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Decreased Metabolism and Viability of *Mycoplasma hominis* Induced by Monoclonal Antibody-Mediated Agglutination

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Monoclonal antibodies (MAbs) were generated against lysates of clinical *Mycoplasma hominis* isolates. Three of these, designated BG2, BA10, and FE6, recognized an integral membrane protein of *M. hominis* with an apparent molecular weight of 50,000 (p50). Electron microscopy studies demonstrated that this protein is distributed evenly over the cell surface. These anti-p50 MAbs were species specific for *M. hominis*; they reacted with 42% of 126 tested clinical *M. hominis* isolates and showed no reactivity to heterologous mycoplasma species. Immunoblot analysis after limited proteolysis of purified p50 demonstrated that the three MAbs reacted with different epitopes of the protein. Unlike BA10 and FE6, MAb BG2 induced a decrease in arginine metabolism and a reduction of CFU in metabolic inhibition tests. $F(ab)_2$ fragments of MAb BG2 showed the same inhibitory effect as the intact MAb molecule, while Fab and Fc fragments had no influence on vital functions. Preincubation of the mycoplasmas with MAb BG2 followed by trypsin treatment yielded the same amount of CFU as the control without antibodies. In conclusion, the cell aggregates were resolved by the trypsin treatment. These experiments and tests with the antibody fragments led to the conclusion that only the intact MAb structure or the $F(ab)_2$ structure had metabolic inhibition potential and that the observed metabolism inhibition as well as the apparent decrease in viability were a result of agglutination by MAb BG2.

In common with other Mollicutes, *Mycoplasma hominis* is an extracellular parasite which lacks a cell wall. A variety of disorders such as urinary tract infections and gynecological diseases are caused by this organism in humans (15). In terms of pathogenicity, the mycoplasma membrane plays a prominent role in the interaction of the organism with the surface of host cells. Interaction as well as adhesion must be mediated in part by surface membrane structures (21). In addition, the immunological response of the host to such mycoplasma surface structures may be an important factor in controlling or exacerbating the disease (e.g., see references 6 and 21). In clinical diagnostics mycoplasmas are commonly identified to the species level by metabolism inhibition (MI) and growth inhibition, using polyclonal antisera (9, 13, 25). Polyclonal antibodies directed against surface structures may be of major importance in causing inhibition of vital functions. However, a problem with these methods is that surface-localized epitopes exhibit strong variation (7). Even though these methods are protracted and insensitive, MI and growth inhibition tests are widely used in diagnostic and epidemiological studies and in the classification and characterization of mycoplasmas (25). The characterization of the surface structures responsible for the induction of antibody-mediated MI and growth inhibition is not possible with sera. Monoclonal antibodies (MAbs) have been used as specific tools in defining potentially important structures for MI and for the reduction of viability of *M. hominis* strains. Inhibition of growth or metabolism is described for different species of mycoplasmas and for antibodies directed against membrane proteins of different molecular weights (2, 11, 17, 28). The basis of the MI reaction in mycoplasmas has not yet been elucidated (2). In our laboratory, a MAb directed against a protein with an apparent molecular weight of 50,000 (p50) has been found to mediate this inhibition in metabolism and decrease in viability of *M. hominis*. The current research program was undertaken in an attempt to elucidate the biological role of p50.

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MATERIALS AND METHODS

Mycoplasmas. For immunization of mice, the human pathogen *M. hominis* FBG was used. This was obtained from W. Bredt, University of Freiburg, Freiburg, Germany. For other preparations, the *M. hominis* type strain PG21 (NCTC 10111) from the National Collection of Type Cultures, London, United Kingdom, was employed. To test the cross-reactivity of the antibodies used, the following type strains were used: *Acholeplasma axanthum* (NCTC 10138), *A. granularum* (NCTC 10128), *A. laidlawii* (NCTC 10116), *A. modicum* (NCTC 10134), *M. arginini* (NCTC 10129), *M. arthritidis* (NCTC 10162), *M. bovis* (NCTC 10131), *M. bovoculi* (NCTC 10141), *M. buccale* (NCTC 10136), *M. canis* (NCTC 10146), *M. capricolum* (NCTC 10154), *M. faucium* (NCTC 10174), *M. fermentans* (NCTC 10117), *M. gallinarum* (NCTC 10120), *M. gallisepticum* (NCTC 10115), *M. hyorhinis* (NCTC 10130), *M. lipophilum* (NCTC 10173), *M. orale* (NCTC 10112), *M. pneumoniae* (NCTC 10119), *M. primatum* (NCTC 10163), and *M. pulmonis* (NCTC 10139). For the investigation of antigen variability, 126 different clinical isolates of *M. hominis* were used; they were obtained from W. Bredt, University of Freiburg; I. Just, University of Münster, Münster, Germany; and the Institut for Medical Microbiology and Virology, University of Düsseldorf, Düsseldorf, Germany. All strains were grown in PPLO broth (Difco Laboratories, Detroit, Mich.) supplemented with heat-inactivated horse serum (obtained from GIBCO, Eggenstein, Germany) to a final concentration of 10% and with yeast extract (obtained from ICN Biomedicals, Meckenheim, Germany) to a final concentration of 10% or on PPLO

agar plates at 37°C, as described previously (24). The mycoplasmas were harvested by centrifugation (15,000 × *g* for 30 min at 4°C), washed, and resuspended in 10 mM K-phosphate (pH 7.4)–150 mM NaCl (phosphate-buffered saline [PBS]) as described previously (3).

