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Antigenic Mimicry of Mammalian Intermediate Filaments by Mycoplasmas

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A murine monoclonal antibody raised against *Mycoplasma hyorhinis* specifically reacted by indirect immunofluorescence with mammalian intermediate filaments. The antibody recognized a related epitope on a 74,000-molecular-weight protein of *M. hyorhinis* and on components of similar size from other pathogenic mycoplasmas. This defines a shared antigenic structure of interest in autoantibody development during mycoplasmal diseases.

Autoantibodies to mammalian cytoskeletal components occur at low levels in the sera of normal animals (19) and humans (10, 17) but can be markedly elevated during certain mycoplasmal (2) and viral (11, 18, 20, 22, 23, 31) infections. Autoantibodies reacting with intermediate filaments (IF) (21, 27) make up a major component of these responses. Although mechanisms by which microbial infections generally influence the appearance of these autoantibodies are not clear, recent demonstrations (8, 14) of specific epitopes shared by viral constituents and components of IF raise the intriguing possibility that mimicry of host antigens contributes to anti-IF antibody induction during viral infections. Comparatively little is known, however, regarding antigenic structures of mycoplasmas or other procaryotes that could play an analogous role by antigenically resembling host cytoskeletal components. An existing library of monoclonal antibodies (36, 37) raised against free-living *Mycoplasma hyorhinis* was screened by immunofluorescence on mammalian fibroblasts. One monoclonal antibody (F147C18) from this library that reacts with IF is identified in this report. The antibody also defines an epitope on a protein of *M. hyorhinis* that is expressed by other human and animal mycoplasmas. These results provide direct evidence for antigenic mimicry of IF by an important group of procaryotic pathogens and suggest one mechanism by which autoantibodies to IF are induced during mycoplasmal infections.

A library of approximately 60 monoclonal antibodies reacting with *M. hyorhinis* was constructed by using lymphoid cells from BALB/c mice immunized with organisms isolated from broth cultures of free-living mycoplasmas. Monoclonal antibodies reacting with *M. hyorhinis* were initially identified by an enzyme-linked immunosorbent assay employing immobilized, broth-grown organisms. The antibodies were utilized in the present study as culture fluids from multiply cloned, stable hybridoma cell lines (see reference 36 for all aspects of hybridoma construction and antibody screening).

To identify possible epitopes shared by mycoplasmas and normal mammalian cellular constituents, we analyzed antibodies of the library by indirect immunofluorescence for their ability to stain fixed baby hamster kidney (BHK21) fibroblasts, rigorously shown to be free of mycoplasmal contaminants by criteria described elsewhere (35). All antibodies of the library failed to react with fibroblasts except one immunoglobulin G1 (IgG1) κ antibody, F147C18 (Fig. 1). This antibody stained a filamentous network (Fig. 1B) that was in striking contrast to the low background staining of other antibodies of the library (Fig. 1A). Treatment of fibroblasts with colchicine before fixation resulted in staining of condensed, juxtanuclear coils characteristic of IF (21, 27) (Fig. 1C). The antibody gave analogous staining patterns with both embryonic mouse fibroblasts and PtK2 (27) marsupial epithelial cells. These results suggested that antibody F147C18 recognizes a similar epitope expressed by both *M. hyorhinis* and mammalian IF. This was further assessed by absorbing the antibody with broth-grown *M. hyorhinis*, which resulted in complete abrogation of fluorescent staining (Fig. 1D and E). To ensure that absorption was specific, we made an attempt to absorb another monoclonal antibody to IF (F134C34). This antibody was obtained from a separate library of monoclonal autoantibodies derived from autoimmune MRL/Mp mice (K. S. Wise, V. Lucaites, and R. K. Watson, Fed. Proc. **42**:1212, 1983). Immunofluorescent staining of IF by this unrelated antibody was not affected by absorption with *M. hyorhinis* (Fig. 1F and G), confirming that F147C18 specifically recognized a determinant on IF shared by *M. hyorhinis* and that the epitope recognized on IF by this antibody was distinct from that recognized by the unrelated monoclonal autoantibody F134C34.

