Mycoplasma pulmonis Inhibits Electrogenic Ion Transport across Murine Tracheal Epithelial Cell Monolayers

LINDA C. LAMBERT,1 HOA Q. TRUMMELL,2 ASHVANI SINGH,2 GAIL H. CASSELL,1 AND ROBERT J. BRIDGES2*

Department of Microbiology1 and Department of Physiology and Biophysics,2 University of Alabama at Birmingham, Birmingham, Alabama 35294

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Murine chronic respiratory disease is characterized by persistent colonization of tracheal and bronchial epithelial cell surfaces by Mycoplasma pulmonis, submucosal and intraluminal immune and inflammatory cells, and altered airway activity. To determine the direct effect of M. pulmonis upon transepithelial ion transport in the absence of immune and inflammatory cell responses, primary mouse tracheal epithelial cell monolayers (MTEs) were apically infected and assayed in Ussing chambers. M. pulmonis-infected MTEs, but not those infected with a nonmurine mycoplasma, demonstrated reductions in amiloride-sensitive Na⁺ absorption, cyclic AMP, and cholinergic-stimulated Cl⁻ secretion and transepithelial resistance. These effects were shown to require interaction of viable organisms with the apical surface of the monolayer and to be dependent upon organism number and duration of infection. Altered transport due to M. pulmonis was not merely a result of epithelial cell death as evidenced by the following: (i) active transport of Na⁺ and Cl⁻, albeit at reduced rates; (ii) normal cell morphology, including intact tight junctions, as demonstrated by electron microscopy; (iii) maintenance of a mean transepithelial resistance of 440 Ω/cm²; and (iv) lack of leakage of fluid from the basolateral to the apical surface of the monolayer. Alteration in epithelial ion transport in vitro is consistent with impaired pulmonary clearance and altered airway function in M. pulmonis-infected animals. Furthermore, the ability of M. pulmonis to alter transport without killing the host cell may explain its successful parasitism and long-term persistence in the host. Further study of the MTE-M. pulmonis model should elucidate the molecular mechanisms which mediate this reduction in transepithelial ion transport.

Mycoplasmas, the smallest free-living prokaryotes, continue to be a significant cause of respiratory infections in a variety of animals, including humans (44). Their limited biosynthetic capability dictates that these organisms must both colonize and parasitize epithelial cell surfaces. It is therefore surprising that mycoplasmas produce diseases that are slowly progressing and chronic and yet often clinically inconspicuous. The molecular mechanisms responsible for this tenacious truce between the pathogen and the host cell have not yet been identified. Murine respiratory mycoplasmosis, a naturally occurring respiratory disease in laboratory rats and mice caused by Mycoplasma pulmonis (6, 8) and characterized by chronic, often lifelong tracheitis, bronchopneumonia, and bronchiectasis (7, 23, 28), would seem to be an ideal model with which to study these processes, Na⁺ absorption, and accumulation of mucus (23) suggest that M. pulmonis may compromise the ability of the epithelial cells to absorb and secrete fluid and electrolytes.

Airway epithelial cells possess two major active transport processes, Na⁺ absorption and Cl⁻ section. Water, in turn, osmotically follows the transepithelial movement of these ions, thereby providing a fluid film between the mucus layer and the epithelial cell surface. The depth and composition of this fluid microenvironment must be carefully regulated to allow the exchange of gases and the humidification of the airway epithelium and to ensure proper mucociliary clearance (47, 49). The consequences of impaired fluid and electrolyte transport in the airways are strikingly apparent in cases of cystic fibrosis, a recessive genetic disease resulting from the loss of epithelial chloride channels (31, 48). The studies reported here were designed to determine whether M. pulmonis infection alters electrolyte transport across the murine tracheal epithelium.

The effect of bacteria upon mammalian epithelia in the absence of immune and inflammatory cells can be systematically evaluated by using the Ussing chamber model. Short-circuit current (Isc) studies have helped determine the mechanism by which cholera toxin (18) and other enterotoxins affect the intestinal mucosa (17, 35). Study of various types of epithelial cells by using the Ussing chamber model has also resulted in the identification of numerous microbial substances capable of directly altering the ion transport capacity of the airway epithelium (3, 20, 41). In the present study, control and M. pulmonis-infected mouse tracheal epithelial cells (MTEs) were evaluated in Ussing chambers. The results clearly show that M. pulmonis infection directly alters epithelial ion transport and that this alteration is species specific, dose and time related, and dependent upon the association of viable organisms with the apical cell surface.