Immunization, fusion, selection of hybridomas, ELISA, and antibody preparation. The procedures for immunization of mice and subsequent fusion and selection of hybridomas have been described previously (3). Female BALB/c mice were immunized intraperitoneally with mycoplasma protein, emulsified in complete Freund adjuvant (Difco). After the final immunization, the spleen cells were fused by the polyethylene glycol-mediated technique with the myeloma cell line X63Ag8 653. After fusion, the cells were distributed into 96-well microtiter plates (Greiner, Nürtingen, Germany), and the supernatant fluids of the cell cultures were screened for antibodies by enzyme-linked immunosorbent assay (ELISA). Freeze-thaw-disrupted mycoplasmas were diluted to 20 μg of protein per ml in coating buffer (borate-buffered saline [50 mM $Na_4B_4O_7$, 150 mM NaCl], pH 9.0); 50-μl volumes were then applied to each well of a microtiter plate (Falcon; Becton Dickinson, Oxnard, Calif.) and plates were incubated for 2 h at room temperature. Free binding capacity was blocked with 1% bovine serum albumin in PBS for 30 min. Fifty microliters of the antibody solution to be tested was added to each well, and wells were incubated for 1 h. The bound antibody was detected by goat anti-mouse peroxidase-labeled immunoglobulin (Dianova, Hamburg, Germany), using H_2O_2-ABTS (Boehringer, Mannheim, Germany) as substrate.

The MAbs (BG2, BA10, and FE6) were prepared from culture supernatants. The immunoglobulin fractions were precipitated from the culture supernatants by addition of 1 volume of a saturated ammonium sulfate solution and further purified by affinity chromatography on protein G-Superose in accordance with the instructions of the manufacturer (Pharmacia, Freiburg, Germany).

The immunoglobulin class of the generated MAbs was determined by ELISA, as described previously (3).

Preparation of rabbit serum. Two-month-old New Zealand White rabbits were immunized subcutaneously with *M. hominis* FBG protein (1 mg/ml of PBS). Further booster injections were given at 10- to 14-day intervals. The sera were screened for anti-mycoplasma antibodies by ELISA. When sufficient antibodies had developed, the rabbits were bled and the sera were inactivated for 30 min at 56°C.

Preparation and purification of active Fab and $F(ab)_2$ fragments. For preparation of Fab or $F(ab)_2$ fragments, the MAbs were treated with papain or with preactivated papain, respectively, as described by Parham (19). The fragments were purified by affinity chromatography on protein G-Superose. The purity and identity were assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. The activity of these fragments in binding the epitope was tested by ELISA.

SDS-PAGE, immunoblot analysis, and immunobinding assay. The proteins, which were detected by the MAbs, were identified by SDS-PAGE and immunoblotting by the methods of Laemmli (12) and Towbin et al. (26) with the modifications of Blazek et al. (3). The immunobinding assay was modified by the procedure of Kotani and McGarrity (10), as described previously (3).

Cell lysis and isolation of membranes. Mycoplasma cell membranes were prepared by osmotic lysis as described by Razin (20) with the following modifications. A 100-ml broth culture of *M. hominis* grown to logarithmic phase was centrifuged at 12,000 × *g* for 15 min, washed twice in PBS, and resuspended in 2 ml of PBS. The cells were transferred rapidly into 100 volumes of deionized water, which was preheated to 37°C, and incubated for 15 min. The membranes were collected by centrifugation at 34,000 × *g* for 30 min, washed in deionized water, and finally resuspended in 5 ml of PBS–0.01 M β-mercaptoethanol. The preparation was analyzed by the Bradford protein assay (5), SDS-PAGE, and immunoblotting.

Triton X-114 phase fractionation of mycoplasma components. The *M. hominis* proteins were separated by Triton X-114 phase fractionation as described by Bordier (4) with the modifications of Riethman et al. (21) into hydrophobic, hydrophilic, and nonsoluble fractions. The distribution of the proteins in these fractions was determined by SDS-PAGE and immunoblot analysis.

Preparation and purification of the protein with an apparent molecular weight of 50,000 (p50). A three-liter broth culture of *M. hominis* FBG grown to logarithmic phase was harvested by centrifugation at 12,000 × *g* for 30 min and washed twice with PBS. The mycoplasma cells were resuspended in PBS, clarified by sonication, and diluted to a concentration of 2 mg of protein per ml. After the addition of Nonidet P-40 (20 mg/mg of protein), the lysate was incubated for 5 min at 37°C and centrifuged for 30 min at 30,000 × *g*, and the resulting supernatant was diluted to a final concentration of 0.1% Nonidet P-40. p50 was purified from this solution by affinity chromatography, using MAb BG2 coupled to Tresyl-activated Sepharose 4B (Pharmacia). Bound protein was eluted with 3 M NaSCN–0.1% Nonidet P-40 in PBS. The fractions which contained the p50 were pooled and dialyzed against PBS. Finally, the fraction was concentrated sevenfold by ultrafiltration, using a collodium bag, SM 13200 (Sartorius, Göttingen, Germany).

Immunoelectron microscopy. Mycoplasmas were prepared for electron microscopy as described by Christiansen et al. (7) with the following modifications. A 25-ml broth culture of mycoplasmas grown to the logarithmic phase was harvested by centrifugation (3,000 × *g*, 15 min) and washed twice in 25 mM Tris HCl (pH 7.0)–150 mM NaCl (TBS). For fixation, cells were resuspended in 1 ml of TBS–0.5% glutaraldehyde, centrifuged (3,000 × *g*, 15 min), and resuspended in 1 ml of TBS. The cell suspension (10 μl) was placed on top of a Formvar-carbon-coated, glow-discharged copper grid. Nonspecific binding sites were blocked with 0.5% bovine serum albumin (BSA); this was followed by incubation with MAb supernatants (BG2, BA10, FE6, and CCM2 [3]) diluted 1:20 for 1 h. The grids were washed three times in TBS–0.5% BSA and then incubated with secondary antibodies (goat anti-mouse immunoglobulin G [IgG]; Dianova) labeled with colloidal gold (5 nm) diluted 1:50 in TBS–0.5% BSA for 1 h. The grids were then washed five times in TBS and, finally, stained with 1% phosphotungstic acid and dried. The grids were examined in a Philips EM 3000 electron microscope.