An *M. hyorhinis* constituent recognized by antibody F147C18 was identified by immunologically staining protein blots of *M. hyorhinis* components separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 2). Although hyperimmune serum to *M. hyorhinis* specifically stained numerous components on protein blots of the organisms (Fig. 2A), the monoclonal antibody stained a prominent component migrating with an apparent molecular weight of 74,000 (Fig. 2B, lane 1). Inclusion of the appropriate serum in all steps of immunoblotting as previously described (36) ensured that F147C18 was not reacting with a serum component. Supplementation of this incubation medium with 20% (vol/vol) yeast extract (36) also had no effect on the binding of the antibody to the 74,000-molecular-weight component, nor could direct protein blots of serum or yeast extract be stained with F147C18. This ruled out the possibility that F147C18 was directed to components of the medium used to grow the organisms. Sixty other monoclonal antibodies against *M. hyorhinis* either failed to stain mycoplasma protein blots or stained discrete components of different sizes (36, 37). To determine the nature of the 74,000-molecular-weight component recognized by F147C18,

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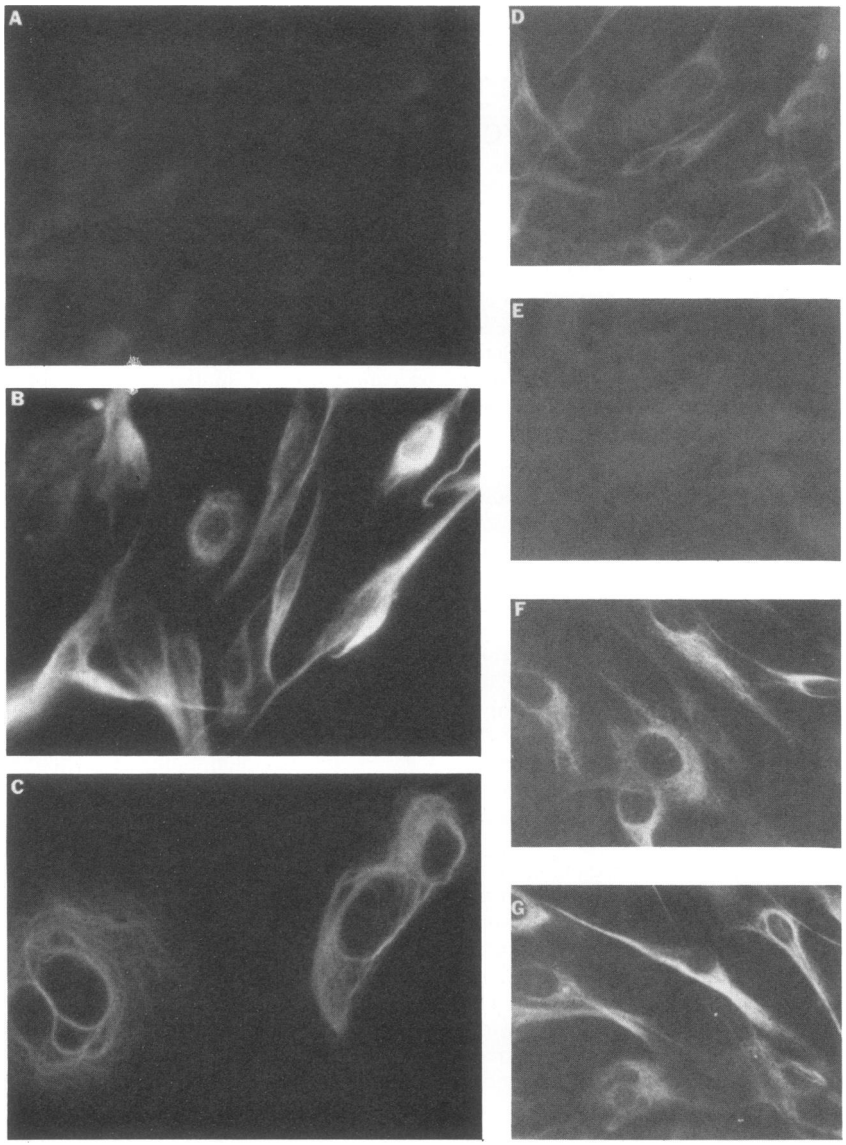

FIG. 1. Indirect immunofluorescent staining of mammalian fibroblasts with monoclonal antibodies. Mycoplasma-free BHK21 fibroblasts were grown in minimal essential medium supplemented with 10% fetal calf serum (FCS) and fixed with 4% formaldehyde and cold methanol. Cells were incubated (30 min, 4°C) with hybridoma culture fluids (RPMI 1640 with 20% FCS) containing approximately 1 μg of monoclonal antibody ml^{-1}, followed by fluorescein-conjugated goat antibody to mouse immunoglobulin diluted 1:200 in FCS-supplemented RPMI 1640 (36). (A) Cells stained with control (IgG1 κ) monoclonal antibody (F22C32D) previously shown to recognize an unrelated protein component (p73) of *M. hyorhinis* (36). (B and C) Cells grown in the absence (B) or presence (C) of colchicine (48 h, 20 μg ml^{-1}) and stained with monoclonal antibody F147C18. (D and E) Cells stained with F147C18 before (D) or after (E) absorption of monoclonal antibody with *M. hyorhinis* (performed by incubating hybridoma culture fluid for 18 h at 4°C with 0.8 mg of freeze-thaw-disrupted, broth-grown mycoplasma ml^{-1}, followed by centrifugation at 10,000 × *g* for 10 min to remove absorbing material). (F and G) Cells stained with unrelated F134C34 monoclonal autoantibody to IF (Wise et al., Fed. Proc. **42**:1212, 1983) before (F) or after (G) identical absorption with *M. hyorhinis*.

