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Monoclonal Antibodies to Surface Antigens of a Pathogenic *Mycoplasma hominis* Strain

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Three monoclonal antibodies (MAbs) were prepared against an arthritogenic strain of *Mycoplasma hominis* isolated from the joint aspirates of a patient with chronic septic arthritis. Immunoblots of polyacrylamide gel-electrophoresed proteins before and after surface proteolysis showed that the predominant antigenic determinants were on surface-exposed polypeptides. These polypeptides have extensive hydrophobic characteristics, as demonstrated by Triton X-114 phase partitioning. The electrophoresed proteins from cells grown in medium containing [^{14}C]palmitate were blotted onto nitrocellulose which was both reacted with the MAbs and exposed to X-ray film. Superimposable bands on both the immunoblots and the exposed film suggested that the proteins might be acylated. The MAbs were further tested for reactivity with 16 other strains of *M. hominis* isolated from patients with septic arthritis (1 strain), septicemia (10 strains), or nongonococcal urethritis (1 strain); from the cervix (1 strain), rectum (1 strain), or surgical wound (1 strain) of patients; and from a contaminated cell culture. No single protein was consistently recognized from strain to strain, although a 94-kDa protein from 16 of the 17 strains tested was bound by at least one of the MAbs. The apparent antigenic heterogeneity among strains of *M. hominis*, including those isolated from the same tissue source and/or from patients with the same type of clinical disease, might be misleading in that all strains express epitopes associated with a discrete number of proteins to which one, two, or all three MAbs bind. The expression of the epitopes on multiple proteins from the same or different strains may reflect a mechanism for generating antigenic diversity.

Strains of *Mycoplasma hominis* isolated from various healthy tissues and from patients with clinically diverse diseases exhibit marked phenotypic and genotypic heterogeneity (2, 5, 34). Antigenic variability among strains has been demonstrated by using protein analysis, direct or indirect hemagglutination, complement-dependent mycoplasmicidal activity, metabolic inhibition, and growth inhibition procedures (2, 19, 22, 31, 32, 34, 39). Many attempts have been made to find common antigenic features, especially features related to pathogenicity, among the different strains of *M. hominis*. Barile and coworkers reported that strains isolated from the same tissue source of either healthy or infected patients or from contaminated-cell cultures appeared to form genomically related clusters on the basis of DNA-DNA homology and DNA restriction pattern analyses (5). These findings were not, however, supported by other investigators using highly conserved (rRNA and ATPase) gene probes (11–13). Others researchers have attempted to classify *M. hominis* strains into serogroups on the basis of observed antigenic properties (29–32).

Monoclonal antibodies (MAbs) may be useful in helping to define antigenic and pathogenic variability of *M. hominis* strains. Polyclonal sera have been and continue to be helpful reagents for identifying mycoplasmas to the species level but are not as discriminating as MAbs for studying differences among strains, especially with respect to defining pathogenic properties of an isolate. For example, rabbit hyperimmune polyclonal antisera or human convalescent sera reacted with 77 to 99% of two-dimensional electrophoresed proteins expressed by 14 *M. hominis* strains (1, 9), whereas MAbs raised against the type strain PG21 reacted with only a few of the other strains tested (13).

We have shown earlier that pathogenic *M. hominis* 1620 isolated from synovial exudates of a patient with chronic septic arthritis (38) has strong attachment activity (23) and produces severe septic arthritis in experimentally infected chimpanzees (5). In contrast, type strain PG21, isolated from the rectum of a patient and maintained in continuous broth passage for more than 30 years, shows very poor attachment activity (23) and fails to produce arthritic disease in chimpanzees.

We prepared MAbs against the cytadsorbing, pathogenic strain 1620 because we are interested in identifying virulence and protective immunogenic components of pathogenic mycoplasmas. We proposed that specific strains might be capable of colonizing target tissues with distinct attachment components, thus resulting in different clinical illnesses. In order to test this hypothesis and to try to identify important virulence, attachment, and/or protective immunogenic components, we analyzed the binding specificity of MAbs to various pathogenic and nonpathogenic *M. hominis* strains.

We report here the reactivities of three MAbs produced against surface proteins of the cytadsorbing, arthritogenic strain 1620. The variation in number and size of MAb-reactive proteins from 16 other *M. hominis* strains isolated from clinical and nonclinical sources suggests that this mycoplasma might be capable of expressing antigenic variability.

