies. These data indicate that the calf's humoral immune system recognizes the capsulelike material obtained from cell-grown *M. dispar*.

The capsulelike carbohydrate produced during culture with lung fibroblasts was produced by the mycoplasmas. Antibodies to capsulelike material and those directed against BLF precipitated <300 cpm of a total of 10,000 to 15,000 cpm from noninfected BLF preparations. In contrast, antibodies to capsulelike material precipitated >13,000 cpm (80%) from *M. dispar*-infected BLF cell culture preparations.

DISCUSSION

Previous studies have established a correlation between virulence and capsule production in mycoplasmas (4, 25–27), but the evidence is at best circumstantial in nature. Increased capsule production was observed when *M. gallisepticum* was grown in chicken embryo cell cultures (30). Yagihashi et al. demonstrated increased capsule production after 10 cell culture passages. Our studies, however, demonstrate that capsulelike production can be regained in *M. dispar* after a single passage in cell cultures even with a high-passage-number strain.

Differentiation between capsule and slime polysaccharides is often operationally defined by the degree of association after centrifugation (29). The extracellular material regained

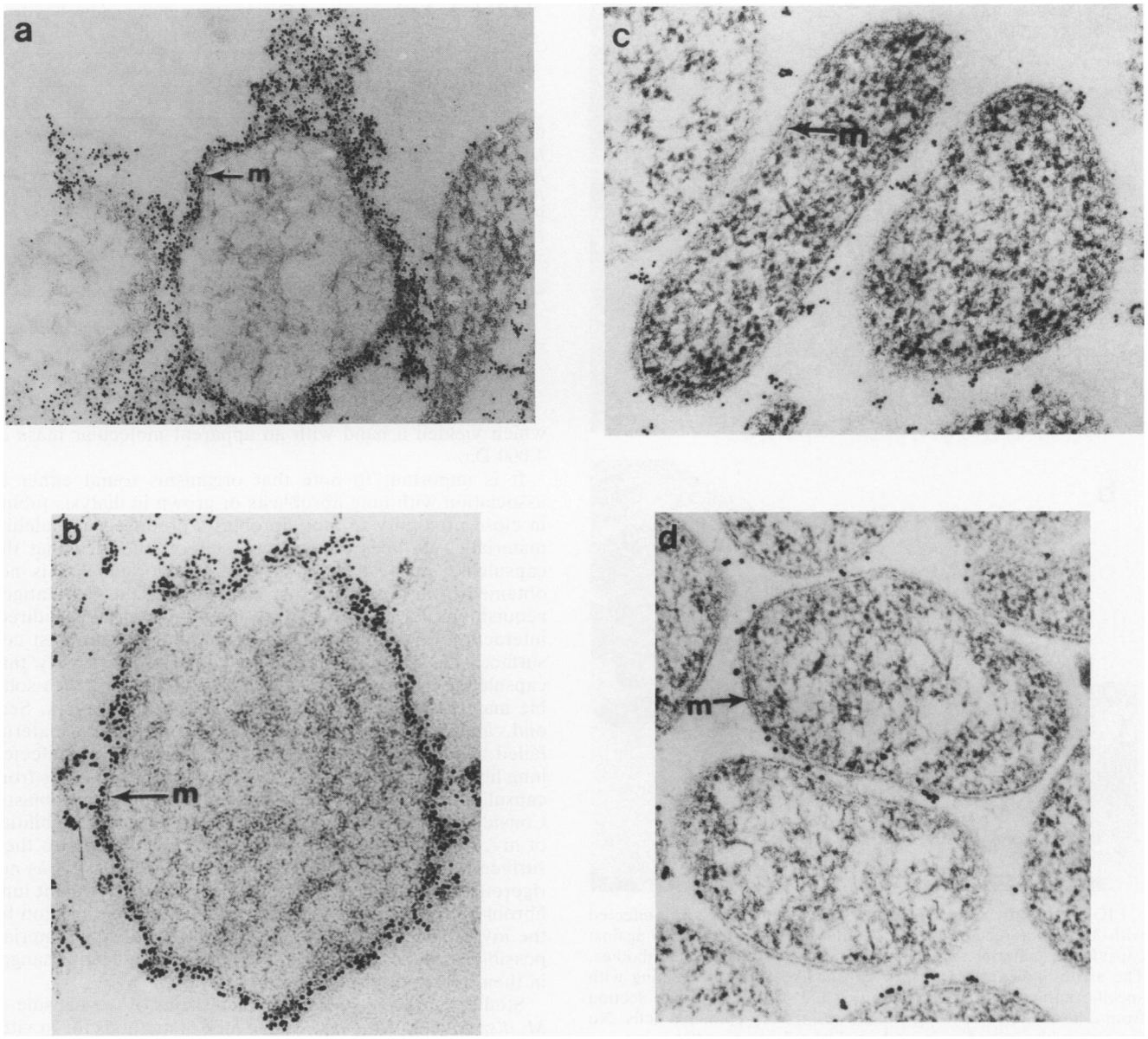

FIG. 3. Immunogold labeling of cell-grown *M. dispar* reacted with 1:10 diluted lung lavage antibodies from a convalescent calf (a), 1:10 diluted rabbit antibodies against the capsulelike material (b), lung lavage antibodies from a control calf (c), and normal rabbit globulins (d). Magnification, ×49,200. m, membrane.

