Monoclonal Antibodies to *Mycoplasma hyorhinis* Surface Antigens: Tools for Analyzing Mycoplasma-Lymphoid Cell Interactions

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A library has been constructed of approximately 50 monoclonal antibodies that recognize antigens of *Mycoplasma hyorhinis*. Characteristics of six antibodies are discussed. Each reacts with a discrete determinant borne on a protein-containing molecule of distinct molecular size. Three of these respective antigens are expressed at the surface of mycoplasmas colonizing lymphoblastoid cells in culture. Of these three surface antigens, two are selectively expressed on strain GDL but not on strain BTS-7 of *M. hyorhinis*, thus defining strain-restricted immunological specificities within these species. One monoclonal antibody of the IgM class (μ,κ) mediated marked complement-dependent growth inhibition of *M. hyorhinis* in broth culture. These monoclonal reagents should facilitate analysis of mycoplasma surface architecture, and the molecular interactions of these organisms with the host cell surface.

INTRODUCTION

Surface constituents of mycoplasmas are centrally important in the immunopathology of mycoplasmal disease. As potential targets of immunopathological host responses, mediators of organism attachment, and elements involved in possible functional and structural rearrangements of the host cell surface, structures at the periphery of mycoplasmas deserve special consideration in the host-parasite interaction. Elucidation of the molecular characteristics of these components should contribute significantly to the understanding of mycoplasma immunopathology.

We have used an *in vitro* model of lymphoblastoid cells experimentally infected with *M. hyorhinis* to elucidate numerous processes occurring during surface colonization of host cells with this organism. Complement-mediated cytolysis of host cells by antibodies to *M. hyorhinis*, spontaneous distribution of organisms into "caps" or "patches" at the cell periphery, selective association of host cell surface glycoproteins, and transfer of a lipophilic fluorescent probe from host cells to these organisms have been investigated [1,2,3]. Features of these interactions may reflect mycoplasma properties important in establishing disease processes associated with chronic arthritis caused by *M. hyorhinis*.

To understand more fully the detailed antigenic and structural surface features of *M. hyorhinis* essential to its interaction with lymphoid cells, we have developed a library of monoclonal antibodies to this mycoplasma, a portion of which recognizes surface antigens. This report describes characteristics of selected monoclonal an-
tibodies from this library and their cognate antigens. The size of these antigens, their partial chemical characterization, expression at the surface of organisms colonizing lymphoblastoid cells, and restricted expression among strains of *M. hyorhinis* are presented.

**MATERIALS AND METHODS**

*Mycoplasma Strains and Cultures*

*M. hyorhinis*, strain GDL [1] and strain BTS-7 (Research Resources Branch, NIH, catalogue M-718-002-084) were grown in broth culture as previously described [1,4]. Chronic infection of the murine T-lymphoblastoid cell line BW5147 with strain GDL is described elsewhere [1].

*Generation of Monoclonal Antibodies*

Immunization of BALB/c mice with *M. hyorhinis*, fusion of lymphoid cells with the P3 X63 Ag 8.653 non-secreting myeloma line, screening of resultant “hybridomas” for antibody to *M. hyorhinis* by an enzyme-linked immunosorbent assay (ELISA), cloning of hybridomas, and isotyping of monoclonal antibodies have been described in detail elsewhere [5].

**Protein Blot Technique**

Separation of mycoplasma components by sodium dodecyl sulfate polyacrylamide gradient gel electrophoresis (SDS-PAGE), electrophoretic transfer of separated components to nitrocellulose paper [6], and immunoperoxidase staining of protein blots following conventional and monoclonal antibody “probes” to detect separated antigens are described elsewhere [4,5].

**Double Antibody Immunofluorescent Staining**

A previously described indirect fluorescent antibody technique was utilized to stain intact (unfixed) BW5147 cells (either uninfected or infected with *M. hyorhinis*) with monoclonal antibodies to mycoplasma antigens [3,5].

**Immunoferritin Staining**

Intact mycoplasma-infected BW5147 cells were sequentially treated with monoclonal antibodies (or control preparations) and ferritin-conjugated antibody to mouse immunoglobulin. Cells were then fixed with glutaraldehyde and processed for transmission electron microscopy as described previously [3,5].