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TABLE 1. *Mycoplasma hominis* strains and *Mycoplasma* species[a] analyzed

Strain	Site of isolation	Clinical condition	Source[b]
Mycoplasma hominis			
1620	Joint	Septic arthritis	MFB
2101	Joint	Septic arthritis	MFB
PG21 (type strain)	Rectum		DE
LBD4	Blood	Septicemia	JGT
LBD5	Blood	Septicemia	JGT
R. Taub	Blood	Septicemia	JGT
W1458	Blood	Septicemia	MFB
1611	Blood	Endocarditis	O
F4238	Blood	Septicemia	MFB
M5039	Blood	Septicemia	MFB
H5488	Blood	Septicemia	MFB
11085	Blood	Postpartum fever	DTR
13428	Blood	Septicemia	DTR
1184	Cell culture	None	MFB
1888	Chimp vagina	None	RAD
11932	Cervix	Unknown	DTR
13408	Urethra	Nongonococcal urethritis	DTR

[a] Additional *Mycoplasma* species analyzed include *M. arginini*, *M. arthritidis*, *M. buccale*, *M. faucium*, *M. fermentans* PG18, *M. genitalium* G37C, *M. lipophilum*, *M. orale*, *M. pneumoniae* M129, *M. primatum*, and *M. salivarium* PG20.

[b] MFB, M. F. Barile (Food and Drug Administration, Bethesda, Md.); JGT, J. G. Tully (National Institutes of Health, Bethesda, Md.); DTR, D. Taylor-Robinson (Medical Research Council, London, United Kingdom); O, Major Ohashi (Walter Reed Army Institute of Research, Washington, D.C.); RAD, R. A. DelGuidice (PRI, Frederick, Md.); DE, D. Edwards.

MATERIALS AND METHODS

***Mycoplasma* species and strains.** *M. hominis* strains and sources are listed in Table 1. All other species used in the studies are maintained as frozen stock cultures in our laboratory (18). Arginine-utilizing *Mycoplasma* species were grown in PPLO broth with 0.25% glucose and 0.25% arginine; glycolytic species of *Mycoplasma* were grown in PPLO broth with 0.25% glucose. The broth media were supplemented with 10% heat-inactivated (56°C, 30 min) horse serum, 5% yeast extract, and 100 U of penicillin per ml (10).

***M. hominis* 1620 antigen preparation.** Broth-grown cultures were centrifuged at 12,000 × *g*. The cell pellets were washed three times in 0.25 M NaCl and frozen in aliquots at −20°C until used for Western immunoblotting analyses. To prepare antigen for immunization and for enzyme-linked immunosorbent assay (ELISA) experiments, washed-cell pellets were first suspended in phosphate-buffered saline (PBS), pH 7.2. The cells were disrupted by rapid freeze-thaw for three cycles, which was followed by sonication (Heat Systems Ultrasonics, Farmington, N.Y.) using a microtip probe at maximum energy for 10 bursts of 15 s each. The protein concentration of the antigen preparation was determined by using a modified Bio-Rad protein assay (16) with bovine serum albumin as the standard. Aliquots of the sonicated antigen preparation were stored frozen at −40°C until used.

Preparation of hyperimmune rabbit serum. Two rabbits were each immunized with 250 μg of the *M. hominis* 1620 antigen preparation emulsified in complete Freund adjuvant (100 μl into each of two subcutaneous and two intramuscular sites). A second and a third immunizing dose of 250 μg (0.5 ml) were given intramuscularly at 21-day intervals with antigen emulsified in incomplete Freund adjuvant. Ten days following the last immunizing dose, animals were exsanguinated and the serum was stored at −70°C.

Production of hybridomas. BALB/c female mice were inoculated intraperitoneally three times per week for 3 weeks with 50 μg of the strain 1620 antigen preparation in a volume of 100 μl of PBS. Sera were obtained and measured for antibody activity by the ELISA and Western blotting procedures. The mice were rested for 3 months, and then each was given final intravenous immunizing doses of 75 μg (150 μl) at 3 and 2 days prior to spleen removal.

Spleens were recovered from the two immunized mice, and the cells were dissociated, washed in Dulbecco modified Eagle medium, and combined with nonsecreting Sp2/0-Ag14 myeloma cells. Fusions were performed by using a modification of a method described earlier (25, 37). Selected hybridomas were cloned by limiting dilution, and selected clones were inoculated intraperitoneally into BALB/c mice primed with pristane. Ascites fluids were collected and stored frozen.