by the mycoplasmas as a consequence of the coculture with cells behaved like a capsule, since it remained tightly associated to the mycoplasma surface after several washes, rather than like a slime, which, under the same circumstances, would be shed into the supernatant.

The nature of the capsulelike material appears to be a negatively charged (acidic) carbohydrate or polysaccharide with a significant proportion of galactose. This is supported by the fact that polycationic ferritin (3) and ruthenium red (19) have a high affinity for the material (Fig. 2a and b), by the ability of agarose-bound lectin to bind the material through a lectin that recognizes galactose, and by the fact that this reaction was inhibited by the addition of D-galactose (Table 2). Additional evidence for its carbohydrate nature is presented through the staining characteristics of material

TABLE 1. Effect of culture conditions on the percentage of capsulated *M. dispar*

Staining procedure	% (SEM) of capsulated organisms[a]			
	A	B	C	D
Expt 1				
Ruthenium red	4b (1)	–[b]	29b (5)	–
Polycationic ferritin	7d (1)	–	30d (4)	–
Expt 3				
Ruthenium red	–	9e (1)	–	62e (9)

[a] Means of three replications. *M. dispar* was grown in Friis medium (A) or MEM (B) or cultured with bovine lung fibroblasts without (C) or with (D) inclusion in dialysis tubing. Mycoplasma suspensions were prepared for TEM as described in the text. Letters after numbers indicate significant differences between numbers with the same letters ($P < 0.05$).

[b] –, Not compared.