MATERIALS AND METHODS

Mycoplasmas. Experiments were performed with either a virulent strain of M. pulmonis, CT, originally isolated from a mouse with respiratory mycoplasmatisis (14), or with Mycoplasma fermentans incognitus, a mycoplasma of human origin provided by Shyh-Ching Lo at the Armed Forces Institute of Pathology, Washington, D.C. Organisms were grown, harvested, and stored as previously described (46). M. pulmonis was cultured in mycoplasma broth base (Difco) supplemented with heat-inactivated and filtered horse serum (HyClone Laborat-
Isolation and culture of mouse tracheal epithelial cells. To study the effect of \textit{M. pulmonis} on epithelial electrolyte transport, mouse tracheal epithelial cell cultures were prepared as previously described \cite{51,52}. Animals were sacrificed by \textit{CO}_2\textit{ }inhalation and opened surgically with a ventral midline incision. The intact trachea was stripped of remaining connective tissue and cut longitudinally to expose the epithelial cells. Tissues were washed twice with minimal essential medium (MEM; GIBCO/BRL, Grand Island, N.Y.) and placed in solution A (MEM, 1% fetal bovine serum [HyClone Laboratories], 0.1% protease XIV [Sigma Chemical Co., St. Louis, Mo.], 8 U of DNase [Sigma]) at 4°C for 14 h with intermittent vortexing. Cells were recovered by vigorously vortexing the tracheas in solution A followed by centrifugation at 500 \textit{g} for 5 min. The pelleted cells were resuspended in MEM medium, which was made by mixing equal volumes of preconditioned medium (Dulbecco's MEM [GIBCO/BRL], 10% fetal bovine serum [recollected after feeding a commercial diet to the donor rats], 1 U of DNase [Sigma]) and solution B (250 mM NaCl, 20 mM KCl, 1.8 mM CaCl_2, 1.2 mM MgCl_2, 5 mM K_2HPO_4, 1.2 mM CaCl_2, and 1.2 mM MgCl_2). Glucose (10 mM) was added to the serosal bathing solution. The composition of the mucosal bathing fluid was: 150 mM NaCl, 2.5 mM KCl, 1.2 mM CaCl_2, 1.2 mM MgCl_2, 5 mM K_2HPO_4, 1.2 mM CaCl_2, and 1.2 mM MgCl_2. Glucose (10 mM) was added to the mucosal bathing solution. The concentration of continuous current which is required to nullify the PD is referred to as the \textit{Isc} and is equal to the sum of all net ionic active-transport processes across the epithelium. The fluid resistance was determined for paired and unpaired data, as appropriate. Experimental treatment effects were considered statistically significant if the probability of a type I error was < 0.05 or as otherwise noted.

Electron microscopy. Preparation of ultrathin sections was performed as previously described \cite{39}. Briefly, mice were killed and the tracheas were excised. The tracheas were cut longitudinally and postfixed with 1% osmium tetroxide at room temperature. Dehydration was performed for a graded series of ethanol-water solutions, and specimens were transferred to propylene oxide and embedded in epoxy resin. Ultrathin sections were cut and stained by standard ethanol-water solutions, and specimens were transferred to propylene oxide and embedded in epoxy resin.
RESULTS