MAb purification and isotyping. High-pressure liquid chromatography (HPLC) purification of MAbs from mouse ascites fluids was performed on a 15-μm Bakerbond ABx column (10 by 250 nm; J. T. Baker, Inc.) (24). The column was equilibrated with 50 mM morpholineethanesulfonic acid (MES), pH 5.6 (buffer A), and the MAb was eluted with a linear gradient of buffer A and 500 mM $(NH_4)_2SO_4$–20 mM sodium acetate, pH 7.0. Protein elution was monitored at 280 nm.

Determination of immunoglobulin class and subclass was accomplished by ELISA with the Screening/Isotyping Kit for Murine Monoclonal Antibodies (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Costar Serocluster EIA microtiter 96-well plates (Costar Corporation, Cambridge, Mass.) were coated with 0.6 μg of *M. hominis* 1620 sonicate (30 μg of protein per well) diluted in a 1:4 (vol/vol) dilution of Dulbecco PBS (DPBS; S&S Media, Inc., Rockville, Md.) for 3 h at room temperature. Wells were blocked with 200 μl of a 0.1% solution of Brij 35 (Sigma Chemical Co., St. Louis, Mo.) for 3 h at room temperature or 4°C overnight. Wash procedures were carried out with 0.1% Brij 35 solution at 200 μl per well. The remaining protocol was performed as recommended by the manufacturer, and the reagents used were those supplied with the kit.

ELISA. Hybridoma culture fluids were screened for antibody activity by ELISA. Microtiter wells were coated with

600 ng (60 μl) of *M. hominis* 1620 whole cells in a 1:4 dilution of DPBS, permitted to incubate for 2 h, and washed three times with DPBS. Hybridoma culture fluid (100 μl) was added to each well and incubated at room temperature overnight. After three washes with DPBS, the wells were then incubated for 2 h with a 1:500 dilution of peroxidase-conjugated, affinity-purified goat antiserum to mouse immunoglobulins (Organon Teknika-Cappel, Malvern, Pa.) and washed. A 0.1% solution of Brij 35 (Sigma) in DPBS was used as the blocking and washing solution and as the diluent for the anti-mouse immunoglobulin. The color reaction was initiated by adding (per well) 100 μl of 2,2-azino-di(3-ethylbenzthiazoline sulfonic acid) diluted in 0.1 M citrate buffer (pH 4.2) containing 0.03% hydrogen peroxide and was measured by using a Dynatech MR650 ELISA reader at 410 nm (Dynatech, Alexandria, Va.).

PAGE and immunoblot analyses. The polypeptide patterns were examined by using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE; 10 to 20% gradient gels or 12% nongradient gels) by the method of Laemmli (28). All lanes of each gel were consistently loaded with 10 μg of protein. Proteins were transferred to nitrocellulose membranes (BA85; Schleicher and Schuell, Keene, N.H.) at 100 mA for 1 h. The nitrocellulose blots were incubated overnight with dilutions of MAbs in 50 mM Tris (pH 6.5)–0.15 M NaCl (TBS) containing 2% powdered milk. Blots were rinsed three times with 50 mM Tris (pH 6.5)–0.15 M NaCl, incubated for 2 h with alkaline phosphatase-conjugated affinity-purified goat antiserum to mouse immunoglobulins, and reacted with substrates from the Protoblot Immunoscreening System according to the manufacturer's recommendations (Promega Corp., Madison, Wis.). After a final rinse in water, the blots were dried and stored until photographed.

Surface proteolysis. A modification of the method reported by Barbour and coworkers was used (3). Sedimented-cell pellets were washed in 0.25 M NaCl and resuspended to a final protein concentration of 1 mg/ml. Trypsin or proteinase K (Boehringer Mannheim) was added to the cells in a final concentration of 100 or 50 μg/ml, respectively, and incubated with occasional mixing for 25 min at room temperature. To terminate trypsin surface proteolysis, 25 μl of a saturated solution of phenylmethylsulfonyl fluoride in isopropanol was added to a final concentration of 100 μg/ml and the cells were sedimented at 12,600 × *g* at 4°C. Proteinase K proteolysis was terminated by the addition of EDTA to a final concentration of 10 mM; the cells were pelleted as described above. Cell pellets were washed once with 0.25 M NaCl containing 50 μg of phenylmethylsulfonyl fluoride, centrifuged at 12,600 × *g*, and stored at −40°C. When 10^7 *M. hominis* cells were treated with the protease concentrations and under the conditions used in this modified procedure and compared with nonproteased controls, survival was not affected, as determined by titration into 10-fold dilutions of media.

