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# Antigenic Mimicry of a Human Cellular Polypeptide by *Mycoplasma hyorhinis*

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**A 46-kilodalton (kDa) polypeptide was immunoprecipitated from radiolabeled extracts of human cell lines infected with *Mycoplasma hyorhinis* by murine monoclonal antibodies PF/2A and ML77. Both of these antibodies also reacted in an enzyme-linked immunosorbent assay (ELISA) with *M. hyorhinis* cells and with human and nonhuman cell lines infected with *M. hyorhinis* but failed to react with A7573 cells infected with any of 10 other species of the order *Mycoplasmatales*. PF/2A also reacted in the ELISA with certain human cell lines that were demonstrated to be free of mycoplasma infection. From extracts of these lines, a polypeptide antigen that appeared as a 24-kDa doublet on polyacrylamide gels was immunoprecipitated by PF/2A. When the PF/2A-reactive human cell lines were infected by *M. hyorhinis*, both the 46- and 24-kDa antigens were immunoprecipitated by PF/2A. ML77 did not react in the ELISA with any noninfected human cells tested and failed to immunoprecipitate a 24-kDa component from any human cells. In Western blotting analyses of extracts of *M. hyorhinis* cells, both PF/2A and ML77 stained a 46-kDa band. PF/2A also stained 24-kDa bands in Western blotting analyses of reactive human cells and *M. hyorhinis* cells, although a 24-kDa component was not precipitated from extracts of *M. hyorhinis* cells by PF/2A.**

The etiologic and pathogenetic implications of cross-reactions between human tissue antigens and microbial antigens have been recognized for several decades. The presentation by an infectious agent of epitopes that are identical or nearly identical to host determinants may break self-tolerance, resulting in the appearance of autoantibodies or autoreactive effector cells. The infectious agent itself need not necessarily persist in the host during the subsequent autoimmune sequelae. Autoantibodies are detectable following many infections in humans; cross-reactions between group A streptococcal antigens and human myocardium elicit autoantibodies that have long been recognized to be associated with acute rheumatic fever (12, 33, 34, 67). Similarly, evidence has acccumulated that coxsackievirus B3 elicits autoreactive antibodies and effector cells that may be largely responsible for the myocarditis associated with coxsackievirus B3 infection (29, 44, 64). *Trypanasoma cruzi* also elicits autoantibodies that may be responsible for the neuropathy and degeneration of the myocardium that characterize Chagas' disease (65). However, antigenic similarities between host and microbial components apparently may also result in specific nonresponsiveness to the microbial antigen. The serogroup-specific capsular polysaccharides from the group A and C meningococci elicit protective responses (26, 46). However, the capsular polysaccharides of the group B meningococci and *Escherichia coli* type K1, which have been demonstrated to cross-react with polysialylated glycopeptides from human brain (21), are poorly immunogenic (26, 31, 66, 68). Tolerance of these antigens may therefore be a factor in the pathogenesis of meningitis caused by these agents. A more complete understanding of the molecular homologies between host constituents and infectious agents is clearly required to fully elucidate the pathogenic mechanisms involved.

Monoclonal antibody technology and protein sequence data bases have facilitated the recognition of the specific epitopes shared between host components and infectious agents suspected of provoking autoimmune responses (25, 39, 65). Thus, a 70-kilodalton (kDa) phosphoprotein of measles virus, a 146-kDa protein of herpes simplex virus type 1, and the hemagglutinin of vaccinia virus all share epitopes with mammalian intermediate filaments (13, 24). Similarly, *E. coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae* share epitopes with the alpha subunit of the nicotinic acetylcholine receptor (54), and the 72-kDa Epstein-Barr virus nuclear antigen shares an epitope with a 62-kDa cellular protein expressed in the synovial membranes of patients with rheumatoid arthritis (22, 42). In some instances, e.g., the common epitope shared by the encephalitogenic site of myelin basic protein and hepatitis B virus polymerase, the homologous amino acid sequences have been defined (23).