Inhibition of epithelial ion transport by M. pulmonis. Strikingly different \( I_{sc} \) profiles were observed when control (mock-infected) and M. pulmonis-infected MTEs were evaluated for \( \text{Na}^+ \) absorption and \( \text{Cl}^- \) secretion in Ussing chambers (Fig. 1). Prior to the addition of amiloride, the control MTE monolayer mounted in Ussing chambers displayed a resistance of 2,336 \( \Omega/cm^2 \) and an \( I_{sc} \) across the monolayer of 27.0 \( \mu A/cm^2 \) (Fig. 1A). The addition of amiloride to the apical buffer rapidly reduced the \( I_{sc} \) to 7.1 \( \mu A/cm^2 \), resulting in the identification of an \( I_{Na} \) of 19.9 \( \mu A/cm^2 \). Forskolin caused an increase in the \( I_{sc} \) of 11.4 \( \mu A/cm^2 \), referred to here as the \( I_{Cl} \). Carbachol, a \( \text{Ca}^{2+} \)-mediated agonist, rapidly increased the \( I_{sc} \) to a peak value of 78.6 \( \mu A/cm^2 \). Subtraction of the \( I_{Cl} \) from this total increase resulted in a \( I_{Cl} \) peak of 67.2 \( \mu A/cm^2 \). The \( I_{Cl} \) peak was similarly calculated and found to be 38.6 \( \mu A/cm^2 \). After an additional 10 to 15 min, the addition of furosemide to the basolateral solution reduced the \( I_{sc} \) to 21.4 \( \mu A/cm^2 \), verifying that the \( I_{sc} \) was due to net \( \text{Cl}^- \) secretion. MTE monolayers that had been infected with M. pulmonis were similarly evaluated and demonstrated a dramatic decrease in transepithelial ion transport (Fig. 1A and D). After monolayers were infected with an initial inoculum of \( 1.9 \times 10^7 \) CFU (Fig. 1D), the \( I_{Na} \) was 1.8 \( \mu A/cm^2 \), and the \( I_{Cl} \) and \( I_{Cl} \) peak values were 4.29, 11.4, and 6.8 \( \mu A/cm^2 \), respectively. The results of 13 to 16 additional experiments are summarized in Table 1. In addition, the transepithelial resistance of infected monolayers was also substantially altered. These monolayers displayed a resistance of only 440 ± 87 \( \Omega/cm^2 \) (mean ± SEM). Unidirectional [\( ^3\text{H} \)]mannitol flux studies revealed a 2.1-fold-higher flux across M. pulmonis-inoculated monolayers (2.3 ± 0.33 nmol, \( n = 4 \)) than across control monolayers (1.1 ± 0.21 nmol, \( n = 4 \)) (\( P < 0.01 \)). These results are consistent with the 5.4-fold-lower resistance of the M. pulmonis-infected monolayers described above and confirm that M. pulmonis significantly decreases the paracellular resistance of the MTE monolayers.

In contrast to the results described above in which the apical surface was infected, paired experiments in which the basolateral surface of the MTEs was exposed to either the control medium or the mycoplasma inoculum (\( 2 \times 10^7 \) to \( 2 \times 10^8 \) CFU) were performed. These experiments revealed a similar inhibition of ion transport in the basolateral direction. The results of these experiments are summarized in Table 2.

FIG. 1. Dose-dependent effect of M. pulmonis on \( I_{sc} \) from MTE monolayers. Representative \( I_{sc} \) traces showing \( 
\text{Na}^+ \) and \( \text{Cl}^- \) currents in control or M. pulmonis-infected MTE monolayers are presented. (A) \( I_{sc} \) trace from medium-inoculated MTE monolayer (control); (B to D) \( I_{sc} \) trace from MTE monolayers inoculated with increasing numbers of M. pulmonis cells (\( 1.9 \times 10^6 \) [B], \( 6.3 \times 10^6 \) [C], and \( 1.9 \times 10^7 \) [D] CFU). Additions to the Ussing chamber were 10 \( \mu M \) amiloride, mucosal side (a), 10 \( \mu M \) forskolin, mucosal and serosal sides (b), 100 \( \mu M \) carbachol, serosal side (c), and 100 \( \mu M \) furosemide, serosal side (d). Deflections represent the change in current resulting from the ±5-mV pulse, and their values are used to determine the transepithelial resistance.
CFU, (n = 6) were also performed. The \( I_{sc} \) values for monolayers exposed basolaterally to the control inoculum included an \( I_{Na}^{+} \) of 23.4 ± 2.8 \( \mu A/cm^{2} \), an \( I_{Cl-CAMP} \) of 11.9 ± 1.5 \( \mu A/cm^{2} \), and an \( I_{CI-peak} \) of 108.0 ± 12.5 \( \mu A/cm^{2} \) at 48 h. Prior to the assessment of the transport capacity of the MTE filters that had been basolaterally infected with \( M. \) \textit{pulmonis}, the basolateral feeding medium was cultured to determine the number of CCU of \( M. \) \textit{pulmonis} present. Approximately 10\(^6\) CCU of \( M. \) \textit{pulmonis} were routinely recovered from the basolaterally infected filters which displayed an \( I_{Na}^{+} \) of 24.5 ± 1.7 \( \mu A/cm^{2} \), an \( I_{Cl-CAMP} \) of 11.5 ± 1.6 \( \mu A/cm^{2} \), and an \( I_{CI-peak} \) of 118.8 ± 8.2 \( \mu A/cm^{2} \). These results indicate that exposure of the basolateral surface of the monolayer of \( M. \) \textit{pulmonis} did not alter electrogenic ion transport.