Attachment assays. Attachment assays with MRC-5 cell cultures (untransformed human fibroblasts) on glass coverslips were performed as previously described (27) with MAb dilutions of 1/500, 1/2,000, and 1/8,000. Polyclonal rabbit serum was used at 1/100, 1/500, and 1/1,000.

RESULTS

Reactivity of MAbs with *M. hominis* strains 1620, 2101, and PG21. Three MAbs, 3313H3 (MAb-H3; isotype immunoglobulin G2a [IgG2a]), 3349A8 (MAb-A8; isotype IgG1), and 3323G5 (Mab-G5; isotype IgG2a), were prepared against the arthritogenic *M. hominis* 1620. These MAbs were specific for *M. hominis* and had no cross-reactivity with the 11 heterologous *Mycoplasma* species listed in Table 1, as determined by Western analysis.

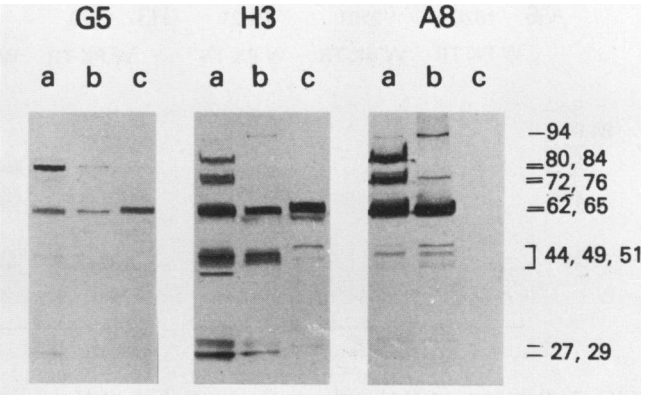

FIG. 1. Immunoblots of MAbs with *M. hominis* strains. Immunoblots of proteins from *M. hominis* strains 1620 (lanes a), 2101 (lanes b), and PG21 (lanes c) reacted with MAb-G5, MAb-H3, and MAb-A8. Numbers indicate molecular mass in kilodaltons.

Immunoblots of proteins from *M. hominis* 1620 and 2101, both of which were isolated from the synovial fluids of the same patient during different periods of exacerbation, and the nonpathogenic reference strain PG21 with the three MAbs are shown in Fig. 1.

MAb-G5 bound to a 62-kDa protein present in all three strains. It also bound to an 80-kDa protein in both arthritogenic strains. MAb-G5 bound weakly to 84-, 76-, 51-, and 27-kDa proteins from strain 1620 and to a 94-kDa protein from strain 2101.

MAb-H3 bound intensely to a 62-kDa protein from all three strains (Fig. 1). Binding by MAb-H3 to several other proteins from *M. hominis* 1620 was also observed; the molecular masses of these proteins were 94, 84, 76, 72, 51, 49, 44, 42, 29, and 27 kDa. The 51-, 49-, and 44-kDa proteins of strain 2101 were likewise bound by MAb-H3, as were weakly reacting bands at 94, 29, and 27 kDa. MAb H3 also bound to 59-, 51-, 46-, and 29-kDa bands from the *M. hominis* type strain PG21.

MAb-A8 bound to a 62-kDa protein from strains 1620 and 2101, as did the other two MAbs (Fig. 1). MAb-A8 also bound avidly to 84- and 72-kDa proteins and weakly, but consistently, to proteins with molecular masses of 94, 51, 49, 44, and 29 kDa from strain 1620. Proteins other than the 62-kDa protein from strain 2101 observed to be bound by MAb-A8 included the 94-, 72-, and 29-kDa proteins, as well as a cluster of proteins with molecular masses ranging from 51 to 44 kDa. No binding of the MAb to proteins from strain PG21 was observed.

