Interaction of Mycoplasmas With Host Cells

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Rottem, Shlomo. Interaction of Mycoplasmas With Host Cells. Physiol Rev 83: 417–432, 2003; 10.1152/physrev.00030.2002.—The mycoplasmas form a large group of prokaryotic microorganisms with over 190 species distinguished from ordinary bacteria by their small size, minute genome, and total lack of a cell wall. Owing to their limited biosynthetic capabilities, most mycoplasmas are parasites exhibiting strict host and tissue specificities. The aim of this review is to collate present knowledge on the strategies employed by mycoplasmas while interacting with their host eukaryotic cells. Prominant among these strategies is the adherence of mycoplasma to host cells, identifying the mycoplasmal adhesins as well as the mammalian membrane receptors; the invasion of mycoplasmas into host cells including studies on the role of mycoplasmal surface molecules and signaling mechanisms in the invasion; the fusion of mycoplasmas with host cells, a novel process that raises intriguing questions of how microinjection of mycoplasma components into eukaryotic cells subvert and damage the host cells. The observations of diverse interactions of mycoplasmas with cells of the immune system and their immunomodulatory effects and the discovery of genetic systems that enable mycoplasmas to rapidly change their surface antigenic composition have been important developments in mycoplasma research over the past decade, showing that mycoplasmas possess an impressive capability of maintaining a dynamic surface architecture that is antigenically and functionally versatile, contributing to the capability of the mycoplasmas to adapt to a large range of habitats and cause diseases that are often chronic in nature.
I. INTRODUCTION

Mycoplasmas (class Mollicutes) are the smallest and simplest self-replicating bacteria (82). These microorganisms lack a rigid cell wall and are bound by a single membrane, the plasma membrane. Wall-less bacteria are known (88). The lack of a cell wall is used to distinguish these microorganisms from ordinary bacteria and to include them in a separate class named Mollicutes. Most human and animal mollicutes are Mycoplasma and Ureaplasma species of the family Mycoplasmataceae. Because mycoplasmas have an extremely small genome (0.58 Mb; Ref. 34), these organisms have limited metabolic options for replication and survival. Phylogenetically, the mollicutes are related to Gram-positive bacteria from which they developed by genome reduction (43). Therefore, the mollicutes are not at the root of the phylogenetic tree but are most probably late evolutionary products (43, 61). The smallest genome of a self-replicating organism known at present is the genome of Mycoplasma genitalium (0.58 Mb; Ref. 34). Comparative genomic studies suggested that the genome of this organism still carries almost double the number of genes included in the minimal gene set essential for cellular function (61, 74).

Owing to their limited biosynthetic capabilities, most mycoplasmas are parasites exhibiting strict host and tissue specificities (82, 89). The mycoplasmas enter an appropriate host in which they multiply and survive for long periods of time. These microorganisms have evolved molecular mechanisms needed to deal with the host immune response and the transfer and colonization in a new host. These mechanisms include mimicry of host antigens, survival within phagocytic and nonphagocytic cells, and generation of phenotypic plasticity. The major question is whether mycoplasmas cause damage to the host cells and to what extent the damage is clinically apparent. Mycoplasmas have long resisted detailed analyses because of complex nutritional requirements, poor growth yields, and a paucity of useful genetic tools. Although questions still far outnumber answers, significant progress has been made in identifying the mechanisms by which mycoplasmas interact and damage eukaryotic host cells.

II. ADHERENCE TO HOST TISSUES

Many animal mycoplasmas depend on adhesion to host tissues for colonization and infection. In these mycoplasmas adherence is the major virulence factor, and adherence-deficient mutants are avirulent (6, 81). The best studied adherence systems are those of M. pneumoniae, the causative agent of primary atypical pneumonia in humans, which inhabits the respiratory tract, and M. genitalium, which preferentially colonize the urogenital tract. These organisms exhibit the typical polymorphism of mycoplasmas, with the most common flask and filamentous shaped (Fig. 1). Cytadherence of these organisms to cells in the respiratory or urogenital epithelium is an initial and essential step in tissue colonization and subsequent disease pathogenesis (5).

A. Adhesins

M. pneumoniae is the most extensively studied system with respect to the adhesins and receptors. This organism has a polar, tapered cell extension at one of the poles containing an electron-dense core in the cytoplasm (Fig. 1). This structure, termed the tip organelle, functions both as an attachment organelle and as the leading end in gliding-type motility. A surface 169-kDa protein designated P1 (47) and a 30-kDa protein designated P30 (19) are densely clustered at the tip organelle of virulent M. pneumoniae (Fig. 2), providing polarity to the cytadherence event. Both proteins elicit a strong immunological response in convalescent-phase sera from humans and experimentally infected hamsters, and anti-P1 or anti-P30 monoclonal antibodies block this cytadherence (4, 81). Neither P1 nor P30 mutants cytadhere (50, 103). At present, it is apparent that P1 is the adhesin of M. pneumoniae and P30 is a protein associated with the adherence process. Nonetheless, the exact role of P30 in ad-
herence is not yet fully understood. The P30 mutants appear to localize the adhesin P1 to the tip organelle, suggesting that P30 is required for P1 function rather than trafficking to the tip. Recently, it has been shown that P30 plays a role in cell development because its absence leads to morphological abnormalities, notably ovoid or multilobed cells (85). These cells, having poorly defined tip organelles, were associated with the loss of P30 (85). It is reasonable to assume that mutations in P30 will confer a defect in cell development, motility, and the potential to cytadhere to respiratory epithelium. Most importantly, the homologies predicted to be shared between P30 and mammalian structural proteins are implicating molecular mimicry as a basis for mycoplasma-mediated postinfectious autoimmune immunity (20). Recent findings demonstrate that M. pneumoniae interacts in the respiratory system also with the surfactant protein D (SP-D, Ref. 15), a major constituent of the alveolar environment that acts to keep alveoli from collapsing during the expiratory phase of the respiratory cycle. The primary mycoplasmal determinants participating in the interaction of M. pneumoniae with human SP-D were membrane glycolipids (15), suggesting that there is more than one type of adhesin on the cell surface of M. pneumoniae.