Altered transport following exposure of the apical surface to \( M. \) \textit{pulmonis} was not merely a result of epithelial cell death, as evidenced by the following: (i) maintenance of a mean transepithelial resistance of 440 \( \Omega/cm^{2} \), which is 20 times greater than fluid resistance; (ii) lack of leakage of fluid from the basolateral to the apical surface; and (iii) normal cell morphology, including intact tight junctions, as demonstrated by electron microscopy performed at the time of altered ion transport. Transmission electron micrographs of control MTEs revealed a confluent monolayer of uniform, columnar epithelial cells characterized by tight junctions, microvilli, and a highly convoluted basolateral membrane (data not shown). These characteristics are consistent with the high electrical resistance measured in the Ussing chambers. MTEs examined as late as 48 h after \( M. \) \textit{pulmonis} infection were morphologically identical to the control monolayers (data not shown). Mycoplasmas were not observed in association with the epithelial cells; however, quantitative cultures of infected monolayers documented that the organisms were not only present but also replicating (Table 2). Since the number of epithelial cells comprising the confluent monolayer was estimated to be 3.0 × 10\(^5\), at 48 h postinoculation, the number of mycoplasmas per cell (i.e., approximately 17) may not be easily detectable by electron microscopy.

Effect of organism number and duration of exposure. Increasing the number of \( M. \) \textit{pulmonis} cells in the infecting inoculum resulted in a dose-dependent decrease in both ion transport and transepithelial resistance at 48 h (Fig. 1). The length of exposure of the monolayer to the \( M. \) \textit{pulmonis} inoculum was also varied so that shorter incubation times could be evaluated. Monolayers that were inoculated with \( M. \) \textit{pulmonis} for 1, 3, or 6 h, washed, and immediately assayed displayed a dramatic time-dependent decline in their \( I_{sc} \) profile compared to that of the control filters (Fig. 2). The monolayers exposed for 6 h displayed a mean transepithelial resistance of only 53 \( \Omega/cm^{2} \), whereas the controls maintained a resistance of 1,571 \( \Omega/cm^{2} \). In addition, the infected monolayers did not display \( Na^{+} \) transport and no longer responded to pharmacological stimulators of \( Cl^{-} \)secretion. Interestingly, 6-h-infected MTEs that were washed and allowed to recover for an additional 18 h displayed resistance values of 1,434 ± 439 \( \Omega/cm^{2} \) (n = 10). These values were similar to those of the control MTEs of 1,746 ± 312 \( \Omega/cm^{2} \) (n = 14) (Fig. 2).

### Table 1. Effect of mycoplasma on MTE epithelial ion transport

<table>
<thead>
<tr>
<th>Inoculum (no. of exps)</th>
<th>( I_{Na}^{+} ) value (( \mu A/cm^{2} ))</th>
<th>Resistances (( \Omega/cm^{2} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( I_{Na}^{+} )</td>
<td>( I_{Cl-CAMP} )</td>
</tr>
<tr>
<td>Hayflick’s medium (control) (n = 16)</td>
<td>35.1 ± 2.8</td>
<td>8.42 ± 1.1</td>
</tr>
<tr>
<td>( M. ) \textit{pulmonis} CT (n = 13)</td>
<td>8.7 ± 1.6</td>
<td>4.4 ± 0.7</td>
</tr>
<tr>
<td>SP4 medium (control) (n = 4)</td>
<td>43.1 ± 5.7</td>
<td>21.9 ± 4.3</td>
</tr>
<tr>
<td>( M. ) \textit{fermentans} incognitus (n = 6)</td>
<td>34.2 ± 3.8</td>
<td>17.7 ± 2.0</td>
</tr>
</tbody>
</table>

* MTEs were inoculated for 6 h, washed, and immediately assayed.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>M. \textit{pulmonis}</th>
<th>M. \textit{fermentans}</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>((3.0 ± 0.01) \times 10^4)</td>
<td>ND</td>
</tr>
<tr>
<td>48</td>
<td>((5.1 ± 1.1) \times 10^5)</td>
<td>((8.2 ± 2.9) \times 10^5)</td>
</tr>
<tr>
<td>72</td>
<td>((8.6 ± 3.5) \times 10^5)</td>
<td>ND</td>
</tr>
<tr>
<td>96</td>
<td>((1.2 ± 1.9) \times 10^7)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Values are means ± standard errors for numbers of CFU recovered from infected filters 48 h postinoculation. ND, not determined. n, numbers of experiments.
DISCUSSION