Limited proteolysis. The purified p50 protein was proteolytically digested by V-8 protease (EC 3.4.21.19) as described by Cleveland et al. (8). p50 (4 μg per slot) and V-8 protease (Boehringer) of different concentrations (5, 50, or 100 ng per slot) in 60 mM Tris HCl (pH 6.8)–0.1% SDS–20% (vol/vol) glycerin–1 mM sodium EDTA were loaded onto a 15 to 18% SDS-polyacrylamide gel. The proteins were electrophoresed up to 1 cm over the end of the stacking gel. The protein and the protease were then incubated at room temperature for 45 min, and the gel was then run in the normal manner. The peptide bands were detected by Coomassie blue staining and immunoblot analysis.

Ouchterlony immunodiffusion. Agarose, 1% in PBS buffer containing 0.05% sodium azide, was poured onto precoated slides, and agar plugs were withdrawn with a Pasteur pipette as described by Ouchterlony (18). The wells were filled with 5 or 10 μl of antibody (1 mg/ml of PBS) and purified p50 (1 mg/ml). The slides were placed in a humid chamber and incubated overnight at 37°C. The precipitation rings were detected by Coomassie blue staining.

Inhibition of *M. hominis* metabolism by MAbs. The complement-independent metabolic inhibition potential of the anti-p50 antibodies was examined on the basis of two parameters: (i) the reduction of the metabolism of arginine via the arginine deiminase pathway, resulting in an alkaline shift in the pH of the medium; and (ii) reduction in *M. hominis* CFU. Each parameter will be considered in turn.

Inhibition of *M. hominis* metabolism of arginine by MAbs was analyzed by the MI tests of Washburn (27) and Taylor-Robinson (25), with modifications. *M. hominis* broth cultures were incubated in sterile microtiter plates (Greiner) (2.3×10^3 or 4.5×10^2 CFU in 150 μl of PPLO broth per well) after the addition of antibody preparations in PBS (50 μl per well) of various concentrations at 37°C. After suitable incubation (24 to 48 h), the extinction was read in an Immuno Reader, NJ2000 (Nunc, Wiesbaden, Germany), by dual mode at 550 and 620 nm against a medium control.

To evaluate the effect of different MAbs on mycoplasma viability, mycoplasmas were incubated in 200 μl of broth culture medium with different MAb preparations in PBS (20 μl), including fragments of MAb BG2, in various concentrations at 37°C for 24 h. The *M. hominis* cultures were thoroughly resuspended and serially diluted, plated on PPLO agar plates, and incubated for 4 days at 37°C; then CFU were quantified.

For microscopy studies of the mycoplasma cultures in PPLO broth, mycoplasma cultures grown in broth culture medium in microtiter plates were visualized with a Zeiss photomicroscope, IM35 (Zeiss, Oberkochen, Germany).

Trypsin treatment of intact mycoplasmas. Mycoplasmas, grown for 24 h in the presence or absence of MAbs, were centrifuged and washed in PBS. The cell sediment was resuspended in 1 ml of 2.5% trypsin solution in PBS (Boehringer). The suspension was incubated at 4°C for 10 min. Mycoplasmas were sedimented by centrifugation at 13,000 × *g* for 10 min, the sediment was resuspended in broth culture medium and serially diluted, and CFU were quantified.

RESULTS

Specificity of MAbs. From a pool of MAbs generated after immunization of BALB/c mice with *M. hominis* FBG protein, three MAbs which reacted specifically with a protein with an apparent molecular weight of 50,000 were characterized [BG2, BA10, and FE6; all isotype IgG1(κ)]. As shown in Fig. 1, MAbs BA10 and FE6 recognized an additional protein with an apparent molecular weight of 48,000, whereas MAb BG2 bound exclusively to p50.

The three MAbs were specific for *M. hominis* and showed no cross-reactivity with 21 heterologous mycoplasma species tested in this study by immunobinding assays. Furthermore, the reactivity of the three MAbs to 126 clinical isolates of *M. hominis* was determined. MAb BG2 reacted with 6% of these clinical isolates and not with the type strain PG21 (NCTC 10111), whereas MAbs BA10 and FE6 reacted with 42% of the tested clinical *M. hominis* isolates as well as with PG21. FE6 and BA10 recognized, in part, different isolates, which indicates that the MAbs react with different epitopes.

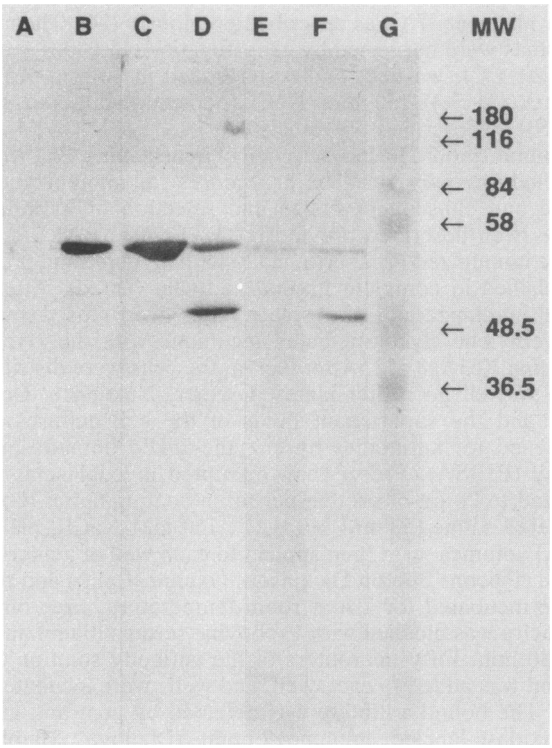

FIG. 1. Immunoblot analysis of the reactivity of MAbs BG2, BA10, and FE6. Samples prepared under reducing conditions of mycoplasma proteins of *M. hominis* type strain PG21 (NCTC 10111) (lanes A, C, and E) or of clinical isolate FBG (lanes B, D, and F) were subjected to SDS-PAGE on 12.5% acrylamide gels. The proteins were transferred to nitrocellulose membranes and immunostained with the detecting MAbs BG2 (lanes A and B), BA10 (lanes C and D), and FE6 (lanes E and F). Lane G, Prestained molecular weight (MW) marker standards (α-macroglobulin, 180,000; β-galactosidase, 116,000; fructose-6-phosphate kinase, 84,000; pyruvate kinase, 58,000; fumarase, 48,500; lactic dehydrogenase, 36,500).