B. Accessory Proteins

The isolation and characterization of M. pneumoniae mutants that possess P1 and P30, yet fail to cytadhere, suggest that the tip-mediated adherence of M. pneumoniae to eukaryotic target cells is more complex (50, 81). Two groups of accessory proteins, the first including a 40-kDa and a 90-kDa proteins (P40 and P90), and the second, proteins HMW1-HMW3, were described (25, 50, 51). These proteins are not adhesins, but the loss of one of them is associated with the inability of M. pneumoniae to cytadhere, whereas its regain results in reversion to a cytadherence-positive phenotype (81). P40 and P90 were found to be surface proteins localized mainly at the tip organelle (Fig. 2). These proteins are closely associated with P1 (53, 54) but are not directly involved in receptor binding. Although P1 is present on the surface of strains lacking P40 and/or P90, it is not clustered at the tip organelle, but scattered on the surface of the mycoplasma (50). Since P40 and P90 were responsible for the association of P1 with M. pneumoniae Triton shell, it has been suggested that the P1 is kept in the membrane by P40 and P90 in a way that allows it to closely interact with the cytoskeleton forming or associated proteins.

In certain noncytadhering mutant strains another group of proteins, designated HMW1, HMW2, and HMW3, is missing, while in the corresponding revertants, cytadherence was regained. These proteins are coded by two unlinked genetic loci in the M. pneumoniae chromosome. HMW1 (112 kDa) and HMW3 (74 kDa) are members of a family of mycoplasma proteins that share acidic residues and an internal proline-rich domain in repeated motifs (25, 50). The proline-rich domains probably impart an extended conformation to the protein backbone, while the hydrophobic surface provided by the proline residues may contribute to interactions with other mycoplasma proteins. Similar to their counterparts lacking P40 and P90, mutants devoid of HMW1-HMW3 are avirulent, cytadhere very poorly, and fail to cluster P1 at the tip organelle (40, 79). Furthermore, their tip organelle lacks the characteristic truncated appearance seen in wild-type M. pneumoniae.
pneumoniae, suggesting that one or more of the HMW proteins is required both to anchor P1 at the attachment organelle and to maintain the proper architecture of the tip organelle (102). Consistent with this interpretation is the observation that these proteins are components of the Triton X-100-insoluble mycoplasma cytoskeleton and as such may have a structural role in the localization of P1 (90, 101). The deduced amino acid sequence of HMW3 shows that this protein is largely hydrophilic. Results of whole cell radioimmunoprecipitation experiments revealed that HMW3 is not exposed on the surface of M. pneumoniae cells (50; Fig. 2).

Because the tip organelle in mutants having reduced levels of HMW3 lacks the truncated tip appearance characteristic of wild-type M. pneumoniae (102), and loss of HMW3 resulted in subtle changes in morphology, inability to cluster the adhesion P1 and reduced cytadherence (109), it has been suggested that HMW3 contribute to the architecture and stability of the tip organelle (50, 109). Similar to HMW3, HMW1 has an unusual subcellular distribution. This protein is a peripheral membrane protein that is antibody accessible on the outer surface of wild-type M. pneumoniae, (3, Fig. 2). The subcellular location of HMW2 is yet unknown, but there are suggestions that this protein is present near the base of the tip organelle (40).

How do the accessory proteins promote adherence? As already mentioned, clustering of P1 at the tip organelle appears to be necessary to attachment providing a critical concentration of the adhesion molecule required for securing a stable primary association with receptor molecules on the host cells. In the nonadhering mutants, P1 fails to cluster at the tip organelle (4). It is conceivable that one or more of the accessory proteins plays a role in the lateral movement and concentration of the adhesion molecules at the tip organelle. How this is accomplished is still not fully understood. A plausible hypothesis is that to fulfill this role, the accessory proteins must be associated with the mycoplasma cytoskeleton, which is responsible not only for the lateral movement and proper orientation of P1, but also for changes in cell shape, cell division, and motility (82, 88). Recent studies showed that the loss of HMW2 from wild-type M. pneumoniae cells resulted in accelerated turnover of HMW1 and other cytadherence-accessory proteins, probably by proteolysis (3). These findings suggest a role for HMW2 in promoting the export of HMW1 to the cell surface, where it is stable and fully functional (3).

C. Receptors

The role of host cell surface sialoglycoconjugates as receptors for mycoplasmas has long been established (81). The carbohydrate moiety of the glycoprotein, which serves as a receptor for M. pneumoniae on human erythrocytes, has been identified as having a terminal NeuAc(a2–3)Gal(b1–4)GlcNAc sequence (84). Nevertheless, neuraminidase treatment has frequently failed to abolish the ability of various eukaryotic cells to bind M. pneumoniae (36). A sialic acid-free glycoprotein, isolated from cultured human lung fibroblasts, which serves as a receptor for M. pneumoniae, has been isolated by Geary et al. (37). Sulfated glycolipids containing terminal Gal(3SO₄)β1 residues were also found to function as receptors (52). Clearly, there is more than one type of receptor for M. pneumoniae and apparently for other adhering mycoplasmas as well. It is interesting to note that at the primary site of M. pneumoniae infection, the apical microvillar border and the cilia carry the sialo-glycoconjugate-type receptors, whereas the secretory cells and mucus lack them, favoring attachment of M. pneumoniae to the ciliated cells (59).

III. INVASION INTO HOST CELLS

Current theory holds that mycoplasmas remain attached to the surface of epithelial cells (82), although some mycoplasmas have evolved mechanisms for entering host cells that are not naturally phagocytic. The intracellular location is obviously a privileged niche, well protected from the immune system and from the action of many antibiotics. The ability of M. penetrans, isolated from the urogenital tract of acquired immunodeficiency syndrome (AIDS) patients (56, 57), to invade and survive within host cells has been intensively studied. This microorganism has invasive properties and localizes in the cytoplasm and perinuclear regions (2, 12, 38). Other mycoplasmas known to be surface parasites such as M. fermentans (100, 105), M. pneumoniae (4), M. genitalium (48), and M. gallisepticum (110), under certain circumstances, reside within nonphagocytic cells.