Surface proteolysis. In order to determine if the proteins bound by the MAbs were surface proteins, *M. hominis* strains 1620, 2101, and PG21 were subjected to surface proteolysis. As can be seen in Fig. 2, binding to most of the immunoblotted proteins by the three MAbs is significantly reduced or abolished following 25 min of surface proteolysis with either proteinase K or trypsin, regardless of the strain. The 27-kDa protein from strains 1620 and PG21 that was bound by MAb-H3 following proteinase K treatment appears to be a limit digest. The results demonstrate that the MAbs

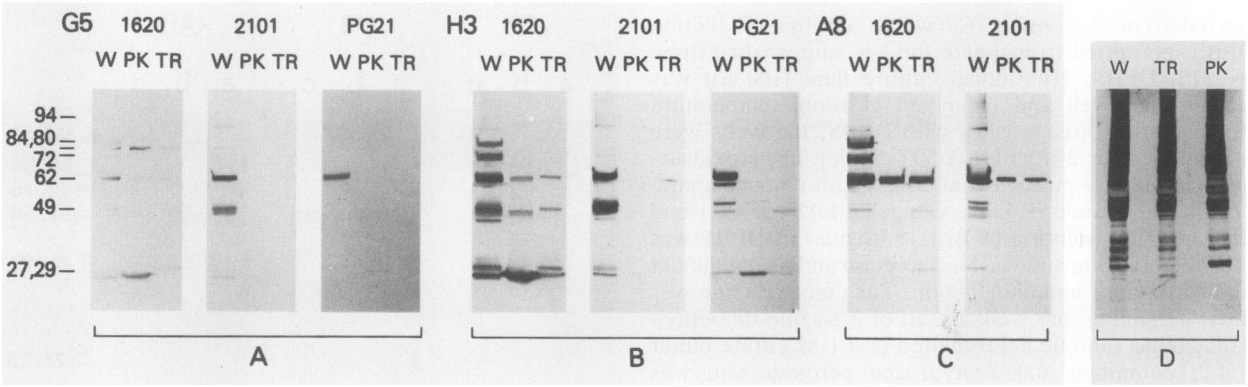

FIG. 2. Reactivity of MAbs after surface proteolysis of *M. hominis* strains 1620, 2101, and PG21. W, Whole-cell protein; PK, proteinase K treatment of whole cells; TR, trypsin treatment of whole cells. Treated and untreated cell proteins were separated by SDS-PAGE, immunoblotted, and reacted with MAb-G5 (A), MAb-H3 (B), MAb-A8 (C), and polyclonal rabbit serum (D). Numbers indicate molecular mass in kilodaltons. The apparent resistance of the 62-kDa protein bound by MAb-A8 to proteolysis is discussed in the text.

are truly binding to *M. hominis* proteins and that these proteins are surface exposed.

The 62-kDa protein bound by MAb-A8 is an exception. One hour of surface proteolysis was required to decrease or abolish binding by MAb-A8 to this protein from strain 1620 (data not shown). Nevertheless, the binding of MAb-A8 to a comigrating band from strain 2101 was somewhat reduced but not abolished even after 3 h of proteolysis by the two proteases.

Triton X-114 partitioning. Triton X-114 partitioning (14) was performed on the three *M. hominis* strains in order to assess the hydrophobicity of MAb-reactive proteins. Following partitioning, the proteins in the various fractions were immunoblotted and reacted with the MAbs. The results are shown in Fig. 3.

All proteins bound by the three MAbs were found in the hydrophobic detergent phase following the extraction procedure, suggesting that the surface-exposed proteins to which the MAbs bind are all integral membrane proteins. Strain PG21 extraction results with MAb-A8 are not presented, since the MAb does not bind to any proteins from this strain.

Palmitate-labeling studies. The animal pathogen *M. hyorhinis* has been shown to possess surface proteins that are acylated (8). To analyze the possibility that the *M. hominis* surface proteins under study are also lipoproteins, strain 1620 was grown with 0.2 mCi of [^{14}C]palmitate (NEN-Dupont, Boston, Mass.) added to each liter of medium. Immunoblots were prepared from SDS-polyacrylamide gels of the labeled cells, reacted with each MAb, and then exposed to X-ray film for 2 days. The results, presented in Fig. 4, show that superimposable bands were both bound by the MAbs and labeled with [^{14}C]palmitate. This suggests that the proteins to which the MAbs bind are acylated. Furthermore, it can also be seen from the autoradiogram that *M. hominis* 1620 appears to possess a "ladder" of potentially acylated low-molecular-weight proteins. The results are reminiscent of the size-variable *M. hyorhinis* surface proteins observed by Rosengarten and Wise (36) and the pattern of immunoblotted *M. pulmonis* surface proteins reported by Watson and coworkers (43).