**RESULTS**

*Identification of Antigens Recognized by Monoclonal Antibodies*

A library has been constructed comprising approximately 50 stable, cloned hybridoma lines secreting monoclonal antibodies that react in an ELISA system [5] with *M. hyorhinis*. The reaction of six of these antibodies with individual mycoplasma components is shown in Fig. 1. While numerous components in protein blots of whole organisms were stained with conventional polyspecific mouse antiserum to *M. hyorhinis*, each of the six monoclonal antibodies specifically reacted only with discrete components of this organism. The antigens recognized were designated p120, p73, p51, p46, p38, and p23/17, corresponding to their apparent molecular weights in kilodaltons. While most staining patterns were sharply defined,
two antigens (p120 and p23/17) showed heterogeneity in their banding pattern. Similar heterogeneity was not observed in the corresponding region of gels stained for proteins with Coomassie blue or silver stain (not shown). In addition, the monoclonal antibody recognizing p23/17 showed reaction with two separate components. That numerous subclones of this hybridoma yielded antibodies giving the same staining pattern suggested that the single determinant recognized resided on different molecular species.

**Expression of p120, p23/17, and p46 at the Surface of M. hyorhinis**

Since most of the monoclonal antibodies within our library were probably directed against internal mycoplasma components, a means of identifying those reacting with surface-associated antigens was sought. In screening antibodies for their ability to react with *M. hyorhinis*-colonized BW5147 cells, it was found that relatively few members of the library were able to stain cell-associated organisms, although all reacted in an ELISA using disrupted mycoplasmas as target antigen, and many antibodies reacted in the protein blot assay. Fluorescent staining with three monoclonal antibodies (recognizing p120, p46, and p23/17) is shown in Fig. 2. These demonstrated typical staining of mycoplasmas frequently distributed in restricted areas ("caps" or "patches") at the surface of BW5147 cells [2,3]. Control culture fluid concentrates showed no staining of mycoplasma-infected cells. In addition, nearly 20 monoclonal antibodies tested (including those recognizing p73, p51, and p38) failed to stain infected cells, nor did antibodies to p120, p46, and p23/17 stain uninfected cells (not shown).

The possibility that the antibodies reacting with cell-associated organisms might be directed toward mycoplasma surface components was further explored using indirect immunoferritin staining (Fig. 2). Treatment of cells with antibody to p120 resulted in intense ferritin deposition at the surface of virtually all mycoplasmas (Fig. 2-9). Staining was uniform and was restricted to the periphery of organisms ex-
Expression of *M. hyorhinis* antigens on organisms colonizing BW5147 lymphoblastoid cells.

Indirect fluorescent antibody staining (*top row*) and corresponding light field images (*middle row*) of intact BW5147 cells chronically infected with *M. hyorhinis* strain GDL, using monoclonal antibodies (culture fluid concentrates) against p23/17 (1 and 5); p120 (2 and 6); p46 (3 and 7) or control myeloma culture fluid concentrate (4 and 8). Indirect immunoferritin staining (*bottom row*), after treatment of cells with monoclonal antibody against p120 (9), p23/17 (10) or with myeloma culture fluid concentrate (11). Bars = 10 microns

Internal to their plasma membrane. No staining of the BW5147 cell surface was observed. The staining pattern observed with monoclonal antibody recognizing p23/17 also indicated a surface staining of mycoplasmas (Fig. 2-10). This was less dense and non-uniform, often showing clustered ferritin granules at the mycoplasma surface or between adjacent organisms. Staining with control supernatant concentrate (Fig. 2-11) yielded only very sparse, occasional ferritin deposition which was not limited to the mycoplasma surface.

These experiments provided direct visual evidence that p120 and p23/17 antigenic determinants resided at the surface of *M. hyorhinis*, and that they were expressed not only on organisms grown in broth medium, but also during long-term colonization of lymphoid cells in culture. (Although immunoferritin staining of p46 antigen remains to be done, this antigen has also been provisionally classified as a mycoplasma surface constituent.)