In studying bacterial invasion, it is essential to differentiate between microorganisms adhering to a host cell and those which have penetrated the cell. The early light microscopic and electron microscopic observations of mycoplasmas engulfed in membrane vesicles lead to conflicting interpretations. Are the mycoplasmas intracytoplasmatic, or are they at the bottom of crypts formed by the invagination of the cell membrane (117)? A more sophisticated ultrastructural study was based on a combined immunochemistry and electron microscopy approach. Staining surface polysaccharides of the host cell with ruthenium red allows a better differentiation between intracellular and extracellular mycoplasmas (105). Currently, the gentamicin resistance assay is the most common assay to differentiate intracellular from extracellular bacteria (28, 94). In this assay, the extracellular bacteria are killed by gentamicin, but the intracellular...
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Bacteria are shielded from the antibiotic because of the limited penetration of the gentamicin into eukaryotic cells. The gentamicin procedure was successfully adapted to mycoplasma systems (2, 110). "M. penetrans and M. gallisepticum are relatively susceptible to gentamicin. In the case of M. penetrans, the susceptibility to the antibiotic can be markedly increased by adding low concentrations of Triton X-100 to the medium (2). For example, a combination of 200 μg gentamicin and 0.01% Triton X-100 resulted in an 8 log decrease in CFU within 1 h (38). An increase in the invasive capacity of M. fermentans, which arises from the potential of this organism to bind plasminogen and activate it by urokinase to plasmin, has been recently described (113). Plasmin, a protease with broad substrate specificity, may alter M. fermentans-cell surface proteins and thereby promotes its internalization. Proteolytic modification of bacterial and/or host cell surface protein(s) is an emerging theme in the study of bacterial pathogenicity. For example, the plasminogen activator of Yersinia pestis degrades bacterial outer membrane proteins triggering virulence (98). Similarly, a secreted protease was shown to stimulate the fibronectin-dependent uptake of Streptococcus pyogenes into eukaryotic cells (14).

A. Invasins and Receptors

Bacterial invasion of eukaryotic cells involves complex bacterial and host cell processes. Invasion is associated with adhesins as well as host cell receptors that mediate interaction of the bacteria with the host cell (14, 94). It is likely that surface molecules (proteins and lipids) that facilitate the adhesion process will have an effect on the invasion. Nevertheless, adherence to the surface of host cells is not sufficient to trigger events that lead to invasion. The signals generated by the interaction of host cells with invasive mycoplasmas have yet to be investigated. Bacterial invasion is based on the ability of several bacteria to bind fibronectin (27) or sulfated polysaccharides (26, 27). These compounds form a molecular bridge between the bacteria and different types of host cell surface proteins that enables invasion (14, 26). Fibronectin binding activity was detected in M. penetrans. This organism, which contains a 65-kDa fibronectin binding protein, binds selectively immobilized fibronectin (38). An increase in the invasive capacity of M. fermentans, which arises from the potential of this organism to bind plasminogen and activate it by urokinase to plasmin, has been recently described (113). Plasmin, a protease with broad substrate specificity, may alter M. fermentans-cell surface proteins and thereby promotes its internalization. Proteolytic modification of bacterial and/or host cell surface protein(s) is an emerging theme in the study of bacterial pathogenicity. For example, the plasminogen activator of Yersinia pestis degrades bacterial outer membrane proteins triggering virulence (98). Similarly, a secreted protease was shown to stimulate the fibronectin-dependent uptake of Streptococcus pyogenes into eukaryotic cells (14).

B. Changes in the Host Cell Cytoskeleton

Almost all invasive bacteria that come into contact with the host cell surface trigger cytoskeletal rearrangements that facilitate bacterial internalization (31, 39, 75). M. penetrans invasion of HeLa cells depends on the capacity of the cells to assemble actin microfilaments, as treatment with cytochalasin D has a dramatic effect on the invasion of HeLa cells by M. penetrans (2). Furthermore, both vinblastine, which disrupts microtubules, and taxol, which freezes microtubules, virtually abolish penetration of M. penetrans (12). These findings suggest that alterations in the polymerization dynamics and stability of microtubules inhibits the invasion of M. penetrans into HeLa cells. On the other hand, the entry of M. gallisepticum into chicken embryo fibroblasts is inhibited by the microtubule inhibitor nocodazole, but not by cytochalasin D, suggesting that M. gallisepticum may use a different strategy from that of M. penetrans for reaching the intracellular space (110).

C. Signal Transduction

Involvement of the host cell cytoskeleton in internalization is considered to be the result of a host cell signal transduction cascade induced by the invasive bacterium. As in many signal transduction processes initiated by bacteria, kinases and/or phosphatases are usually involved (75, 83). The invading mycoplasmas generate up- take signals that trigger the assembly of highly organized cytoskeletal structures in the host cells (38). Yet, the nature of these signals and the mechanisms used to transduce them are not fully understood. Specific activation of protein kinases occurs during the internalization of most of the bacteria taken up by microtubule-dependent mechanisms (86). It has been shown that invasion of HeLa cells...
by *M. penetrans* is associated with tyrosine phosphorylation of a 145-kDa host cell protein (2). Tyrosine phosphorylation activates phospholipase C to generate two second messengers: phosphatidylinositol metabolites and diacylglycerol (DAG). Changes in host cell lipid turnover occur as a result of *M. penetrans* binding and/or invasion of Molt-3 lymphocytes (91). These changes include the accumulation of DAG and the release of unsaturated fatty acids, predominantly long-chain polyunsaturated ones such as docosahexanoic acid (*C*\(_{22:6}\), Ref. 91). Nonetheless, metabolites of phosphatidylinositol were not detected. These observations support the hypothesis that *M. penetrans* stimulates host phospholipases to cleave membrane phospholipids, thereby initiating the signal transduction cascade. Because in HeLa cells, which are invaded by *M. penetrans*, DAG is generated, it is likely that the protein kinase C is activated in the host cells. Indeed, transient protein kinase C activation was demonstrated in invaded HeLa cells by several methods, including translocation to the plasma membrane and enzymatic activity (12). However, activation was weak and transient, peaking at 20 min postinfection. How any of these different signal transduction events lead to specific microtubule activity resulting in mycoplasmal internalization is unknown. The role of these signals in the penetration, survival, and proliferation of mycoplasmas within host cells, as well as the involvement of the lipid intermediates in the pathobiological alterations taking place in the host cells, merit further investigation.