Immunofluoresence staining activities. Each of the three MAbs produced positive immunofluorescence reactions (4) with agar-grown colonies of arthritogenic strains 1620 and 2101 (data not shown). This supports the contention that the reactive determinants were surface-exposed polypeptides. MAb-H3, but not MAb-A8 or MAb-G5, also produced a weak immunofluorescence reaction with colonies of the nonpathogenic type strain PG21.

Metabolic inhibition activities. MAb-H3 inhibited the metabolism of *M. hominis* 1620 (4). The other two MAbs had no effect on metabolism.

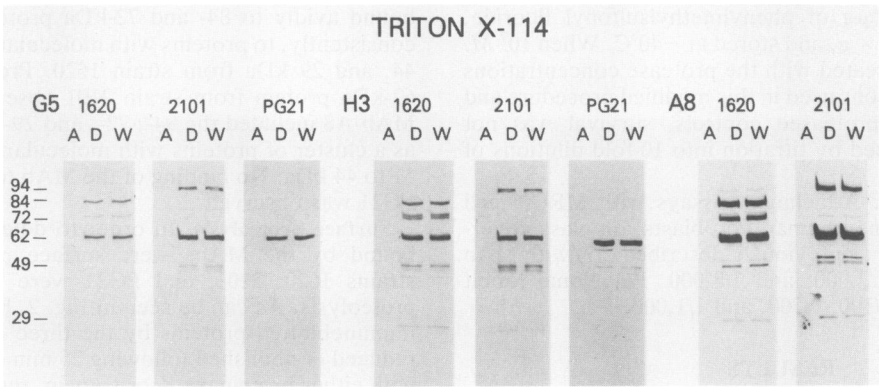

FIG. 3. Fractionation of whole cells of *M. hominis* strains 1620, 2101, and PG21 in Triton X-114. Lanes: A, aqueous phase of Triton X-114 fractionation; D, Triton X-114 detergent phase; W, whole-cell protein. Protein from each preparation was separated by SDS-PAGE and reacted with MAb-G5, MAb-H3, or MAb-A8. Numbers indicate molecular mass in kilodaltons.

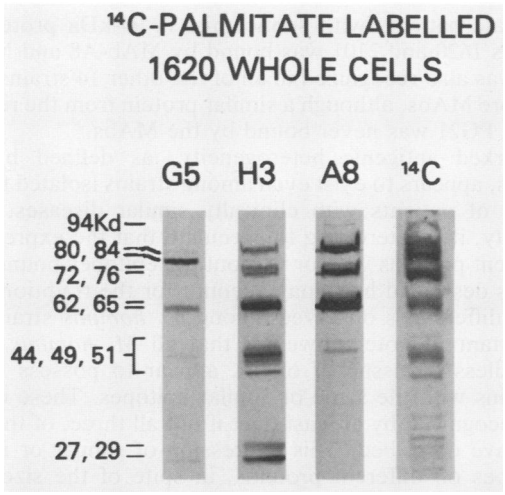

FIG. 4. Autoradiograph and immunoblots of *M. hominis* 1620 grown with [^{14}C]palmitate in medium. After SDS-PAGE and immunoblotting, each blot of proteins from *M. hominis* 1620 grown in [^{14}C]palmitate was exposed to X-ray film for 2 days. Blots of strain 1620 were subsequently reacted with MAbs as indicated. All autoradiographs for the respective MAbs were identical; hence, only one for each MAb is shown. Numbers indicate molecular mass in kilodaltons.

Attachment assay. MAb-H3 and MAb-G5 diluted 1:500 inhibited attachment of *M. hominis* 1620 to MRC-5 untransformed human fibroblast cells up to 40 and 34%, respectively, whereas rabbit hyperimmune polyclonal serum diluted 1:100 inhibited strain 1620 attachment by 71%. Heat-inactivated normal rabbit serum, mouse ascites fluids, and MAbs to nonmycoplasmal proteins, which served as negative controls, failed to inhibit attachment.

Variation in MAb reactivity among *M. hominis* strains. The reactivity of the MAbs with immunoblots of proteins from 14 additional *M. hominis* strains is shown in Fig. 5. The Western analysis clearly demonstrates antigenic variability of *M. hominis*.