This study identifies an epitope shared by a 46-kDa polypeptide of *Mycoplasma hyorhinis* and a 24-kDa human cellular polypeptide. The involvement of mycoplasmas in respiratory and genitourinary diseases has been recognized for many years (53, 56). *M. pneumoniae*, a human respiratory pathogen, is associated with the development of both direct Coombs-positive and cold agglutinin erythrocyte autoantibodies, which may be manifested as hemolytic anemia in patients (17, 18). In addition, a small fraction of the antibodies elicited by *M. pneumoniae* infections may cross-react with human brain, lung, and liver (3, 4). Other mycoplasmas are known to cause naturally occurring arthritic diseases in a wide variety of animal species, including cattle (*M. bovis* and *M. mycoides*) (2, 30, 52, 57), goats and sheep (*M. mycoides* and *M. agalactiae* ) (11, 41), swine (*M. hyorhinis* and *M. hyosynoviae*) (1, 14, 15, 47–50, 55), rats (*M. arthritidis*) (20, 58), and fowl (*M. gallisepticum* and *M. synoviae*) (16, 32, 35, 36, 45). Experimental infections of rodents and rabbits with *M. arthritidis* or *M. pulmonis* progress to chronic phases that resemble rheumatoid arthritis in humans (9, 10, 27, 59), and antigenic cross-reactions between *M. arthritidis* and rodent tissues have been reported (7, 37). In the case of *M. hyorhinis*-infected swine, a chronic inflammation also persists in the joints long after the organisms can be recovered

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(1, 14, 49). Although there is no compelling evidence linking *M. hyorhinis* to human disease states, an epitope shared by a 74-kDa polypeptide of *M. hyorhinis* and mammalian intermediate filaments (63) has previously been described.

## MATERIALS AND METHODS

**Cells and antibodies.** Cell lines derived from tumors were cultured in RPMI 1640 medium (Flow Laboratories, Inc., McLean, Va.) supplemented with 10% fetal calf serum, 2 mM glutamine, Eagle minimal essential medium nonessential amino acids, 1 mM sodium pyruvate, and 25 μg of gentamicin per ml. Hybridomas were cultured either in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 2 mM glutamine, and 25 μg of gentamicin per ml or as ascites in pristane-primed BALB/c mice.

The FaDu (squamous cell carcinoma of pharynx), ME-180 (squamous cell cervical carcinoma), and 5637 (squamous cell carcinoma of bladder) human cell lines were obtained from the American Type Culture Collection, Rockville, Md. The USCLS-1 cell line (human squamous cell lung carcinoma) was provided by June Kan-Mitchell, University of Southern California. The M14 and M21 cell lines (human melanoma) were provided by D. L. Morton, University of California at Los Angeles. The T-222 cell line (human squamous cell lung carcinoma) was provided by H. Masui and F. Rosen, University of California, San Diego. The A7573 cell line (canine thymus) was provided by R. S. Metzgar, Duke University. These lines were confirmed to be noninfected with mycoplasmas by the following assays: the fluorescent DNA-staining method of Chen (8), screening with a tritiated cDNA probe specific for mycoplasma rRNA (Gen-Probe, Inc., San Diego, Calif.), and direct cultivation on solid and liquid media (carried out at Bionique Laboratories, Inc., Saranac Lake, N.Y.).

The following strains were obtained from the American Type Culture Collection: *M. hyorhinis* (23234 [BTS-7], 23839 [GDL], 29052 [DBS 1050]); *M. arginini* (23838); *M. orale* (15539); *M. hominis* type 1 (23114); *M. salivarium* (23064); *Mycoplasma arthritidis* (14152); *M. fermentans* (19989); *M. gallisepticum* (19610); *M. pneumoniae* (29343); *Acholeplasma laidlawii* (29804); and *Ureaplasma urealyticum* (27618). These strains were maintained in cocultures with A7573 cells. The persistence of these organisms in the cocultures was monitored by the fluorescent DNA-staining method of Chen (8).