### D. Survival and Multiplication Within Host Cells

The intracellular fate of invading bacteria can vary greatly. Most invasive bacteria appear to be able to survive intracellularly for extended periods of time, at least if they have reached a suitable host cell (29). Other engulfed bacteria are degraded intracellularly via phagosome-lysosome fusion. The invasive bacteria either remain and multiply within the endosomes after invasion or are released via exocytosis and/or the lysis of the endosomes which may allow multiplication within the cytoplasm. Most ultrastructural studies performed with engulfed mycoplasmas revealed mycoplasmas within membrane-bound vesicles (48, 100, 105). Persistence of *M. penetrans* within NIH/3T3 cells, Vero cells, human endothelial cells, HeLa cells, WI-38 cells, and HEp-2 cells has been observed over a 48–96 h postinfection (38, 57). *M. gallisepticum* remains viable within HeLa cells during 24–48 h of intracellular residence (110). The observation of vesicles stuffed with *M. penetrans* in various host cells was taken as an indication that *M. penetrans* is able to divide within intracellular vesicles of the host cells (57). Nonetheless, the intracellular multiplication of mycoplasmas remains to be convincingly demonstrated.

### IV. FUSION WITH HOST CELLS

The lack of a rigid cell wall allows direct and intimate contact of the mycoplasma membrane with the cytoplasmic membrane of the host cell. Under appropriate conditions, such contact may lead to cell fusion (Fig. 3). Fusion of mycoplasmas with eukaryotic host cells has been first observed in electron microscopic studies (88). The development of energy transfer and fluorescence methods has enabled investigation of the fusion process on a quantitative basis in an experimental cell culture-mycoplasma system and has also allowed the identification of fusogenic mycoplasmas.

**FIG. 3.** Schematic diagram of the fusion of a mycoplasma with a eukaryotic cell.
A. Factors Mediating Fusion

In all the fusogenic Mycoplasma species tested, fusogenicity is dependent on the unesterified cholesterol content of the cell membrane (104). Fusogenic activity can be found only among mycoplasmas requiring unesterified cholesterol for growth, whereas Acholeplasma species, which do not require cholesterol, are nonfusogenic. Furthermore, adaptation of M. capricolum to grow in the absence of cholesterol results in a marked reduction in membrane-cholesterol content and renders the organism nonfusogenic (104). Fusogenicity of M. fermentans with Molt-3 cells is markedly stimulated by Ca\(^{2+}\) and depends on the proton gradient across the mycoplasma cell membrane, decreasing markedly when the proton gradient is collapsed by proton ionophores (24).

B. Molecules Implicated in Fusion

Among the Mycoplasma species, the human mycoplasma, M. fermentans, is highly fusogenic, capable of fusing with a variety of cells (33). Furthermore, it has been shown that the polar lipid fraction of this organism is capable of enhancing the fusion of small, unilamellar phosphatidylcholine-cholesterol (1:1 molar ratio) vesicles with Molt-3 lymphocytes in a dose-dependent manner, suggesting that a lipid component acts as a fusogen (11, 92). In an attempt to identify the fusogen, detailed lipid analyses of M. fermentans membranes were performed (23, 92, 114), revealing that the polar lipid fraction of this organism is dominated by the presence of unusual choline-containing phosphoglycolipids (Fig. 4). The major type (MfGL-II) has been identified as 6’-O-[3’-phosphorylcholine-2’-amino-1’phospho-1’,3’-propanediol]-α-D-glucopyranosyl (1’-3’)-1,2-diacyl-glycerol with hexadecanoyl (16:0) and octadecanoyl (18:0) in a molar ratio of 3.6:1 constituting the major acyl residues (114). Other choline-containing lipids identified are MfGL-I (65, 66), which is similar to MfGL-II but without the 2-amino-1,3-propanediol-1,3-bisphosphate, and the ether lipids 1-O-alkyl/alkenyl-2-O-acylglycerol-3-phosphocholine (MfEL) and its lysosome 1-O-alkyl/alkenyl-glycero-3-phosphocholine (lyso-MfEL) (108).

It has been proposed that MfGL-II is the fusogenic component in M. fermentans strain PG18 (92), yet a recent study showed that despite the fact that the respiratory isolates of M. fermentans, strains M39 and M52, have no MfGL-II, these strains fused with Molt-3 cells at almost the same rate and to about the same extent as PG18, suggesting that in these strains MfGL-II is not the fusogenic component (11). It is widely accepted that the reorganization of the membrane structure that occurs during fusion requires that the lipid bilayer is broken up and that other inverted configurations, such as reversed

![Image of the structure of the major choline-containing phospholipids of M. fermentans. The MfEL contains a hexadecyl residue. R, acyl. [From Ben-Menachem et al. (11).]
nonbilayer aggregates, are being formed (18, 60, 97). Nevertheless, analyses of the phase behavior of MfGL-II/H2O mixtures by solid state 31P and pulsed-field gradient diffusion NMR spectroscopy revealed that MfGL-II is a bilayer stabilizing lipid incapable of undergoing a phase transition from a lamellar to an inverted configuration (8). This property of MfGL-II is difficult to reconcile with a role in membrane fusion. On the other hand, it is well established that lysolipids can substantially enhance the rate of fusion in model membranes as well as in biomembranes (18), and it is plausible that the lyso ether lipid found in all M. fermentans strains (108) may act as a fusogen. Very little is known about the role of membrane proteins in the fusion process. The observation that fusion of M. fermentans with Molt-3 cells was inhibited by pretreatment of intact M. fermentans with proteolytic enzymes (24) implies that this organism possesses a proteinase-sensitive receptor(s) responsible for binding and/or the establishment of tight contact with the cell surface of the host cell involved in fusion.