Previous in vitro studies have indicated that *M. pulmonis* can attach to eukaryotic cells within 1 h of exposure (25). The results presented here provide evidence that upon interaction with the respiratory epithelium, *M. pulmonis* may rapidly alter the electrolyte composition of the fluid layer which lines the airways of its natural host. A 1-h exposure of MTEs to *M. pulmonis* resulted in a substantial decrease in its ion-transporting capacity and its electrical resistance. As the length of exposure to the inoculum was increased, both transport functions continued to decrease. MTEs inoculated with *M. pulmonis* for 6 h and then immediately assayed had lost all detectable transport function and almost all transepithelial resistance. Those that were exposed to the inoculum for 6 h, washed, and then incubated for an additional 18 h recovered all of their transepithelial resistance and some of their active-transport capacity. Thus, a 6-h exposure severely compromised the barrier integrity and transport capacity but did not kill the epithelial cells. Several explanations may account for the observed recovery of the monolayer resistance as well as the partial recovery of active transport. The first suggests that the number of organisms associated with the monolayer directly influences its transport capacity. Washing the monolayer after a 6-h exposure to the inoculum may have removed most of the *M. pulmonis* cells unassociated with the monolayer, thereby allowing it to recover. Forty-eight hours after inoculation, another significant decrease in the resistance of the monolayers occurred. This was again consistent with a measured increase in the number of organisms associated with the monolayer.
of M. pulmonis cells associated with the monolayer. A second explanation suggests the intriguing possibility that the interaction of M. pulmonis with the monolayer may cause a change in the population of mycoplasma cells. Adherence to the monolayer could induce or select for a less cytopathic population of organisms, allowing the epithelium to survive and reestablish ion transport but at a much reduced level. In these studies, the switching of the organisms from liquid culture media to an air-epithelium interface could trigger a phenotypic change in the cell-associated population. This explanation is consistent with previous studies that have shown that in infected animals M. pulmonis undergoes both phenotypic and genotypic changes which are associated with chronicity (37, 45). In contrast to the transepithelial resistance, when the monolayer's capacity for Na\(^+\) absorption or Cl\(^-\) secretion was inhibited, transport of neither pathway returned to control levels. These results suggest that the decrease in transepithelial resistance and the inhibition of electrogenic ion transport by M. pulmonis may be independently mediated.

Together, these studies indicate that the addition of M. pulmonis to the apical surface of MTEs rapidly alters both epithelial barrier integrity and transport function. The failure of M. pulmonis to affect ion transport from the basolateral side reinforces the importance of interaction with the apical surface and is consistent with in vivo studies of mice which suggest that M. pulmonis remains limited to the apical surface, i.e., it is rarely found in the submucosa (39). The epithelial cells were grown on a 0.4-μm-pore-size filter, and it is possible that the filter precluded the interaction of the mycoplasmas with the basolateral cell surface despite their small cellular size (180 to 300 nm) and their plasticity associated with the lack of a cell wall (44). M. pulmonis cells (10^6/ml) were recovered from the basolateral medium after a 48-h incubation, indicating that their presence on the basolateral side was insufficient to alter transport. We were unsuccessful in our attempts to grow MTEs on a more porous support (4.0-μm pore size) to test further whether MTE-M. pulmonis contact at the basolateral membrane would alter transport.

Other investigators have previously shown that M. fermentans, a mycoplasma of human origin, does not cause cytopathic effects in mice following intranasal inoculation or in murine tracheal organ cultures (40). Consistent with these results, ion transport was also not altered when the MTE monolayers were infected with M. fermentans, despite the large numbers of M. fermentans cells recovered from the monolayers at 48 h postinfection. Thus, the presence of mycoplasma cells per se does not lead to the alterations observed in the MTEs but rather suggests that a species-specific interaction between M. pulmonis and the MTE monolayer would seem to be required. To verify this, additional species of mycoplasma must be studied in the MTE monolayer system.