*A. laidlawii* and *M. hyorhinis* were also produced in liquid cultures by Bionique Laboratories. Cells were cultured in commercial mycoplasma broth (Difco Laboratories, Detroit, Mich) fortified with 20% horse serum, 10% fresh yeast extract, and vitamin supplement X (43) for 3 to 5 days at 33°C under aerobic conditions. Cells were then pelleted at 1,500 × *g*, washed three times in calcium- and magnesium-free phosphate-buffered saline (0.15 M NaCl, 10 mM sodium phosphate [pH 7.2]; PBS) and stored at −70°C until used in enzyme-linked immunosorbent assay (ELISA) and Western blotting experiments.

**Production and isotyping of monoclonal antibodies.** Monoclonal antibody PF/2A was raised against the USCLS-1 cell line, and ML77 was elicited by *M. hyorhinis*-infected M21 cells. Monoclonal antibodies were produced by using standard hybridoma technology (38). BALB/c mice were given six injections of $2 \times 10^6$ cells at weekly intervals. Three days after the final injection, splenocytes were fused with the murine myeloma line P3X63Ag8 and cultured in 96-well plates with $2 \times 10^6$ murine thymocytes per ml. Screening of monoclonal antibodies was carried out by an ELISA as previously described (51). The isotypes of the monoclonal antibodies were determined by an ELISA using diluted affinity-purified rabbit antisera specific for different murine heavy and light chains (Southern Biotechnology Associates, Birmingham, Ala.) dried in 96-well microtiter plates as the targets. Both PF/2A and ML77 are of the immunoglobulin G3 subclass, kappa type.

**ELISA.** The ELISA was carried out as previously described (51) with hybridoma supernatants as the primary antibody source, horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (Bio-Rad Laboratories, Richmond, Calif.) as the secondary antibody, and *o*-phenylenediamine–$H_2O_2$ as the substrate. Approximately $2 \times 10^4$ cells per well were cultured overnight in sterile 96-well microtiter plates, washed, air dried, and tested in situ. Alternatively, $5 \times 10^4$ cultured human cells per well were suspended in 50 μl of PBS, air-dried in 96-well vinyl microtiter plates, and used as the target antigen. For ELISA testing of mycoplasmas, 2.5 ng (wet weight) of washed mycoplasma cells per well was suspended in 50 μl of PBS, air dried in 96-well vinyl microtiter plates, and used as the target antigen.

**Radiolabeling.** Cell lines, approximately 50% confluent in 75-$cm^2$ flasks (roughly $2.5 \times 10^6$ to $1.0 \times 10^7$ cells), were washed twice with PBS and incubated for 12 h in 20 ml of leucine-free RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, 25 μg of gentamicin per ml, and 2 mCi of L-[$^3$H]leucine (121 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). The labeled cultures were then extracted with the nonionic detergent Renex-30 (Accurate Chemical, Westbury, N.Y.). as previously described (19). Briefly, labeled cells were washed twice with cold PBS and extracted for 20 min on ice in 0.15 M NaCl containing 50 mM Tris (pH 8.5), 0.02% sodium azide, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, and 2% Renex-30. Following centrifugations at 15,000 × *g* for 20 min at 4°C and at 100,000 × *g* for 1 h at 4°C, the radiolabeled supernatant extracts were stored at 70°C until further use. Generally, $2.5 \times 10^8$ to $1.0 \times 10^9$ cpm, representing approximately 10.9 to 43.6% of the input label, was incorporated into the Renex-30-soluble, trichloracetic acid-precipitable fraction.

**Indirect immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.** Aliquots (1 ml) of hybridoma supernatants were incubated for 1 h at 4°C with 100 μl of 1 M Tris (pH 8.5) and 100 μl of a 10% suspension of protein A-Sepharose conjugate (Pharmacia, Uppsala, Sweden) in buffer containing 0.15 M NaCl, 10 mM Tris (pH 8.5) 0.5 mM EDTA, 0.25% Renex-30, 0.02% sodium azide, and 1 mg of ovalbumin per ml (IP buffer). Following two washes with IP buffer, the antibody-coated protein A-Sepharose was incubated for one additional hour at 4°C with 0.5 ml of IP buffer and $10^7$ cpm of radiolabeled cell extract (representing approximately 1 to 4% of the total incorporated counts). The Sepharose beads were then washed with IP buffer until the counts per minute eluted in the supernatant buffer were low and stable and were then washed twice with IP buffer without ovalbumin. Bound antigens were eluted and analyzed as described by Laemmli (40) and visualized by fluorography as described by Bonner and Laskey (5).