**Enzymatic Digestion of Antigens**

Immobilization of separated antigens on nitrocellulose paper after protein blotting provided a method to test the sensitivity of antigens to various enzymes as a means of partially characterizing the macromolecules recognized by monoclonal antibodies. Treatment of protein blots prior to immunological staining with trypsin
and/or pronase (see [4] and [5] for details) resulted in the abrogation of subsequent staining by any of the six monoclonal antibodies (Table 1). Primary monoclonal antibodies incubated with enzyme-treated blots were subsequently capable of staining untreated blots, thus insuring that abrogation of binding was not due to enzyme digestion of the monoclonal antibody. These results suggested that the macromolecules bearing the defined determinants contained polypeptide, though the chemical nature of the precise determinant recognized could not be determined by this approach.

**Restricted Expression of p120 and p23/17 on M. hyorhinis Strain GDL**

The existing library of monoclonal antibodies represents clones from mice immunized with either GDL or BTS-7 strains of *M. hyorhinis*. Initial screening of the library using these two strains as antigen targets in an ELISA system [5] indicated that some monoclonal antibodies might selectively react with only one strain. A more detailed analysis, using (1) a competition ELISA to assess the ability of *M. hyorhinis* strains GDL and BTS-7 to inhibit the binding of monoclonal antibody to immobilized strain GDL, and (2) comparative immunological staining of protein blots of the two strains, indicated that p120 and p23/17 were expressed on GDL but completely absent from BTS-7 [7,8]. Thus, our library contained at least two antibodies defining strain-restricted determinants on distinct surface proteins of *M. hyorhinis*.

**DISCUSSION**

Properties of monoclonal antibodies to *M. hyorhinis* and their cognate antigens are summarized in Table 1. Collectively, the antigens defined by this small antibody library constitute a diverse set of mycoplasma constituents. All the determinants apparently reside on distinct protein-containing macromolecules having a range of molecular weights from 17,000 to 120,000. The actual chemical nature of the determinants has not been established.

Of particular relevance to investigations of mycoplasma-host cell interactions are the three antigens p120, p23/17, and p46. These are expressed at the surface of mycoplasmas and have interesting additional properties. First, the characteristically diffuse banding pattern of p120 and p23 in protein blots may reflect size differences among antigenically related molecules or other properties generating electrophoretic heterogeneity. The use of specific monoclonal antibodies to characterize and isolate
these components should facilitate analysis of this heterogeneity. Glycosylation or other modification of specific mycoplasma surface protein antigens is a particularly intriguing possibility now amenable to experimental manipulation in this system. The presence of the same determinant on p23 and p17 (both of which are resistant to trypsin digestion but antigenically degraded by pronase) is consistent with the expression of a peptide antigen in either a smaller, unmodified form or as a larger molecule containing additional chemical moieties. Since antigens were reduced and dissociated prior to SDS-PAGE and protein blots, the larger p23 band probably does not reflect the covalent linkage of p17 to another component by disulfide bonds.

A second property expressed by the p120 and p23/17 antigens is a marked strain specificity within the species *M. hyorhinis*. The use of monoclonal antibodies to differentiate serogroups of these organisms should allow a correlation of antigenic structure with important functional features that vary among isolates within this species. Differences among isolates in their ability to adsorb to cells and in their tendency toward adaptation to obligate growth on cell surfaces (with concomitant loss of growth capability in normal unsupplemented media) are hallmarks of *M. hyorhinis* for which no detailed explanation presently exists [9,10]. That surface constituents of these organisms reflect marked intraspecies antigenic differences underscores the possibility that structural variants of these components may be involved in dictating important aspects of the mycoplasma-host cell interaction, ultimately including their potential for causing disease.

The nature of the interaction between monoclonal antibodies and surface antigens of mycoplasmas may provide insight into the immunopathological consequences accompanying expression of these antigens in the host. For example, while neither the antibody (IgG) recognizing p120, nor that (IgM) recognizing p23/17 can alone cause growth inhibition of *M. hyorhinis* (GDL) in broth culture, addition of complement to this system provides a marked growth inhibition in the latter case [8]. Monoclonal antibodies may therefore be useful for investigating humoral immunological function associated with immune responses to mycoplasmas. The development of monoclonal antibodies to mycoplasma surface structures has provided the experimental tools to define, characterize, and manipulate mycoplasma constituents involved in the interaction of these organisms with host cells.

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