V. POSSIBLE MECHANISMS OF DAMAGE TO HOST CELLS

A. Competition for Precursors

Genomic analyses of mycoplasmas have revealed the limited biosynthetic capabilities of these microorganisms (45, 46, 78). Mycoplasmas apparently lost almost all the genes involved in the biosynthesis of amino acids, fatty acids, cofactors, and vitamins and therefore depend on the host microenvironment to supply the full spectrum of biochemical precursors required for the biosynthesis of macromolecules (78, 82). Competition for these biosynthetic precursors by mycoplasmas may disrupt host cell integrity and alter host cell function. Nonfermenting Mycoplasma spp. utilize the arginine dihydrolase pathway for generating ATP (78) and rapidly deplete the host's arginine reserves affecting protein synthesis, host cell division, and growth (87). Certain strains of arginine-utilizing Mycoplasma spp. have been shown to induce chromosomal aberrations in host cells, most commonly chromosomal breakage, multiple translocations, a reduction in chromosome number, and the appearance of new and/or additional chromosome varieties (5, 67). Because histones are rich in arginine, it has been suggested that arginine utilization by mycoplasmas inhibits histone synthesis and causes chromosomal damage (5, 67, 87). M. fermentans infection of rat astrocytes has been shown recently to result in a choline-deficient environment and in the induction of apoptosis (9). Choline is an essential dietary component that ensures the structural integrity and signaling functions of the cell membranes; it is the major source of methyl groups in the diet, and it directly affects cholinergic neurotransmission, transmembrane signaling, and lipid transport and metabolism (115).

B. Damage Induced by Adherence

The attachment of mycoplasmas to the surface of host cells may interfere with membrane receptors or alter transport mechanisms of the host cell. The disruption of the K+ channels of ciliated bronchial epithelial cells by Mycoplasma hyopneumoniae that resulted in ciliostasis has been described (21). The host cell membrane is also vulnerable to toxic materials released by the adhering mycoplasmas. Although toxins have not been associated with mycoplasmas, the production of cytotoxic metabolites and the activity of cytolitic enzymes is well established. Oxidative damage to the host cell membrane by peroxide and superoxide radicals excreted by the adhering mycoplasmas appears to be experimentally well-substantiated (1). The intimate contact of the mycoplasma with the host cell membrane may also result in the hydrolysis of host cell phospholipids catalyzed by the potent membrane-bound phospholipases present in many mycoplasma species (96). This could trigger specific signal cascades (86) or release cytolytic lysospholipids capable of disrupting the integrity of the host cell membrane (92, 93).

C. Damage Induced by Fusion

During the fusion process, mycoplasma components are delivered into the host cell and affect the normal functions of the cell. A whole array of potent hydrolytic enzymes has been identified in mycoplasmas (82, 95, 96). Most remarkable are the mycoplasmal nucleases (76, 77, 82) that may degrade host cell DNA. It has recently been shown that M. fermentans contains a potent phosphoprotein phosphatase (95). Phosphorylation of cellular constituents by interacting cascades of serine/threonine and tyrosine protein kinases and phosphatases is a major means by which a eukaryotic cell responds to exogenous stimuli (86). The delivery of an active phosphoprotein phosphatase into the eukaryotic cell upon fusion may interfere with the normal signal transduction cascade of the host cell. In addition to delivery of the mycoplasmal cell content into the host cell, fusion also allows insertion of mycoplasmal membrane components into the membrane of the eukaryotic host cell. This could alter receptor recognition sites as well as affect the induction and expression of cytokines and alter the cross-talk between the various cells in an infected tissue.

D. Cytopathic Effects

Contamination of a cell culture by mycoplasmas may go undetected because mycoplasmal infections do not
produce overt turbid growth commonly associated with bacterial and fungal contamination. The morphological cellular changes can be minimal or unapparent. Frequently, the cellular changes are similar to those caused by nutritional effects such as the depletion of amino acids, sugars, or nucleic acid precursors. These morphological effects can be reversed by changing the medium or by replenishing the medium with fresh nutrients.

Mycoplasma attachment to eukaryotic cells may sometimes lead to a pronounced cytopathic effect. Attachment permits the mycoplasma contaminant to release noxious enzymatic and cytolytic metabolites directly onto the tissue cell membrane. Some mycoplasmas selectively colonize defined areas of the cell culture. This results in microcolony formation producing microlesions and small foci of necrosis, e.g., M. pulmonis, or form plaques, e.g., M. gallisepticum, in an agar overlay system (5). Microcolonization suggests that mycoplasma-specific receptors are localized in defined areas of the cell monolayer. However, other fermenting mycoplasmas, e.g., M. hyorhinis, attach to every cell and destroy the entire monolayer, producing a generalized cytopathic effect. With HeLa cells infected by the invasive M. penetrans, the most pronounced effect was the vacuolation of the host cells (12). The vacuoles appeared to be empty, differing from the described membrane-bound vesicles containing clusters of bacteria (57). The number and size of the vacuoles depended on duration of infection. Because vacuolation is not obtained with M. penetrans cell fractions (12), it is unlikely that a necrotizing cytotoxin is involved in the generation of the cellular lesions. A possible mechanism that leads to vacuolation may be associated with the accumulation of organic peroxides upon invasion of HeLa cells by M. penetrans. Indeed, when HeLa cells were grown with the antioxidant α-tocopherol, the level of accumulated organic peroxides was extremely low, and vacuolation was almost completely abolished (12).

Being unable to synthesize nucleotides, mycoplasmas developed potent nuclease, either soluble ones secreted into the extracellular medium or membrane-bound nucleases (7, 68, 82) apparently as a means of producing nucleic acid precursors required for metabolism. It has been shown that, occasionally, secreted mycoplasmal nucleases are taken up by the host cells (76, 77). Thus it was suggested that the cytotoxicity of M. penetrans is mediated at least in part to a secreted mycoplasmal endonuclease that is cleaving DNA and/or RNA of the host cells (7), and the endonuclease activity of M. bovis was implicated in the increased sensitivity of lymphocytic cell lines to various inducers of apoptosis (99).