Cellular location of the p50 antigen. To determine the subcellular location of the p50 protein, membrane fractions of *M. hominis* FBG and PG21 were prepared by osmotic lysis and purified. As can be seen in Fig. 2, the three MAbs bound to a protein found only in the membrane fraction and not in the cytoplasmic fraction.

Triton X-114 partitioning of cell proteins was performed with *M. hominis* FBG and PG21 to assess the hydrophobicity of the MAb-reactive protein. Following partitioning, the proteins of the resulting fractions were immunoblotted and reacted with the MAbs. As shown in Fig. 2, the p50 protein was found in the hydrophobic detergent phase following the extraction procedure, suggesting that p50 is an intrinsic membrane protein.

Immunoelectron microscopy of negatively stained mycoplasmas demonstrated that MAbs BG2, BA10, and FE6 react with surface-localized epitopes of the p50 protein. As shown in Fig. 3, the p50 protein is distributed evenly over the cell surface. Nonspecific binding of gold-labeled secondary antibody to the cell surface was excluded by controls without detection antibody, with MAb CCM2 directed against the cytoplasmic protein EF-Tu (3), or with an unrelated MAb directed against a nonmycoplasmal protein (Fig. 3).

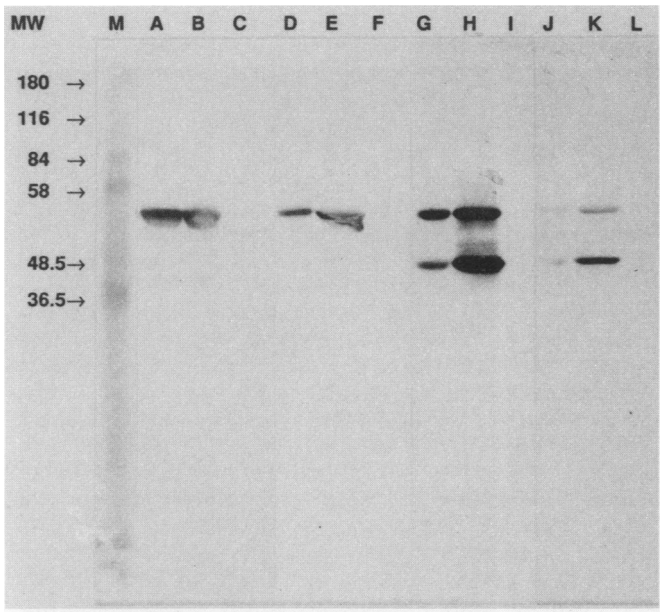

FIG. 2. Immunoblot analysis of the cellular location of p50 protein. For cell lysis and isolation of membranes (lanes A, B, and C), *M. hominis* FBG cells were osmotically lysed and separated into membrane and cytoplasmic fractions. Samples prepared under reducing conditions of whole mycoplasma protein (lane A), membrane fraction (lane B), and cytoplasmic fraction (lane C) were subjected to 12.5% SDS-PAGE. Lane M, Prestained molecular weight standards (MW) as described in the legend to Fig. 1. The proteins were analyzed by immunoblotting. For detection, the anti-p50 MAb BG2 was used (lanes A, B, and C). Immunoblot analysis with MAb CCM2 against cytoplasmic protein EF-Tu (3) served as a control (data not shown). For Triton X-114 phase separation (lanes D to L), lysates of *M. hominis* FBG were partitioned by Triton X-114 phase fractionation (4, 21). The samples were treated as described previously. Lanes D, G, and J contained total mycoplasma protein, lanes E, H, and K contained the Triton X-114 phase, and lanes F, I, and L contained the aqueous phase. For detection, MAbs BG2 (lanes D, E, and F), BA10 (lanes G, H, and I), and FE6 (lanes J, K, and L) were used. As a control, immunoblot analysis was performed with MAb CCM2. The recognized EF-Tu partitioned into the aqueous phase (3) (data not shown).

Mapping of epitopes. The p50 protein was purified by affinity chromatography as described in Materials and Methods. The purified p50 protein was treated with V-8 protease (the enzyme hydrolyzes peptides at the carboxy-terminal side of glutamic acid), and the polypeptides thus produced were subjected to SDS-PAGE and immunoblot analysis. MAbs BG2, BA10, and FE6 were used for immunostaining of the protein blots, thereby identifying only those molecules which retain the epitope. In experiments with limited and complete V-8 protease digestion, p50 was degraded through intermediate products to fragments with molecular weights of <5,000. MAbs BA10, FE6, and BG2 recognized a polypeptide with a molecular weight of 12,000 as the smallest immunoreactive fragment. As shown in Fig. 4, the patterns of the immunoreactive fragments are different for the three antibodies. This indicates again that the investigated MAbs recognize different epitopes on the same protein.

For further characterization of the MAb-reactive epitopes, an Ouchterlony double-diffusion assay was performed. If MAb BG2 was applied around the well containing the purified p50, a line of precipitation would be formed closely around the p50-containing well.

Effects of MAbs on metabolism and viability. Incubating *M. hominis* FBG in broth culture medium with different concentrations of MAb BG2 led to an apparent decrease in viability and also to MI. As shown in Fig. 5, MAb BG2, as well as the corresponding $F(ab)_2$ fragment, inhibited the metabolism of *M. hominis* FBG. As a control, rabbit anti-*M. hominis* FBG serum was used. The polyclonal antibodies of the serum inhibited mycoplasma metabolism in the same way as MAb BG2. The control MAbs, directed against other membrane proteins of *M. hominis* of an apparent molecular weight of 60,000 (p60) or 100,000 (p100) (CG4 = anti-p60 MAb; DC10 = anti-p100 MAb), did not affect the metabolic activity (Fig. 5).