detergent-disrupted organisms were treated with increasing concentrations of trypsin and applied to sodium dodecyl sulfate-polyacrylamide gels, and subsequent protein blots were probed with this antibody. Disappearance of the stained component with trypsin treatment indicated that either the epitope or the molecule on which it resided was made up at least in part of protein (Fig. 2B, lanes 2 and 3). Since degradation of a protein without destruction of an epitope could have yielded the observed results, the precise nature of the antigenic structure recognized could not be determined by this experiment. Nevertheless, the protein p74 was identified as a component of *M. hyorhinis* bearing the antigenic structure shared by mammalian IF.

Preliminary screening of additional mycoplasma species with F147C18 in an enzyme-linked immunosorbent assay (36) indicated the presence of a corresponding epitope in organisms other than *M. hyorhinis* (data not shown). This was further analyzed by immunological staining of protein blots to determine the distribution and size characteristics of components bearing related epitopes in five mycoplasma species (*M. hyorhinis*, *M. pulmonis*, *M. fermentans*, *M. hyopneumoniae*, and *M. pneumoniae*). Although complex

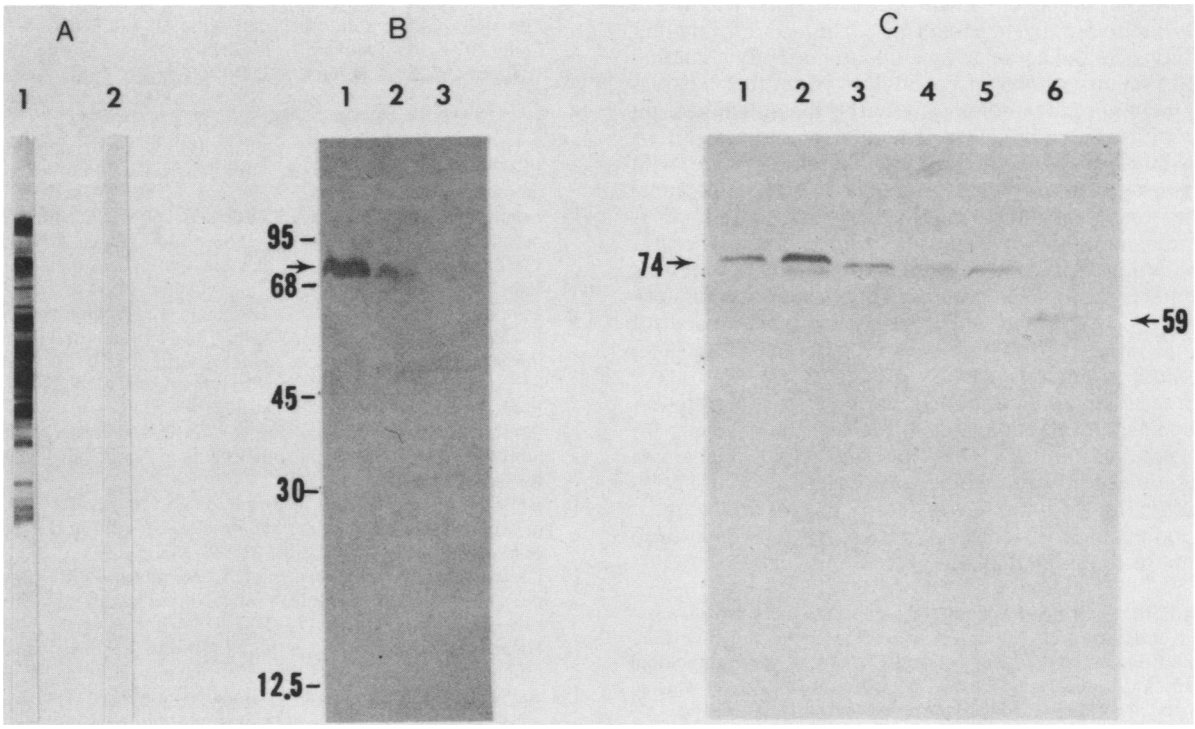

FIG. 2. Immunostaining of mycoplasma protein blots. Broth-grown mycoplasmas were applied (50 μg per lane) to 9 to 14% linear gradient sodium dodecyl sulfate-polyacrylamide gels containing 0.5 M urea and electrophoresed under reducing conditions as described previously (36). Separated components were electrophoretically transferred to nitrocellulose paper, and blots were stained by sequential incubations with mouse antibody, followed by peroxidase-conjugated goat antibody to mouse immunoglobulin as described previously (36). (A) Blots of *M. hyorhinis* stained with hyperimmune mouse serum to *M. hyorhinis* (lane 1) or preimmune serum (lane 2). (B) Blot of *M. hyorhinis* stained with monoclonal antibody F147C18 after treatment (37°C, 30 min) of detergent-disrupted (0.2% sodium dodecyl sulfate) organisms before electrophoresis with trypsin concentrations of 0 μg ml^{-1} (lane 1), 1.6 μg ml^{-1} (lane 2), 8 μg ml^{-1} (lane 3). Arrow, Location of 74,000-molecular-weight component. Numbers show molecular weight ($\times 10^3$). (C) Blot of different mycoplasma species stained with monoclonal antibody F147C18. Lanes: 1, *M. hyorhinis* GDL (36); 2, *M. hyorhinis* BTS-7 (National Institutes of Health Research Resources Branch [RRB] M-718-002-084); 3, *M. pulmonis* Ash (RRB M-717-002-084); 4, *M. fermentans* PG18 (RRB M-713-002-084); 5, *M. hyopneumoniae* J (ATCC 25934); 6, *M. pneumoniae* PI1428 (32). Mycoplasmas used for protein blots were harvested from FCS-supplemented broth medium as described previously (36), except for *M. hyopneumoniae* which was grown in FF medium supplemented with swine serum (12) and *M. pneumoniae* which was obtained from FCS-supplemented G1994 medium (32) (and provided by M. Gabridge). Primary and secondary antibodies were incubated with blots in the presence of 20% FCS as described previously (36). Numbers mark the 74,000- and 59,000-molecular-weight components.