VI. CIRCUMVENTING THE HOST IMMUNE SYSTEM

Circumvention of the host immune system is of utmost importance to the survival of a mycoplasma within its host. The major mechanisms that have been studied at length are molecular mimicry and phenotypic plasticity, which ensure that the mycoplasmas are not fully or efficiently recognized by the host’s immune system. Molecular mimicry refers to antigenic epitopes that have been shown to be shared by different mycoplasmas and host cells and were proposed as putative factors involved in evasion of host defense mechanisms and/or induction of autoantibodies observed during infections with certain mycoplasmas. Mycoplasmas are also endowed with phenotypic plasticity defined as the ability of a single genotype to change its antigenic make-up producing more than one alternative form of morphology, physiological state, and/or behavior in response to environmental condition. Phenotypic plasticity can be accomplished by two distinct mechanisms: a response to environmental signals or random changes of expression of single or multiple genes. The use of signal transduction pathways to sense signals in the host environment and respond accordingly by expressing gene products is necessary for survival in the host (107, 112). The apparent scarcity in mycoplasmas of regulatory genes functioning as sensors to environmental stimuli and of genes encoding transcriptional factors suggests, but does not rule out, that adaptation of mycoplasmas to the changing environment is not per se a response to signals. The common way to achieve phenotypic plasticity in mycoplasmas is by “antigenic variation,” a term that refers to the reversible high-frequency gain or loss of surface components that is a common survival strategy used by bacterial pathogens (62, 112). These surface components that in microorganisms include components of flagella, pili, outer membrane, or capsules are the major targets for the host antibody response. Therefore, the ability of a microorganism to rapidly change the surface antigenic repertoire and consequently vary the immunogenicity of these components allows the microorganism to avoid detection or to outpace a host’s immune system. Lacking a cell wall, locomotive organelles, or pili, most of the variable surface components of mycoplasmas are membrane proteins. These proteins are mature, processed prokaryotic lipoproteins anchored to the membrane by DAG and amide-linked fatty acids (13). A mycoplasma population may spontaneously and randomly generate distinct lipoprotein populations with a variety of antigenic phenotypes, “heterotypes,” that will survive the specific host response capable of eliminating the predominant “homotypes.” Notably, the molecular switching events leading to the generation of these heterotypes are reversible, and the escape variants produced through random genetic variation must inherit the ability to produce, at a high frequency, a wide range of antigenic phenotypes. A considerable evolutionary dividend to the microbial pathogen of such random phenotypic switching can be achieved even before the onset of a specific immune response. For example, by “fine tuning” of the specificities.
of variant receptors or adhesion factors throughout the cell population, there is a better chance that a given variant will succeed in finding the preferred receptors on the mosaic of different tissues displayed by the host. It may also provide the mycoplasma, during the course of parasitic life, the flexibility to reach and adapt to different niches within the host where distinctive receptors may be required for colonization. Despite the very limited genetic information that mycoplasmas contain, the number of mycoplasmal genes involved in diversifying the antigenic nature of their cell surface is unexpectedly high. The utilization of multiple variable genes organized as gene families, allowing the generation of an extensive repertoire of antigenic variants, is a common theme in pathogenic bacteria and parasites for maintaining surface variability (107, 112). By oscillating at a high frequency, these genes allow numerous combinatorial antigenic repertoires to be generated. Genetic mechanisms of antigenic variation emerging from the mycoplasma studies can be broadly divided into three categories: 1) variation by homopolymeric repeats, 2) variation by chromosomal re-arrangements, and 3) variation by reiterated coding sequence domains. Comprehensive and comparative reviews on the genetic mechanisms generating antigenic variation of surface proteins in mycoplasmas and other bacteria and on the role of antigenic variation in bacteria-host cell interactions have been recently published (13, 82, 89, 107, 111, 112).

VII. MODULATING THE IMMUNE SYSTEM

A. Modulatory Effects on Monocytes and Macrophages

It is increasingly recognized that for many bacteria induction of cytokines is a major virulence mechanism (41, 112). The induced cytokines have a wide range of effects on the eukaryotic host cell and are recognized as important mediators of tissue pathology in infectious diseases. It appears that although mycoplasmas circumvent phagocytosis, they interact with mononuclear and polymorphonuclear phagocytes stimulating the synthesis of cytokines with proinflammatory action (88, 92). These immunomodulatory influences depend on both the immune cells and the Mycoplasma spp. involved. Macrophage-mediated cytolysis of fibrosarcoma A9HT induced by whole cells of M. orale was first described by Lowenstein et al. (38). Cytolysis of the neoplastic cells was obtained even with macrophages from the lipopolysaccharide (LPS)-unresponsive C3H/HeJ mice, suggesting that the mechanism of activation is different from that of LPS (53). Since then over 20 Mycoplasma spp. have been shown to activate monocytes, macrophages, and brain astrocytes and induce secretion of the proinflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6, chemokines, such as IL-8, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 (MIP-1α), granulocyte-macrophage colony stimulating factors (GM-CSFs), as well as prostaglandins and nitric oxide (82, 92). More recent observations suggest that the mechanisms underlying macrophage activation by whole cells are in many cases identical to those employed by their purified membrane lipoproteins, supporting the notion that lipoproteins are the principle component of intact mycoplasmas activating monocytes/macrophages and playing an important role in the inflammatory response during infection (13, 41).

The potent molecules and mediators released by cells responding to mycoplasmas and mycoplasma-derived cell components enhance expression of major histocompatibility complex (MHC) class I and class II antigens and of costimulatory end cell adhesion molecules in leukocytes and endothelial cells, induce recruitment and extravasation of leukocytes to the site of infection and cause local tissue damage (41, 82, 90). It is interesting to note that mycoplasmal infections are not necessarily associated with a strong inflammatory response, and some mycoplasmas colonize the respiratory and urogenital tracts with no apparent clinical symptoms. It is therefore tempting to speculate that in addition to triggering the production of proinflammatory cytokines, certain organisms have the capacity to downregulate NFκB or to induce anti-inflammatory cytokines such as IL-4, IL-10, IL-13, or transforming growth factor-β, contributing to the complex network of synergistic and antagonistic influences induced by mycoplasmas on cells of the immune system.