The apparent decrease in viability, manifested in the reduction of CFU, is correlated to the concentration of the added MAb BG2 (Fig. 6). Analogous control experiments were performed with three other MAbs directed against membrane proteins p60 and p100 (MAbs CG4 and DC10) or against the intracellular EF-Tu (MAb CCM2) (3). These MAbs did not have any effect on the viability. Interestingly, MAb BA10 or FE6, which both react with the same p50 membrane protein but with different epitopes, had no or only a negligible effect (Fig. 7).

To investigate these effects of MAb BG2 in more detail, the same experiments were performed with active Fab and $F(ab)_2$ fragments. While the $F(ab)_2$ fragments affected the metabolism and viability in the same way as the intact MAb, the Fab fragments did not show a comparable effect on vitality (Fig. 8). Trypsin treatment of intact mycoplasmas after incubation with MAb BG2 or the antibody fragments led to the conclusion that only the intact MAb structure and the $F(ab)_2$ structure had any influence on metabolism and growth (Fig. 6 and 8). Photomicroscopic investigations of mycoplasmas grown in PPLO broth to the late logarithmic or the stationary phase showed that the mycoplasmas had agglutinated to clumps of very large numbers of cells (Fig. 9). In control studies performed without any antibody, or with antibodies directed against other surface proteins, neither agglutination nor accumulation of cells was visible. In mycoplasma cultures incubated with rabbit anti-*M. hominis* FBG serum, the same amounts of aggregates were detectable. In the early logarithmic growth phase there was no agglutination visible. This indicated that the effect of MAb BG2 on the metabolism and viability of mycoplasmas is caused by an agglutination of cells mediated by MAb BG2.

DISCUSSION

Using hybridoma technology, our group has generated MAbs which bind to a variety of *M. hominis* epitopes. This multitude of MAbs has enabled us to analyze mechanisms of antibody-mediated inhibition of growth and metabolism. From these antibodies only MAb BG2, directed against the p50 protein, induced an apparent decrease in viability and an inhibition of metabolism (23). Interestingly, two other MAbs, which also recognized the p50 protein, did not show these distinct effects on viability and metabolism. As shown by electron microscopy, the p50 protein is randomly distributed over the cell membrane and the recognized epitopes are localized on the surface. Cross-reactivity studies with other mycoplasma species and tests of antigen variability within *M. hominis* indicate that p50 is species specific for *M. hominis*. The epitopes recognized by MAbs BA10 and FE6 are frequently detected, whereas the epitope recognized by MAb BG2 is rarely detected on the different isolates. This