**Western blotting analysis.** Aliquots of washed, pelleted USCLS-1 cells, *M. hyorhinis* cells, and *A. laidlawii* cells were extracted for 20 min on ice in buffer containing 2% Renex-30, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 0.02% sodium azide, and 50 mM Tris (pH 8.5) and were centrifuged at 15,000 × *g* for 20 min at 4°C and at 100,000 × *g* for 60 min at 4°C. The $A_{280}$ was determined, and samples

TABLE 1. ELISA reactivity of mycoplasmas and cell lines

| Target | Reactivity with: | |
|---|---|---|
| | PF/2A | ML77 |
| *M. hyorhinis*[b] | + + + | + + + |
| *A. laidlawii*[b] | − | − |
| Noninfected cell lines | | |
| T-222 | + | − |
| 5637 | + | − |
| FaDu | + | − |
| ME-180 | + | − |
| USCLS-1 | + | − |
| M14 | − | − |
| A7573 | − | − |
| Infected cell lines (cell line-agent)[c] | | |
| USCLS-1-*M. hyorhinis* | + + + | + + + |
| M14-*M. hyorhinis* | + + + | + + + |
| A7573-*M. hyorhinis* | + + + | + + + |
| A7573-*M. arginini* | − | − |
| A7573-*M. arthritidis* | − | − |
| A7573-*M. fermentans* | − | − |
| A7573-*M. gallisepticum* | − | − |
| A7573-*M. hominis* | − | − |
| A7573-*M. orale* | − | − |
| A7573-*M. pneumoniae* | − | − |
| A7573-*M. salivarium* | − | − |
| A7573-*A. laidlawii* | − | − |
| A7573-*U. urealyticum* | − | − |

[a] + + +, $A_{490} \geq 1.0$; −, $A_{490} \leq 0.1$; +, $0.3 \leq A_{490} \leq 0.5$.
[b] Produced in broth cultures. See Materials and Methods.
[c] The infected status of cell lines was confirmed by the fluorescent DNA-staining method of Chen (8). See Materials and Methods.

containing 25 μg of total protein were separated on polyacrylamide gels by the method of Laemmli (40). The antigens were then transferred to nitrocellulose paper overnight at 20 V in buffer containing 20% methanol, 25 mM Tris, and 200 mM glycine (pH 8.5). The nitrocellulose paper was then blocked with buffer containing 0.5 M NaCl, 10 mM Tris, (pH 7.4), 0.1% thimerosal, 3% bovine serum albumin, and 10% normal goat serum for 1 h at 4°C. The nitrocellulose paper was next incubated at 4°C for 2.5 h with hybridoma culture supernatants diluted 1:5 in buffer D (0.15 M NaCl, 10 mM sodium phosphate [pH 7.1], 0.1% bovine serum albumin, 0.2% Tween 20, 0.01% thimerosal), washed five times over a 0.5-h period with buffer W (0.15 M NaCl, 10 mM sodium phosphate [pH 7.1], 0.5% Tween 20, 0.1% ovalbumin), incubated for 2 h at 4°C with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (Bio-Rad) diluted 1:1,000 in buffer D, and again washed five times over a 0.5-h period with buffer W. Bands were developed in 25 μg of 3,3′-dimethoxybenzidine dihydrochloride per ml–0.01% $H_2O_2$–10 mM Tris (pH 7.4), and the reaction was terminated by rinsing the nitrocellulose paper in distilled $H_2O$.