MAb-G5 binds to a 94-kDa protein found in all but one of the *M. hominis* strains (Fig. 5, lane 1, strain LBD4) as well as strain 2101 (Fig. 1). The binding of MAb-G5 to the 94-, 84-, 62-, 51-, or 49-kDa proteins varies from strain to strain, with no obvious pattern or relationship to tissue or cell culture of origin. MAb-G5 bound a 27-kDa protein from strain 1620 (Fig. 1) but was not observed to bind similarly migrating low-molecular-weight proteins from these 14 *M. hominis* strains.

MAb-H3 also bound a 94-kDa protein in some of the *M. hominis* strains. However, the epitope on the 94-kDa protein to which MAb-H3 binds only appears in 7 of the 14 strains. In contrast, 13 of these strains possess a protein of similar size which is recognized by MAb-G5.

The other proteins bound by MAb-H3 in the various strains are primarily of 62 and 51 kDa. Furthermore, strain 11932 (Fig. 5, lane 13), isolated from cervical tissue, has bands at 76, 65, 51, and 49 kDa that were bound by MAb-H3.

MAb-A8, like MAb-G5 and MAb-H3, bound to a 94-kDa protein. An epitope recognized by MAb-A8 appears to be on this protein in 12 of the 14 strains, as was the case for strains 1620 and 2101, including the blood isolate in lane 1, strain LBD4 (Fig. 5). It is interesting that the 94-kDa protein of strain LBD4 was not bound by the other two MAbs. MAb-A8 also binds to 62-, 51-, or 49-kDa proteins from different strains. Only the arthritogenic strains 1620 and 2101, however, were observed to have both 51- and 49-kDa proteins bound at the same time. A 90-kDa protein from two blood strains (Fig. 5, strain 11085 in lane 9 and strain 13428 in lane 10) was weakly bound by MAb-A8.

DISCUSSION

Although *M. hominis* is a common inhabitant of the genitourinary tract of healthy men and women (34), there is considerable evidence that this agent is associated with numerous genitourinary and extragenitourinary diseases.

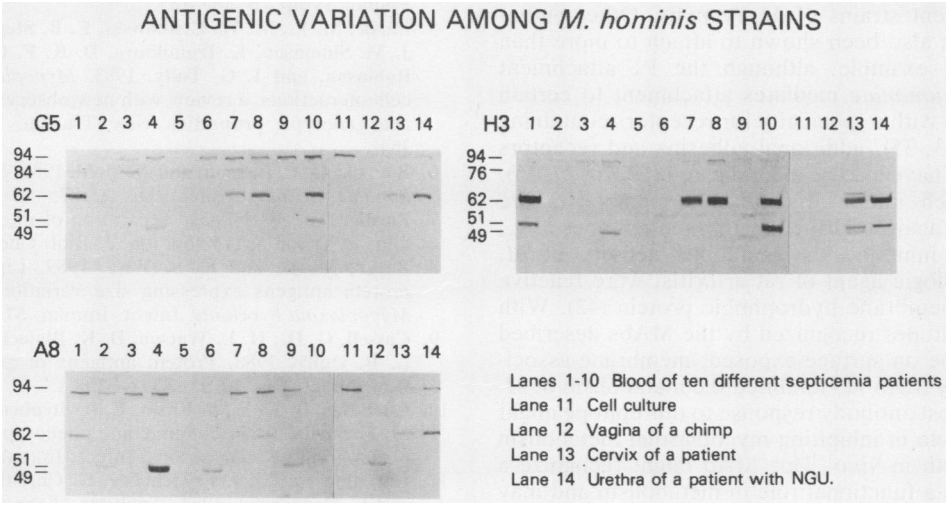

FIG. 5. Reactions of MAbs with 14 additional *M. hominis* strains. Following SDS-PAGE and immunoblotting, each MAb was reacted with the nitrocellulose. Lanes 1 through 10, strains from 10 septisemic patients as follows. Lanes: 1, LBD4; 2, LBD5; 3, R. Taub; 4, 1458; 5, 1611; 6, 4258; 7, 5039; 8, 5488; 9, 11085; 10, 13428. Lane 11, strain 1184, a cell culture contaminant; lane 12, strain 1888, from the vagina of a chimp; lane 13, strain 11032, from the cervix of a patient; lane 14, strain 13408, from the urethra of a patient with nongonococcal urethritis. Numbers indicate molecular mass in kilodaltons.

Pathogenic strains can colonize and infect a number of different tissues, resulting in corresponding urogenital, hematologic, or arthritic disease (5, 6, 15, 40, 41, 44). Since strains of *M. hominis* show considerable antigenic, pathogenic, and genomic variation, we considered the possibility that specific strains are capable of colonizing target tissues with distinct attachment components, thus resulting in different clinical illnesses.