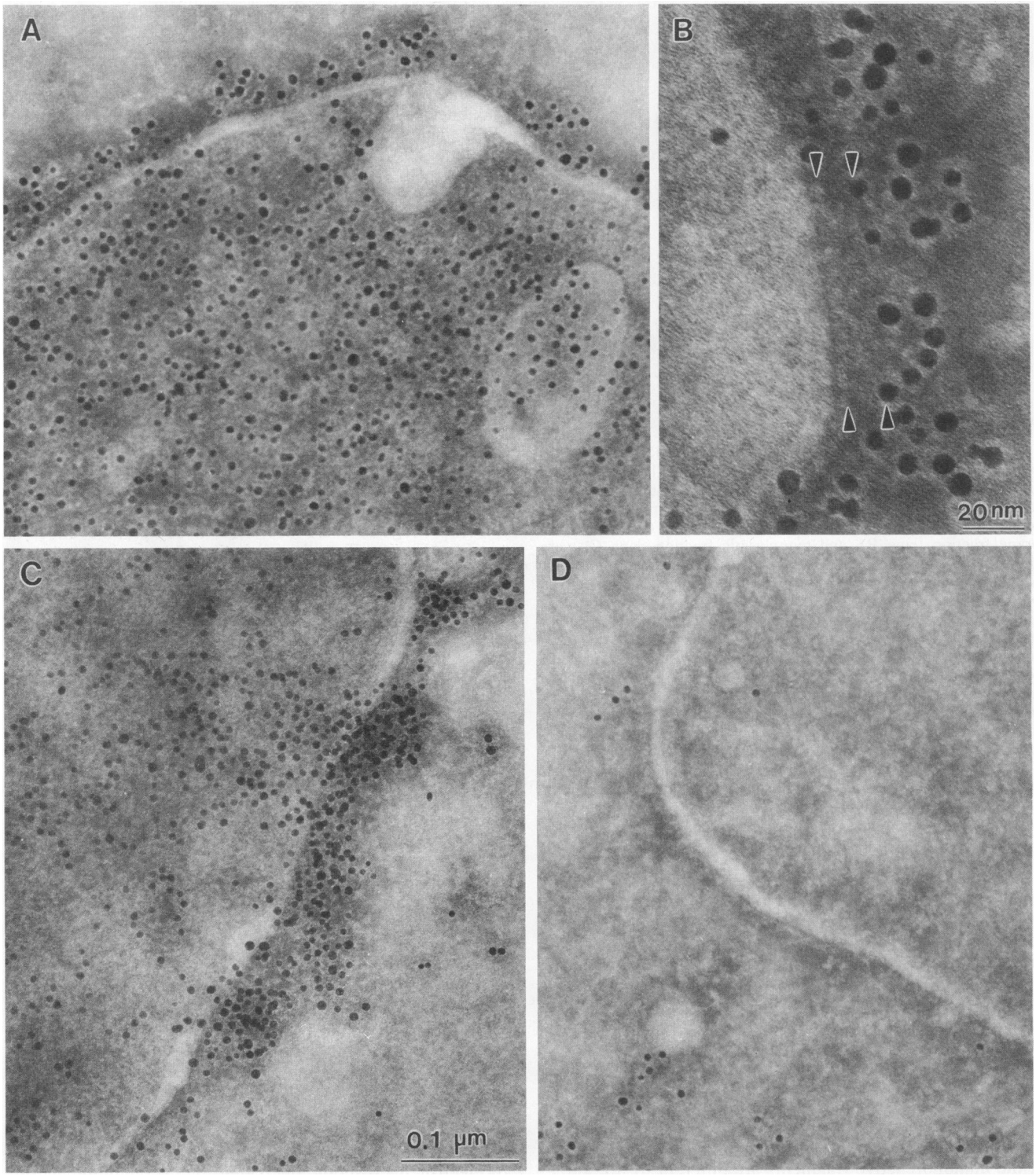

FIG. 3. Immunoelectron microscopy of negatively stained *M. hominis* FBG. Surface-localized epitopes of p50 were detected by MAbs BG2 (A and B) and BA10 (C). The epitope recognized by the MAbs appears to be localized in the membrane periphery as visualized by the relative distance of gold label from the membrane (arrowheads in panel B). Negative control experiments were performed with MAb CCM2, directed against the cytoplasmic protein EF-Tu (D). Control experiments performed without any detection antibody or with an unrelated MAb showed the same result as in panel D. The bar in panel C applies to panels A, C, and D.

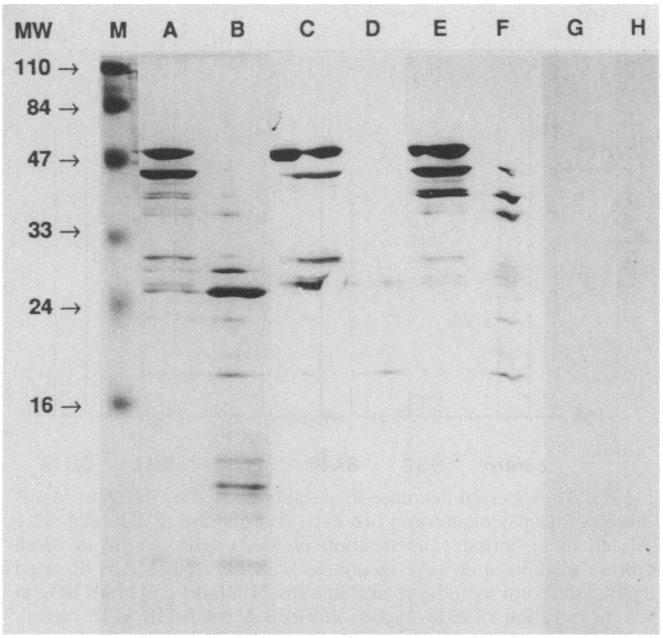

FIG. 4. Limited proteolysis. The purified p50 protein was proteolytically digested by V-8 protease (8). The samples were treated as described in Materials and Methods. Lane M, Prestained molecular weight (MW) standards (phosphorylase *b*, 110,000; bovine serum albumin, 84,000; ovalbumin, 47,000; carbonic anhydrase, 33,000; soybean trypsin inhibitor, 24,000; lysozyme, 16,000). Lanes A and B show the digested peptides in the Coomassie blue-stained SDS-polyacrylamide gel (lane A, 5 ng of V-8 protease; lane B, 100 ng of V-8 protease). The other lanes show the corresponding immunoblot analysis prepared with the anti-p50 MAbs BG2 (lanes C and D), BA10 (lanes E and F), and FE6 (lanes G and H) (lanes C, E, and G, 5 ng of V-8 protease; lanes D, F, and H, 100 ng of V-8 protease).

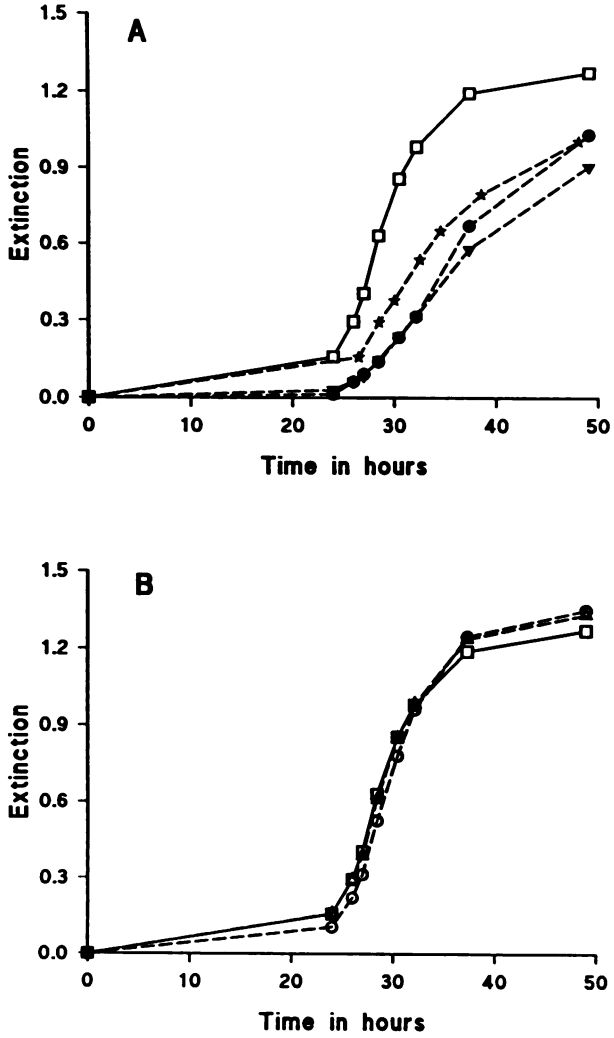

FIG. 5. Inhibition of *M. hominis* metabolism. The metabolism of arginine, resulting in an alkaline shift of pH of the medium, was detected by measurement of the extinction by dual mode at 550 and 620 nm against a medium control. In microtiter plates, 450 *M. hominis* FBG cells per well were incubated with different MAbs or MAb fragments. Shown are the growth phases of the cultures. (A) Inhibition of metabolism by intact anti-p50 MAb BG2 at a concentration of 0.09 mg per well (▼), by $F(ab)_2$ fragments of BG2 at a concentration of 0.07 mg per well (●), or by heat-inactivated rabbit anti-*M. hominis* FBG serum at a concentration of 0.3 mg per well (*). □, control culture without antibody. (B) The same experiment with MAbs directed against other surface proteins. Anti-p60 MAb CG4 at a concentration of 0.07 mg per well (○) and anti-p100 MAb DC10 at a concentration of 0.07 mg per well (△) show no effect on metabolism in comparison with the control without antibody (□). The values represent the means from three reproduced measurements.

indicates that the common epitopes to which BA10 and FE6 bind are more conservative.