B. Characterization of Modulin

The term modulin has been proposed to describe components and products of bacteria that have the capacity to stimulate cytokine synthesis. The first and most widely studied modulin was the LPS of Gram-negative bacteria (41). During the past decade it has been shown that in addition to LPS, other bacterial components, mainly those associated with the cell wall, such as peptidoglycan fragments, lipoteichoic acid, and murein lipoproteins can stimulate mammalian cells to produce cytokines (41). Recent attempts to identify mycoplasmal cytokine-inducing moieties have targeted membrane lipoproteins (13, 70, 90), superantigens (16, 17), and choline-containing phosphoglycolipids (10, 92).

C. Lipoproteins

Lipoproteins are found in the cytoplasmic membrane and in the outer membrane of many Gram-positive and
Gram-negative bacteria. All membrane-anchored bacterial lipoproteins contain a lipoylated amino-terminal cysteinyl residue which, in some cases, is \(N\)-acylated (Fig. 5). Lipoproteins are extremely abundant in the cell membrane of mycoplasmas. In *M. pneumoniae*, for example, of an estimated number of 150 membrane proteins, 46 open reading frames encoding putative lipoprotein genes have been identified (46). Chemical analyses of mycoplasmal lipoproteins have revealed that their lipoylation mechanism is similar to that of Gram-negative and Gram-positive bacteria (13). However, in many mycoplasmas, the lipoproteins are not \(N\)-acylated (Fig. 3), nor has an \(N\)-acyltransferase gene been found in the genome (34, 45, 46). The first reports on the cytokine-inducing ability of mycoplasmal lipoproteins showed that a lipoprotein from *M. fermentans* (49, 70) or *M. arginini* (44) is capable of stimulating the release of proinflammatory cytokines such as IL-1\(\beta\), IL-6, and TNF-\(\alpha\) from human peripheral blood monocytes in a dose-dependent manner. Comparison of the effects of intact lipoproteins with those of proteinase-K-treated lipoproteins reveals that the lipoylated amino terminus is responsible for the immunostimulating properties of the lipoproteins (13). However, it is not certain whether all naturally occurring mycoplasmal membrane lipoproteins are potent macrophage activators. The importance of the lipid residue has been emphasized by the isolation and characterization of naturally occurring lipopeptides with macrophage-activating potential from two mycoplasmas. A macrophage-activating lipopeptide with a molecular mass of 2 kDa (MALP-2) has been identified in the cell membrane of *M. fermentans* (73), and two lipopeptides, derived from the variable lipoproteins VlpA and VlpC, respectively, were characterized in *M. hyorhinis* (72). Further information on the functionally important lipopeptide moieties has been obtained by analyzing the cytokine-inducing potency of a number of synthetic peptides, which are analogs of the MALP-2 of *M. fermentans* (35, 71). These studies clearly demonstrated that the macrophage-activating agents carry a fatty acid-substituted amino-terminal \(S\)-(2,3-bisacyloxypropyl) cysteinyl group, but lack the \(N\)-acyl long-chain fatty acid present in bacterial lipoproteins. This feature renders these compounds exceptionally active as macrophage stimulators in vitro (72, 73).

**D. Superantigens**

Superantigens (SAg) are potent immunoregulatory proteins produced by bacteria, viruses, and mycoplasmas (32, 63, 69). These proteins are able to activate large proportions of the peripheral T-lymphocyte population, inducing them to secrete a large panel of cytokines both in vivo and in vitro (32, 42). The SAg are presented directly to T cells in association with various class II major histocompatibility complex molecules on accessory cell surfaces, usually without the need for processing (22, 32), and are recognized predominantly by T cells bearing specific V-chain segments of the T-cell receptor for antigen (TCR) (42, 63). Because recognition is dependent on fewer restricting elements that are required for traditional antigens, large numbers of naive T cells may be activated. This response contributes to the marked inflammation seen after in vivo administration of SAg, which has clear implications for disease pathogenesis (69). The superantigen of *M. arthritidis* (MAM) was most thoroughly studied (16). MAM has been cloned and sequenced, and the functional regions of the molecule were described (17). There is currently no evidence that other mycoplasmas have SAg.
E. Membrane Lipids

Very few mycoplasmal lipids have been investigated for cytokine stimulating activity. MfGL-II, the major choline-containing phosphoglycolipid of M. fermentans (Fig. 4), has been found to be associated with the secretion of inflammatory mediators by human monocytes (92) and by rat cells of the central nervous system (10). Stimulation of primary rat astrocytes by MfGL-II caused activation of protein kinase C, secretion of nitric oxide and prostaglandine E$_2$, in a dose-dependent manner (10). Deacylation of MfGL-II or treatment with monoclonal anti-phosphocholine antibodies pronouncedly reduced the stimulatory activity, suggesting that the fatty acyl residues are essential for activity and that the terminal phosphocholine moiety plays an important role in MfGL-IIs stimulation (10).

F. Signaling Pathways by Modulins

The monocyte surface molecule CD14 is part of the receptor complex for several microbial products. This 50- to 55-kDa glycoprotein is expressed predominantly on myeloid cells, and in addition to being a high-affinity receptor for LPS, it has been implicated in the response to peptidoglycan and other bacterial cell wall components (41). The failure of anti-CD14 monoclonal antibodies to block cytokine induction by M. fermentans lipoproteins (49, 80) indicates that cytokine induction by M. fermentans lipoproteins does not proceed through CD14 (49). This was further supported by the finding that cytokine synthesis by a human monocyte cell line induced by M. fermentans lipoproteins was not affected by pretreatment of the cells with vitamin D$_3$, known to increase CD14 expression on the cell surface (80). In contrast to CD14, the toll-like receptors (TLRs) contain all of the characteristics that one would expect from a true pattern recognition receptor, including the presence of a true signal-transducing intracellular domain (35). Although only recently described, the list of putative ligands for TLR2 and TLR4 is already impressively large. Both TLR2 and TLR4 have been reported to function as LPS signal transducers (35). However, lipoproteins/lipopeptides from M. fermentans activated cells expressing TLR2 but not those expressing TLR4, suggesting that TLR2 is a central pattern recognition receptor in host response to M. fermentans invasion (35).