Although several potential pathogenic factors have been identified in the mycoplasmas, including peroxidases (10), endonucleases (30, 32), phospholipases (16, 34), and a membrane-associated hemolytic activity (10, 27), the molecular mechanisms by which M. pulmonis colonizes the respiratory epithelium and establishes chronic airway disease are unknown. Exposure of the monolayers to M. pulmonis culture supernatants did not alter Na\(^+\) absorption, Cl\(^-\) secretion, or the resistance of the MTEs. These results indicate that the M. pulmonis component responsible for the alterations in electrogenic ion transport and the decrease in epithelial resistance of the MTE monolayer is not released by M. pulmonis cells under normal laboratory culture conditions but requires the intact organism. Additional studies will be needed, however, to determine if the inhibiting activity is selectively secreted by

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**FIG. 3.** Lack of effect of M. pulmonis culture supernatant or UV-irradiated M. pulmonis cells on epithelial ion transport. (A) Monolayers were incubated with either sterile mycoplasma medium (control) or filtered (0.1-μm pore size) supernatant obtained from M. pulmonis stocks for 6 h. Subsequently, the monolayers were washed twice with MTE conditioned medium and incubated for an additional 42 h prior to evaluation in Ussing chambers. Results are the mean $I_{sc}$ values obtained from three experiments ± SEM. (B) Lack of effect of UV-irradiated M. pulmonis inoculum for 6 h. After incubation, the monolayers were washed twice with MTE conditioned medium and incubated for an additional 42 h in Ussing chambers prior to evaluation. Results are the mean $I_{sc}$ values obtained from five experiments ± SEM.
mycoplasma cells upon contact with the epithelium. Several studies have examined the pathogenic potential of purified mycoplasma membranes, cell lysates, and nonviable organisms (2, 12, 19, 26). In our studies, *M. pulmonis* cells that were killed by UV treatment were no longer able to inhibit the absorption or secretion of ions or to decrease the transepithelial resistance of the monolayer.

Numerous researchers have reported that the infection of tracheal organ cultures with mycoplasmas results in ciliositis, loss of tight junctions, and in most cases the complete exfoliation of the respiratory epithelium (2, 9, 11, 15, 24, 39). This study, in contrast, evaluated the electrogenic ion transport capacity of MTEs infected with *M. pulmonis* and reveals that this organism causes much more subtle changes in the respiratory epithelium than have previously been observed by other investigators. The inhibition of Na⁺ absorption and Cl⁻ secretion and the concomitant loss in resistance of the epithelial monolayer suggest that *M. pulmonis* infection leads to the formation of a new functional steady state in which the monolayer continues to absorb and secrete fluid and electrolytes but at a much reduced level. The pathophysiological consequences of this reduced functional state are a change in the volume and composition of the fluid which bathes the airways. This could, in turn, compromise ciliary movement and mucus hydration and ultimately lead to a reduction in mucociliary clearance.

Ion transport across the epithelial monolayer is a highly complex and tightly regulated molecular cross talk between extracellular signals, intracellular second messengers, and the transport proteins in the apical and basolateral cell membranes. Therefore, the modulation of ion transport and transepithelial resistance by *M. pulmonis* could occur at a variety of sites. The absorption and secretion of fluid and electrolytes across the respiratory epithelium involve the coordinated movement of ions across the apical and basolateral membranes through highly selective Na⁺, Cl⁻, and K⁺ channels, the Na⁺-K⁺ pump, and the Na⁺-K⁺-2Cl⁻ cotransporter, any of which could be directly or indirectly affected during mycoplasmal infection. The ability of *M. pulmonis* to alter host cell second messengers is a logical explanation for the observed decrease in both Na⁺ and Cl⁻ transport; however, the factors mediating these alterations have yet to be clearly addressed for any mycoplasma species (36). Both Na⁺ and Cl⁻ were inhibited in our studies, suggesting that *M. pulmonis* may alter a single step which is central to both pathways (e.g., the basolateral K⁺ channel). This notion is supported by the recent studies of Izutsu et al., who showed that *Mycoplasma orale* infection affects the expression and regulation of K⁺ channels in the surface membrane of a human submandibular gland cell line (22).

Recent studies by Smith et al. (38) indicate that pathogenic microorganisms may persist in the airways of patients with cystic fibrosis because bacteriocidal factors normally produced by the respiratory epithelium are inactivated by abnormal salt concentrations. Our results suggest that the ability of *M. pulmonis* to alter transepithelial ion transport may allow it to escape killing by a similar mechanism. This is further supported by the fact that *M. pulmonis*-infected animals are incapable of clearing the organism from the respiratory epithelium and are also highly susceptible to secondary infections. It is safe to assume that the pathophysiology which underlies chronic mycoplasmal infections is undoubtedly complex and multifactorial in origin. The methodologies described herein may be useful in determining which pathogenic factors are involved and, in turn, guide us toward the most logical approach for therapy against persistent mycoplasmal diseases.

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REFERENCES


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