To examine the cause of decreased metabolism and vitality mediated by the intact MAb BG2 molecule, analogous experiments were performed with the corresponding $F(ab)_2$ and Fab fragments. The $F(ab)_2$ fragments reacted in the same way as the intact BG2 molecule, whereas the Fab fragment had no influence on vital functions. These results show that the inhibition of metabolism and the concomitant apparent decrease in viability as a result of incubation with MAb BG2 are caused by agglutination. The agglutination is very stable since thorough resuspension does not disaggregate the complexes. Like the intact BG2 molecule, the bivalent $F(ab)_2$ fragments retain the ability to agglutinate the mycoplasma cells. The agglutination of large numbers of mycoplasmas was visible in cultures grown to the late logarithmic phase when the concentration of cells was suitable to allow the buildup of such aggregates.

Trypsin treatment of the mycoplasmas after incubation with MAb BG2 causes a reversion to the normal logarithmic growth of mycoplasma cultures. Trypsin treatment leads to a proteolysis of surface-localized protein structures, including the bound BG2 MAb. It is not surprising, therefore, that MAb BG2 after trypsin treatment or the monovalent Fab fragments are capable of binding to the epitope but are not able to cross-link mycoplasma cells and induce a decrease in vital functions. It is important to emphasize that MAb BG2 is of the isotype IgG1. The observed reversion to the normal logarithmic growth demonstrates that this agglutination of *M. hominis* FBG and MAb BG2 permits multiplication of the organisms. As described earlier by Lin and Kass (14), similar phenomena could be observed in MI tests with polyclonal antisera. The described reaction must be distinguished from usual agglutination reactions and effects that are independent of multiplication (14).

Obviously, the other anti-p50 MAbs, BA10 and FE6, have no inhibitory effects on viability and metabolism, unlike

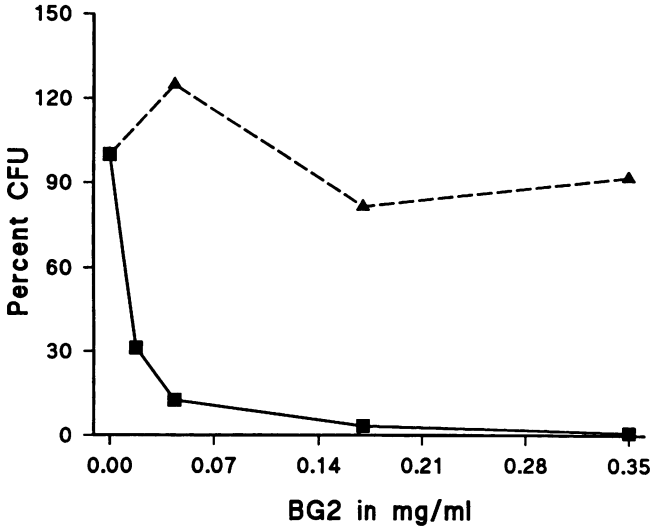

FIG. 6. Apparent decrease in viability caused by different concentrations of MAb BG2. *M. hominis* FBG cells were diluted to an initial concentration of 6,000 cells per ml in broth culture medium and were incubated with different concentrations of MAb BG2 (0.35, 0.18, 0.09, and 0.04 mg/ml) for 24 h. The CFU were quantified by serial dilutions plated on PPLO agar plates. The control without MAb was set to 100%. Quantification after thorough resuspending shows a decrease of CFU depending on the concentration of the added MAb BG2 (■). Trypsin treatment of intact mycoplasmas after incubation with MAb BG2 (▲) resolved the aggregates, finally yielding the same amount of CFU as the control. The values represent the means of three reproduced measurements.

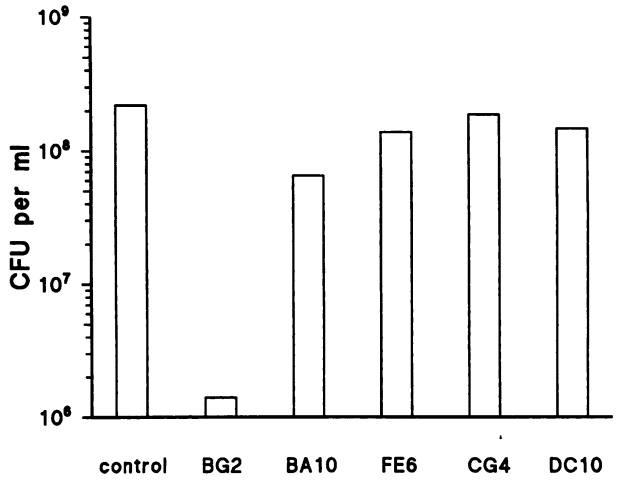

FIG. 7. Apparent decrease in viability caused by different MAbs directed against membrane proteins. *M. hominis* FBG cells were diluted to an initial concentration of 6,000 cells per ml in broth culture medium and were incubated with different MAbs directed against different membrane proteins for 24 h (anti-p50 MAb BG2 at a concentration of 0.18 mg/ml; anti-p50 MAb BA10, 0.16 mg/ml; anti-p50 MAb FE6, 0.06 mg/ml; anti-p60 MAb CG4, 0.1 mg/ml; anti-p100 MAb DC10, 0.1 mg/ml; control without MAb). The CFU were quantified after thorough resuspending by serial dilutions plated on PPLO agar plates. The values represent the means of three reproduced measurements.