In the studies reported here, three MAbs were raised against whole cells of arthritogenic, cytadsorbing *M. hominis* 1620. All three MAbs bound to surface-exposed, membrane-associated proteins (possibly lipoproteins) from strain 1620 as well as from strains 2101 and PG21. This was demonstrated by immunofluorescent staining of agar colonies, susceptibility to trypsin or proteinase K treatment (Fig. 2), Triton X-114 phase partitioning (Fig. 3), and palmitate labeling of the cells (Fig. 4). These reactive proteins might be associated with the lipid bilayer by means of acylation of the protein portion, resulting in a membrane anchor (7, 8, 13). Further studies to characterize the nature of the acylation are under way.

The resistance of the 62-kDa protein bound by MAb-A8 from strains 1620 and 2101 to surface proteolysis was surprising in light of the proteolytic susceptibility of a similarly migrating band bound by MAb-H3 and MAb-G5 (Fig. 2). It is possible that MAb-A8 binds to epitopes on a protein that comigrates with the band(s) bound by the other two MAbs and that the MAb-A8-reactive band is protease resistant. It should be recalled that MAb-A8 does not bind to any epitope from the reference type strain PG21. If MAb-A8 is binding the same protein from both strain 1620 and strain 2101, some change has occurred in the protein from strain 2101, rendering it protease resistant. Analysis of the reactive proteins is currently being performed.

MAb-H3 and MAb-A8 were each able to partially inhibit attachment of *M. hominis* 1620 to human fibroblasts. Thus, these MAbs may be binding to epitopes associated with or adjacent to those involved in attachment. Since neither MAb-H3 nor MAb-A8 completely inhibited attachment, other epitopes are probably also involved in attachment. In fact, Izumikawa and coworkers (23) showed that different proteolytic enzymes have different effects on the attachment activities of different strains of *M. hominis*. Other pathogenic species have also been shown to attach to more than one receptor; for example, although the P1 attachment protein of *M. pneumoniae* mediates attachment to certain cell substrates (22) with a specificity for receptors containing α2-3 sialic acid (33, 35), additional adhesins and receptors without sialic acid have also been implicated (17, 20, 27, 35).

Antibodies which inhibit mycoplasma metabolism are generally directed against surface membrane epitopes, e.g., antibodies which inhibited the metabolic activity of *M. arthritidis*, an etiologic agent of rat arthritis, were reactive with an integral membrane hydrophobic protein (42). With one exception, epitopes recognized by the MAbs described herein appear to be on surface-exposed, membrane-associated proteins. Only MAb-H3 inhibited the metabolism of *M. hominis* 1620. A host antibody response to this epitope might therefore play a role in inhibiting mycoplasmal metabolism and perhaps growth in vivo. This MAb might recognize a protein(s) that has a functional role in metabolism and may be a useful candidate for future evaluation as an important immunogen.

The three MAbs under investigation also bound to protein bands of various molecular masses from each of 14 different strains of *M. hominis*, including 10 isolated from the blood of separate patients with septicemia. A 94-kDa protein from strains 1620 and 2101 was bound by MAb-A8 and MAb-H3 and was also recognized in all of the other 14 strains by one or more MAbs, although a similar protein from the reference strain PG21 was never bound by the MAbs.

Marked antigenic heterogeneity, as defined by these MAbs, appears to exist even among strains isolated from the blood of patients with clinically similar diseases. Consequently, it is interesting to speculate that the expression of different proteins with or without the epitopes bound by the MAbs described here may account for the traditional serologic differences observed among *M. hominis* strains. It is important to note, however, that all *M. hominis* strains, regardless of tissue of origin, appear to possess multiple proteins with the same or similar epitopes. These epitopes are recognized by at least one, if not all three, of the MAbs we have described. This expression of similar or identical epitopes on different proteins, in spite of the size-variant nature of the proteins expressing the undefined epitope(s), might be an important molecular characteristic of all *M. hominis* that can be exploited in studies of virulence and immunologic protection.

We have generated three MAbs that detect surface-exposed, integral membrane protein epitopes on a cytadsorbing, arthritogenic strain of *M. hominis*. The expression of the epitopes defined by the MAbs on multiple proteins from the same or different strains suggests that the ability of the species to express or suppress the epitopes may reflect a mechanism for generating antigenic diversity.

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