The downstream signaling events that follow the TLR-mediated activation by mycoplasmal lipoproteins and lead to cytokine synthesis seem to be similar to the intracellular events induced by LPS. The TLRs have a cytoplasmic domain that is homologous to the IL-1 receptor. Thus it is likely that TLR2 activates the NF-$\kappa$B pathway, and perhaps other proinflammatory pathways as well, via their interactions with IL-1 receptor signaling genes (35). Indeed, M. fermentans lipoprotein-signaling Pathways by Modulins (10), the two transcription factors playing a central role in the induction of proinflammatory cytokines, as well as the mitogen-activated protein kinase family members including extracellular signal-regulated kinases 1 and 2, c-Jun amino-terminal kinase, and p38 (35, 80).

G. Mitogenic Activity

Numerous reports describe the mitogenic stimulation of lymphocytes by mycoplasmas in a nonspecific polyclonal manner both in vitro and in vivo (90), yet many of these mitogenic effects were not immunologically well characterized. These effects may be indirect and due primarily to cytokine release by macrophages and monocytes. Different Mycoplasma spp. have been shown to stimulate B cells, T cells, or both nonspecifically. Antibodies of different specificities, with no affinity for mycoplasmal antigens, are generated both in vitro and in vivo, following exposure of lymphoid cells to different mycoplasmas possessing B cell mitogens (82, 88, 90). It is worth noting that there is a clear distinction between mycoplasma-induced DNA synthesis and differentiation of activated B cells into antibody-producing cells. Thus, on the one hand, stimulation of DNA synthesis is not always a prerequisite for the induction of polyclonal immunoglobulin secretion by B cells exposed to mycoplasmas. On the other hand, stimulation of mitosis by mycoplasmas does not necessarily trigger subsequent differentiation of the dividing lymphocytes into plasma cells (92). Various Mycoplasma spp. are potent stimulators of T-cell-derived cytokines such as IL-2, interferon-$\alpha$, or IL-4. They exert multiple amplifying effects on phagocytes and lymphocytes and affect the balance between Th1 and Th2 populations of CD4$^+$ T cells, thereby influencing the direction of the subsequent effector phases of the immune response, and augmenting natural killer cell activity (82, 88).

Our present knowledge of the biochemical nature of mycoplasma mitogens is very limited. It appears that mycoplasmal B-cell mitogens differ from bacterial LPS in their ability to activate lymphocytes from C3H/HeJ mice that are poor responders to LPS and their inducing potential is unaffected by polymyxin B (82). Furthermore, there are indications that a single mycoplasma may carry more than one cell constituent interacting with lymphocytes. Thus the AIDS-associated M. penetrans mitogens appear to include a glycolipid as well as a major membrane lipoprotein (88).

H. Oncogenic Activity

Mycoplasmas can grow in close interaction with mammalian cells, often silently for a long period of time.
However, prolonged interactions with mycoplasmas with seemingly low virulence could, through a gradual and progressive course, induce chromosomal instability as well as malignant transformation, promoting tumorous growth of mammalian cells (30, 106, 116). This mycoplasma-mediated transformation of cells has a long latency and demonstrates distinct multistage progression (106). Overexpression of H-ras and c-myc oncogenes was found to be closely associated with both the initial reversible and the subsequent irreversible states of the mycoplasma-mediated transformation of cells (116).

VIII. CONCLUSIONS

Over the last decade, intensive studies have been carried out to understand the strategy employed by a mycoplasma pathogen to interact with host cells and to avoid or subvert host protective measures. The identification of mycoplasmal membrane components that participate in the adhesion process will shape and direct future efforts toward understanding the molecular organization of adhesion-associated proteins and to further identify mammalian membrane receptors for mycoplasmas and mycoplasma products. The finding that some mycoplasmas can reside intracellularly opens up new horizons to study the role of mycoplasma and host surface molecules in invasion. Although the ability of internalized mycoplasmas to multiply within the host cell remains to be convincingly demonstrated, the reports of invasive mycoplasmas offer new insights into the potential virulence strategies employed by mycoplasmas. Invasion of nonphagocytic host cells, if only for a short period of time, may provide mycoplasmas with the ability to cross mucosal barrier and gain access to internal tissues. The intracellular organisms are also resistant to the host defense mechanisms as well as to antibiotic treatment and may account for the difficulty to eradicate mycoplasmas from cell cultures. The fusion of mycoplasmas with eukaryotic host cells raises exciting questions on how microinjection of mycoplasmal components into eukaryotic cells affects host cells. The fusion process as well as the invasion of host cells by mycoplasmas brings up an emerging theme in mycoplasma research: the subversion by mycoplasmas of host cell functions mainly in signal-transduction pathways and cytoskeleton organization. Nevertheless, as the invasion and fusion studies rely exclusively on in vitro experiments done with cultured mammalian cells rather than organ cultures of the actual target tissue or intact animals, the interpretation of the results should be cautious. The discovery of genetic systems that enable the mycoplasma cell to rapidly change its antigenic characteristics has been one of the major developments in mycoplasma research over the past decade. It is now clear that these minute wall-less microorganisms possess an impressive capability of maintaining a surface architecture that is antigenically and functionally versatile. These variable surface antigens undoubtedly contribute to the capability of the mycoplasmas to adapt to a large range of habitats and to cause diseases, which are often chronic in nature. Although mycoplasmas circumvent phagocytosis, they interact with mononuclear and polymorphonuclear phagocytes, suppressing or stimulating them by a combination of direct and indirect cytokine-mediated effects. It appears that for many mycoplasmas, induction of cytokines is a major virulence mechanism. The induced cytokines have a wide range of effects on the eukaryotic host cells and are recognized as important mediators of tissue pathology in infectious diseases.

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