MAb BG2. This suggests that only the epitope which is bound by BG2 is located in a sterically suitable position on the cell surface. The ability of MAb BG2, classified as IgG1, to precipitate the p50 protein, as shown by the Ouchterlony double-diffusion assay, indicates that the corresponding epitope is present in more than one copy on the protein molecule. These results demonstrate that the agglutination of the mycoplasmas by MAb BG2 is based on an immunological reaction of high specificity. A binding of immunoglobulin to a surface protein in a nonantigenic manner, as described by Alexander et al. (1), can therefore be excluded. The biological function of p50 is still unclear. The role of the p50 protein in addition to other surface antigens in the pathogenicity of *M. hominis* is under investigation.

The inhibitory effects on vital functions as a consequence of cell agglutination by MAbs described in this study indicate that other similar metabolic inhibition phenomena induced by antisera or MAbs might not only result from inhibition of DNA replication and cessation of growth (11), blocking of a specific metabolic function, or loss of membrane fluidity (2) but also could be caused or intensified by agglutination. Several other effects of MAbs on mycoplasma cells, such as inhibition of growth, adherence, hemadsorption, or monoclonal antibody killing, have been described (11, 16, 17, 21, 22, 28). The agglutination of cells by antibodies could participate in the intensity of these phenomena. Even the inhibition of growth and metabolism for the serological identification of species (9, 25; see also references 29 and 30) could in part be based on agglutination by polyclonal antibodies. In all of these reports, the role of agglutination in inducing or enhancing the observed effects is not thoroughly investigated. It is conceivable that MAbs directed against other epitopes of different surface antigens cause agglutination. The data presented here show that metabolic inhibition and an apparent decrease in viability can be a result of agglutination by a MAb.

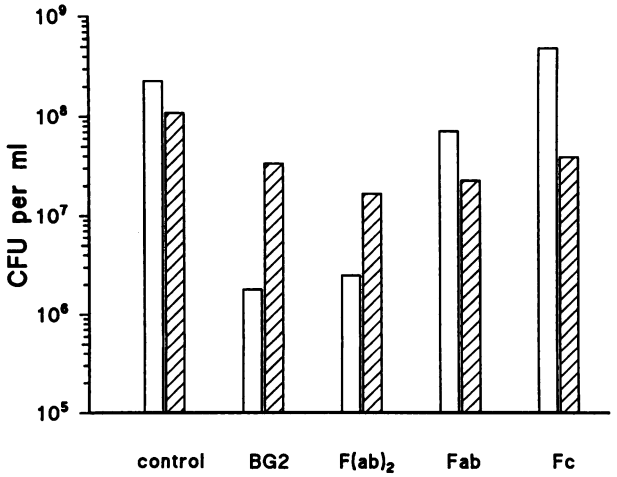

FIG. 8. Apparent decrease in viability caused by active antibody fragments of BG2. *M. hominis* FBG cells were diluted to an initial concentration of 6,000 cells per ml in broth culture medium and were incubated with active antibody fragments of BG2 for 24 h [BG2, 0.15 mg/ml, F(ab)$_2$, 0.06 mg/ml; Fab, 0.13 mg/ml; Fc, 0.05 mg/ml; control without MAb). The CFU were quantified after thorough resuspending (□) or after trypsin treatment (▨) by serial dilutions plated on PPLO agar plates. The values represent the means of three reproduced measurements.

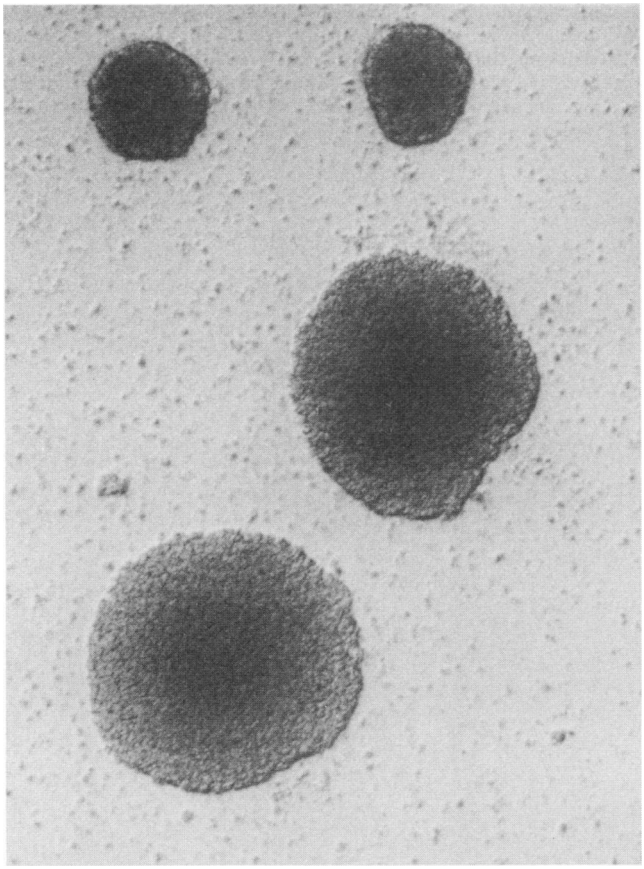

FIG. 9. Photomicroscopic studies of *M. hominis* cultures incubated with MAb BG2. *M. hominis* FBG was grown in PPLO broth culture medium in the presence of MAb BG2 to late logarithmic phase (36 to 48 h). Aggregates of large numbers of mycoplasma cells are clearly visible (initial cell concentration, 6,000 cells per ml; BG2 concentration, 0.35 mg/ml). Magnification, ×270.

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