protein staining patterns of the five species were highly distinctive (not shown), staining of their respective protein blots with F147C18 (Fig. 2C) identified a major component migrating approximately the same distance as p74 in all species examined except *M. pneumoniae*, which demonstrated a component with a molecular weight of 59,000 reacting with this antibody. These results indicate that an antigenic structure defined by antibody F147C18 and present on mammalian IF is also expressed and conserved in a variety of mycoplasma species (including all of those tested). It remains to be determined whether the epitope recognized reflects conservation of related protein structures among these mycoplasma species and whether other epitopes common among IF components (28) are shared by the mycoplasma components identified by this antibody. Proteins with properties resembling eucaryotic cytoskeletal elements have been described in some mycoplasmas with gliding motility (16, 26, 34). However, evidence of a functional mycoplasmal cytoskeletal system, especially one involving components resembling eucaryotic IF, is notably lacking (24). The antibody reported here may be useful in probing the structural relatedness of mycoplasma components antigenically similar to mammalian IF, as well as the distribution and expression of such components in other members of the class *Mollicutes* (13) and in putative eubacterial progenitors of these small wall-less procaryotes (25, 38).

This study defines an antigenic structure shared by mammalian IF and a group of diverse procaryotic microbes involved in a number of chronic, infectious diseases. This may provide a means of systematically assessing the role of these specific microbial components in inducing the autoantibodies often found in association with mycoplasmal diseases such as atypical pneumoniae caused by *M. pneumoniae* (2) or in a variety of other mycoplasmal infections (9). Although most mycoplasmas in this study are pathogens causing chronic respiratory or rheumatic diseases in rodents (*M. pulmonis* [5]), swine (*M. hyopneumoniae* [33] and *M. hyorhinis* [29]), or humans (*M. pneumoniae* [6]), perhaps of equal importance is the expression of this antigen in organisms (represented by *M. fermentans*) making up apparently normal mycoplasma flora (3). The presence of nonpathogenic, commensal mycoplasmas could provide a stimulus for autoantibody production that profoundly affects the expres-

sion of antibodies associated with specific conditions thought to involve autoimmune mechanisms. There is an inherent uncertainty as to the actual source of autoantibody induction in animal systems whose microbial flora is not fully characterized (including mice commonly harboring subclinical infections with *M. pulmonis* or a number of viruses [5]). A recent study (8) showing that inoculation of mice with vaccinia virus resulted in the isolation of IgM monoclonal antibodies reacting with both IF and specific viral components underscores the possibility that microbial mimicry of host components is a widespread phenomenon requiring careful interpretation. (Neither the IgG1 κ monoclonal antibody we report here nor sera from mice routinely used for these studies reacted with vaccinia virus in a sensitive enzyme-linked immunosorbent assay.)

The precise stimulus for anti-IF antibody production during mycoplasmal infections remains to be determined. Although antigenic mimicry offers one attractive mechanism by which this could occur, other processes, including polyclonal activation of lymphocytes (1, 7, 15, 30) and acquisition or modification of host cell antigens (4, 35), may also contribute to the phenomenon.

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