## RESULTS

**ELISA reactivity of mycoplasmas and cell lines.** Table 1 shows the expression of the PF/2A and ML77 epitopes by mycoplasma cells and by a panel of noninfected and mycoplasma-infected cell lines, as determined by ELISA reactivity. *M. hyorhinis* cells expressed the epitope detected by PF/2A and ML77, although *A. laidlawii* cells were nonreactive. The cell lines that were infected with *M. hyorhinis* were also reactive with PF/2A and ML77. The PF/2A and ML77 epitopes were not detectable in the noninfected canine thymus line A7573, in the noninfected human melanoma line M14, or in A7573 cells that were infected with any of eight other mycoplasma species, *A. laidlawii*, or *U. urealyticum* and that subsequently tested positive for mycoplasmas by the fluorescent DNA-staining method of Chen (8). Since the A7573 cells and mycoplasmas were cultured in 96-well plates, washed once, airdried, and tested in situ, it seems unlikely that the lack of reactivity with PF/2A and ML77 was due to a loss of the mycoplasmas during the course of the ELISA. However, five human cell lines (USCLS-1, T-222, ME-180, 5637, and FaDu) that all tested negative for mycoplasmas in three different assays expressed the PF/2A epitope but were nonreactive with the ML77 antibody.

The ML77 epitope was not detected in any of a total of 20 noninfected human cell lines tested (data not shown).

**Immunochemical characterization of antigens.** Figure 1 shows the results of indirect immunoprecipitations of [$^3$H]leucine-labeled extracts of USCLS-1 cells and M14 cells by monoclonal antibodies PF/2A and ML77. A doublet band of approximately 24 kDa was precipitated from extracts of noninfected USCLS-1 cells by PF/2A (lane B). No detectable bands were precipitated by PF/2A from noninfected M14 cells (lane H) or by ML77 from extracts of either noninfected cell line (lanes C and I). However, a 46-kDa band was precipitated by both PF/2A and ML77 from extracts of *M. hyorhinis*-infected USCLS-1 cells (lanes E and F) and M14 cells (lanes K and L). In the case of *M. hyorhinis*-infected USCLS-1 cells (lane E), both the 46-kDa band and the 24-kDa doublet were precipitated by PF/2A. Neither the 24-kDa band (19) nor the 46-kDa band (data not shown) were labeled with [$^3$H] glucosamine, suggesting that neither were glycosylated.

**Western blotting analyses.** To rule out the possibility that the 46-kDa band was produced by the human tumor cells in response to the presence of *M. hyorhinis* we found it necessary to demonstrate the 46-kDa antigen in extracts of *M. hyorhinis* cultured in the absence of mammalian cells. Mycoplasmas were cultured in fortified commercial myco-

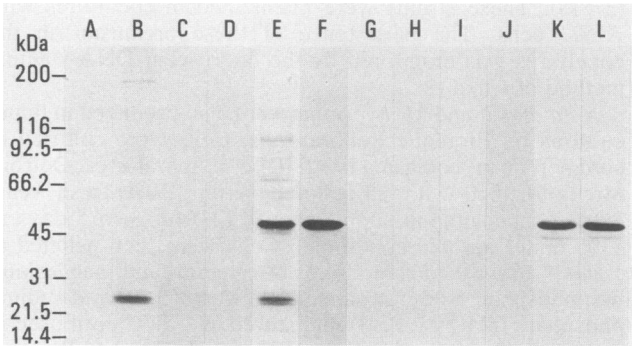


FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of immunoprecipitates obtained by reacting P3X63Ag8 myeloma protein (negative control), monoclonal antibody PF/2A, and monoclonal antibody ML77 with nonionic detergent extracts of [$^3$H]leucine-labeled noninfected and *M. hyorhinis*-infected human cells. Lanes: A, B, and C, noninfected USCLS-1 cells; D, E, and F, *M. hyorhinis*-infected USCLS-1 cells; G, H, and I, noninfected M14 cells; J, K, and L, *M. hyorhinis*-infected M14 cells; A, D, G, and J, P3X63Ag8 myeloma protein (negative control); B, E, H, and K, monoclonal antibody PF/2A; C, F, I, and L, monoclonal antibody ML77.