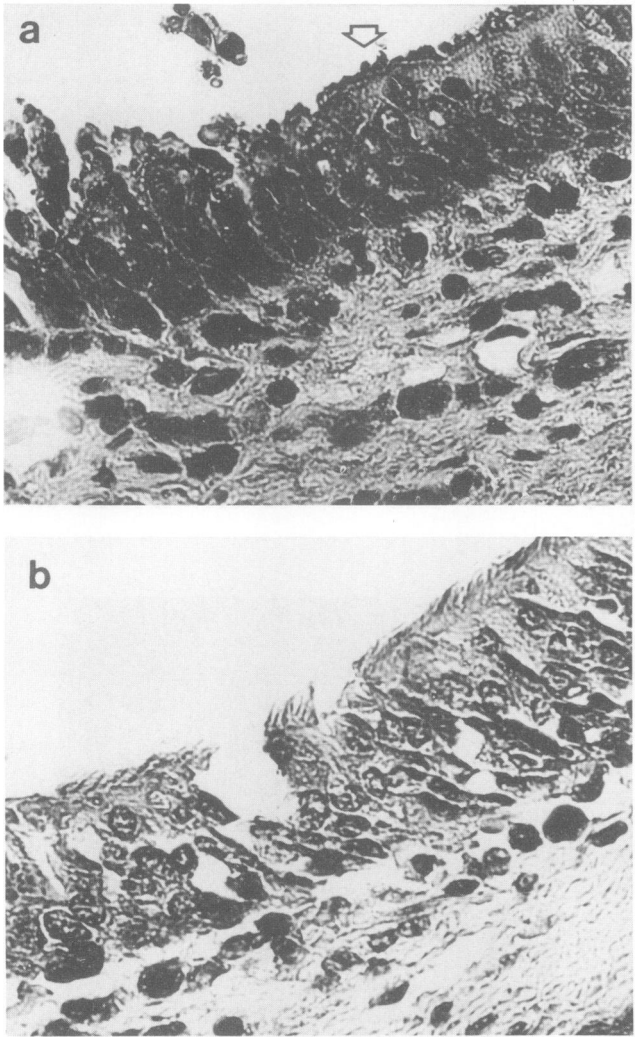

FIG. 4. (a) Micrograph of lung tissue section from a calf infected with *M. dispar* reacted with 1:10 diluted rabbit antibodies against capsulelike material and with anti-rabbit gold-labeled antibodies. The arrow indicates *M. dispar* capsulelike antigens reacting with specific antibodies. Magnification, ×400. (b) Lung tissue section from a normal calf showing ciliated respiratory epithelial cells. No reaction with antibodies is evident. Magnification, ×400.

isolated from cell-grown organisms. A modified silver stain (5, 14), Schiff reagent, and Alcian blue all stain this material, further supporting the idea of a carbohydrate nature (Fig. 1). The presence of galactoselike residues in the capsulelike material may be related to its mode of production, but it should be observed that this carbohydrate is commonly found on mycoplasma surfaces (23, 24).

Typically, bacterial capsules are composed of high-molecular-weight polysaccharides (2). With mycoplasmas, the molecular weight of the capsular carbohydrate of the F-38 strain of *Mycoplasma capricolum* and the galactan from *M. mycoides* can be deduced from their published purification protocols, which include separation through chromatography columns that exclude molecules larger than 200 kDa (Ultrogel ACA 44 and Sephadex G200, respectively) (9, 21). In contrast, in the present report separation of the capsulelike material was performed in denaturing SDS-PAGE, which yielded a band with an apparent molecular mass of 4,000 Da.

TABLE 2. Agglutination of Friis medium-grown and cell-grown *M. dispar* by lectins

Lectin	Agglutination[a]	
	FM	BLF
Concanavalin A	4	8[b] (2)[c]
D. biflorus agglutinin	32	128[d] (4)
R. communis agglutinin I	64	256[d] (0)
R. communis agglutinin II	32	256[d] (2)
Peanut agglutinin	32	64[d] (2)
U. europaeus agglutinin I	32	64

[a] Values indicate the highest lectin dilution causing agglutination. *M. dispar* was grown in modified Friis medium (FM) or cocultured with BLF cells.
[b] Agglutination was inhibited by D-glucose (1%).
[c] Numbers within parentheses indicate the highest lectin dilution causing visible agglutination in the presence of the inhibitor carbohydrate.
[d] Agglutination was inhibited by D-galactose (1%) and lactose (1%).

It is important to note that organisms found either in association with lung fibroblasts or grown in dialysis tubing in close proximity to lung fibroblasts produced capsulelike material. Two lines of evidence support the idea that the capsulelike material is of mycoplasmal origin and is not obtained from lung fibroblast cell surfaces through antigen acquisition. First, the dialysis membrane prevents direct interaction of the mycoplasma and the lung fibroblast cell surface. These studies do not exclude the possibility that capsulelike material production could be induced by a soluble material derived from lung fibroblast monolayers. Second, antibodies directed against the capsulelike material failed to precipitate radiolabeled material from noninfected lung fibroblast cell cultures. This showed that epitopes from capsulelike material were not present in lung fibroblasts. Considering previous studies demonstrating the capabilities of mycoplasmas to tightly bind serum components to their surfaces (25), this was an important observation. It was not rigorously shown, however, that low-molecular-weight lung fibroblast components were not utilized in some fashion by the mycoplasma during production of capsulelike material, possibly as building blocks, resulting in significant changes in their antigenic profiles.

Studies on the biological characteristics of the capsule of *M. dispar* were hampered by the lack of methods for in vitro production of this material. In this report we demonstrate the production of capsulelike material that is antigenically similar to the capsule produced under in vivo conditions. With this material, studies can be designed to investigate the structure and composition of the *M. dispar* capsulelike material, to identify the genes involved in its production, and to determine the nature of the inducing signal and the genetic regulatory mechanisms involved.

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