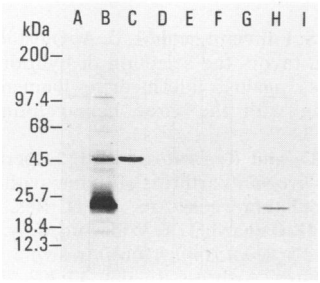


FIG. 2. Western blotting analysis of nonionic detergent extracts of *M. hyorhinis* cells, *A. laidlawii* cells, and USCLS-1 cells. Staining was carried out with P3X63Ag8 myeloma protein (negative control), monoclonal antibody PF/2A, and monoclonal antibody ML77. Lanes: A, B. and C, *M. hyorhinis* cells; D, E, and F, *A. laidlawii* cells; G, H, and I, USCLS-1 cells; A, D, and G, P3X63Ag8 myeloma protein (negative control); B, E, and H, monoclonal antibody PF/2A; C, F, and I, monoclonal antibody ML77.

plasma broth, washed, pelleted, and subjected to Western blotting analysis. Figure 2 shows the results of Western blotting analyses comparing nonionic detergent extracts of washed, pelleted *M. hyorhinis* and *A. laidlawii* cells cultured in the absence of mammalian cells and a nonionic detergent extract of USCLS-1 cells. A 46-kDa band was stained by both PF/2A (lane B) and ML77 (lane C) in the extract of *M. hyorhinis* cells. There was no detectable staining of the extract of the nonreactive *A. laidlawii* cells by either antibody (lanes E and F). In addition, PF/2A stained a 24-kDa band in the extract of noninfected USCLS-1 cells (lane H) and an intense 24-kDa band in the extract of *M. hyorhinis* cells (lane B). In view of this staining of a 24-kDa component in the *M. hyorhinis* cell extract by PF/2A, it is unclear why a 24-kDa component was not immunoprecipitated by PF/2A from extracts of *M. hyorhinis*-infected M14 cells. Possibly, the PF/2A epitope is masked in the 24-kDa antigen from *M. hyorhinis* cells but is exposed following separation, denaturation, or unfolding on the nitrocellulose membrane. Alternatively, the 24-kDa component may not be expressed by *M. hyorhinis* cells cultured in the presence of mammalian cells. It is of interest that only a single band of 24 kDa rather than a doublet was stained in the USCLS-1 cell extract by PF/2A (lane H), suggesting that only one of the doublet components bears the epitope. ML77 failed to stain the USCLS-1 cell extract (lane I) and did not stain a 24-kDa band in the *M. hyorhinis* cell extract (lane C).

## DISCUSSION

The principle observation of this investigation is the demonstration, by use of monoclonal antibody PF/2A, of an epitope shared by a 46-kDa polypeptide of *M. hyorhinis* and a 24-kDa polypeptide that is a constituent of noninfected human cells. The PF/2A epitope is widely expressed in malignant human cells and tissues of various types but exhibits a highly restricted distribution in normal tissues (19). Thus, monoclonal antibody PF/2A, produced against a human squamous cell lung carcinoma-derived line, reacts in indirect immunoperoxidase assays with a variety of fresh-frozen human tumor specimens, including most squamous cell and adenosquamous lung carcinomas, breast carcinomas, colon carcinomas, and gastric carcinomas tested and at least some adenocarcinomas and large cell carcinomas of lung, squamous cell head and neck tumors, cervical carcinomas, renal carcinomas, melanomas, and gliomas (19). PF/2A is nonreactive in indirect immunoperoxidase assays with fresh-frozen samples of normal adult liver, lung, kidney, stomach, colon, pancreas, spleen, skin, adrenal gland, thyroid, and prostate and fetal liver, lung, kidney, colon, intestine, spleen, and skin, but is weakly reactive with Purkinje cells in normal cerebellum (19). The ELISA reactivity of PF/2A with cell lines that have tested negative for mycoplasma contamination in three different assays and the immunoperoxidase reactivity of PF/2A with freshly frozen human tumors and Purkinje cells strongly suggest that the 24-kDa antigen immunoprecipitated from human tumor cells is not mycoplasma associated. The acquisition of ELISA reactivity with PF/2A and ML77 by otherwise nonreactive cells infected by *M. hyorhinis*, the immunoprecipitation of the 46-kDa band by these antibodies only from detergent extracts containing *M. hyorhinis* cells, and the immunoblotting of a 46-kDa band from extracts of washed, pelleted *M. hyorhinis* cells establish the association of the 46-kDa band with *M. hyorhinis*.

*M. hyorhinis* has been demonstrated to acquire Thy-1 and H-2K antigens by translocation during cocultivation with murine T lymphoblastoid cells (6, 60, 61). However, it seems very unlikely that the reactivity of PF/2A and ML77 with *M. hyorhinis* is due to the presence of human antigens acquired during cocultivation on *M. hyorhinis* cells, since M14 cells and A7573 cells are nonreactive with these antibodies until infection with *M. hyorhinis* and since *M. hyorhinis* cells produced in fortified commercial mycoplasma broth are reactive with these antibodies in the ELISA and Western blotting analyses. Furthermore, the apparent molecular mass of the antigen immunoprecipitated from nonreactive human cells infected with *M. hyorhinis* (46 kDa) is distinct from that immunoprecipitated from reactive human cells (24 kDa). Similarly, it appears unlikely that PF/2A and ML77 are reacting with medium components adsorbed to *M. hyorhinis* cells, since no reactivity was detectable when either antibody was used in indirect or competitive immunoassays of the fortified commercial mycoplasma broth components and the mammalian cell culture medium components used in these experiments (data not shown).

A 24-kDa component of *M. hyorhinis* extracts is stained by PF/2A in Western blotting analyses, although this component apparently is not immunprecipated by PF/2A from extracts of *M. hyorhinis*-infected nonreactive human cells. The degree of homology between the 24-kDa component precipitated by PF/2A from human cells and the 24-kDa component stained by PF/2A in extracts of *M. hyorhinis* cells is unclear. The failure of the *M. hyorhinis*-derived 24-kDa component to immunoprecipitate suggests that the two components are nonidentical, however. Wise and Watson (62) have produced a panel of monoclonal antibodies directed against *M. hyorhinis*; one of these antibodies reacts with a surface constituent of *M. hyorhinis* and stains a 23-kDa band in protein blots of *M. hyorhinis*. This antibody has also been demonstrated to stain a second band of approximately 17 kDa and is reactive with *M. hyorhinis* GDL but not with *M. hyorhinis* BTS-7. PF/2A has failed to strongly stain any components of approximately 17 kDa in Western blots of *M. hyorhinis* and has shown approximately equivalent reactivity with three strains of *M. hyorhinis*, GDL, BTS-7, and DBS 1050 (data not shown). If the 24-kDa antigen stained by PF/2A is identical to the 23-kDa antigen reported by Wise and Watson, PF/2A most likely recognizes a different epitope, one that is not strain specific or shared by a 17-kDa component.

ML77 has failed to react in an ELISA with any of a panel of over 20 noninfected human cell lines and has failed to react in immunoperoxidase assays with any of a large panel of fresh-frozen normal and malignant human tissues (unpublished data). If ML77 precipitates the same 46-kDa component as PF/2A does, it must also recognize a distinct epitope, one not shared by the 24-kDa polypeptide.

In humans, the expression of the epitope recognized by PF/2A is highly restricted to tumors, and this appears to be the first report of antigenic mimicry involving a microbial epitope and a non-blood group, tumor-associated epitope. However, if the PF/2A epitope is more widely distributed in tissues of other species, it could perhaps play a role in the pathogenesis of mycoplasma-associated arthritic or autoimmune diseases in animals. It is perhaps worth noting that a form of paraneoplastic cerebellar degeneration involving generalized loss of Purkinje cells from the cerebellar cortex has been associated with several types of human carcinomas, particularly lung, but also including tumors of breast and colon (28). Since PF/2A defines an epitope also shared between these tumors and Purkinje cells, it is possible that this epitope may play a role in the pathogenesis of this paraneoplastic cerebellar degeneration.

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