ROLE OF INNATE IMMUNITY IN RESPIRATORY MYCOPLASMA INFECTION

Judy M. Hickman-Davis

University of Alabama at Birmingham, Department of Anesthesiology, Birmingham, AL

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1. ABSTRACT

Mycoplasmas are unique among respiratory pathogens. They possess very small genomes, lack cell walls and are strictly dependent on the host for survival. These pathogens have developed the ability to quickly adapt to the host environment through attachment to target cells within the host. Mycoplasmas have been identified as commensal microbial flora of healthy persons yet, infection of the upper and lower respiratory tracts can result in acute cough, fever and headache, and even chronic disease involving multiple organs. The lung contains a complex system of defense mechanisms with which to combat these pathogens, including innate (nonspecific) and acquired (specific) immune responses. Innate defenses include mechanical clearance, cellular responses provided by host phagocytes and molecular protection in the form of antimicrobial peptides. The interaction of mycoplasmas with different components of the innate immune system and mechanisms by which they incite pathology has proved elusive. The mechanisms by which pathogenic mycoplasmas evade the innate immune system are unknown. The purpose of this review is to summarize current knowledge of these interactions in the hope of identifying new avenues for research and therapy.

2. INTRODUCTION

2.1. Overview
Mycoplasma pneumoniae is one of the leading causes of pneumonia worldwide. Infections due to this agent occur in smoldering, year round endemics and in cyclic 4-7 year epidemics. In the U.S., *M. pneumoniae* accounts for 20-30% of all pneumonias in the general population, for a total of 8-15 million cases per year (1). *M. pneumoniae* frequently exacerbates asthma, and chronic obstructive pulmonary disease (COPD) (2). Furthermore, it is becoming increasingly apparent that because of the great diversity of clinical manifestations and the special testing required to distinguish active infection, the role of *M. pneumoniae* as a cause of severe disease in the lungs and other organs is much under diagnosed (3).

Efforts to understand protective immunity against mycoplasma infections of the respiratory tract through studies of specific immune mechanisms have proved disappointing for both human (1) and animal mycoplasma infections. It remains unknown whether specific immunity has a role in protection against respiratory mycoplasmas, with one exception. Specific antibody clearly is important in the late stages of *M. pneumoniae* infection as patients with hypogammaglobulinemia develop chronic mycoplasma lung disease and systemic infections such as arthritis (4). Similarly, other mycoplasmas, including *Mycoplasma hominis*, *Mycoplasma salivarium* and *Ureaplasma urealiticum*, cause chronic infections with arthritis in hypogammaglobulinemic patients [reviewed in Cassell (5)]. *M. pneumoniae* also does not cause more severe pneumonia in patients with T-cell deficiencies (4,6). Animal models utilizing T-cell-deficient and severe

combined immunodeficient (SCID) mice experimentally infected with *M. pulmonis* have similar levels of mycoplasmas within whole lung homogenates as compared to immunocompetent controls although SCID mice eventually develop systemic disease including arthritis (7). Thus, innate immunity is important for control of acute infection while specific immunity appears to be important for the control of dissemination of organisms to other organs of the body. The purpose of this review is to summarize current knowledge concerning innate immunity and mycoplasma infection, emphasizing recent advances in the understanding of how pulmonary collectins, and reactive oxygen and nitrogen species are involved in mycoplasma clearance.

2.2. Pulmonary innate immunity

The pulmonary innate immune system consists of structural, cellular and protein components that are capable of immediately targeting, removing and/or killing invading organisms whenever they infect the host. These processes are rapid, nonspecific and occur before the development of the acquired or adaptive immune response.

The mechanical or structural host defenses are composed of the cough reflex and mucociliary clearance by respiratory epithelium, and which comprise the initial barrier to invasion of the lung by infectious agents (8). The primary cellular components of the innate system are alveolar macrophages (AMs) which form the first line of phagocytic defense, polymorphonuclear cells (PMNs) which are rapidly recruited from the lung vasculature in response to mycoplasma infection (9), and natural killer (NK) cells which are thought to be of primary importance for mycoplasma killing in the respiratory tract of mice (10). Cells of the upper and lower respiratory tract also produce a wide range of antimicrobial peptides including collectins, defensins, lysozyme, complement, fibronectin, lactoferrin, transferrin and cathelicidins [recently reviewed in (11)]. A number of innate antimicrobial mechanisms require transcriptional induction of genes important for host defense, such as inducible nitric oxide synthase (iNOS). iNOS produces nitric oxide (NO•), an important antimicrobial product of AMs and PMNs (12).

2.3. Animal models

Most of the reports describing the interaction between mycoplasmas and the innate immune system have utilized the rodent model of *Mycoplasma pulmonis* infection in rats and mice. *M. pulmonis* infection in mice provides an excellent animal model that reproduces the essential features of human respiratory mycoplasmosis. Mouse strains differ markedly in resistance to *M. pulmonis*, with C57BL/6 and C3H/He mice representing the extremes in response to this infection. C57BL/6 mice have a 100-fold higher 50% lethal dose, 50% pneumonia dose, and 50% microscopic lesion dose than C3H/He mice (13). During the first 4 hours post infection (p.i.), the numbers of mycoplasmas decrease by more than 83% and maximum mycoplasmacidal activity occurs within 8 hours p.i. in the lungs of C57BL/6 mice. In contrast, within the lungs of C3H/He mice, mycoplasma numbers increase by 18,000% by 72 hours p.i. Mechanical clearance as well as the

number of AMs, PMNs, or lymphocytes in the lungs does not increase during the first 48 hours p.i., although by 72 hours p.i. C3H/HeN mice exhibit a large increase in PMNs that does not correlate with mycoplasma killing (9). Measurement of *M. pulmonis*-specific serum antibody titers from infected mice have demonstrated immunoglobulin (Ig) M class levels do not appear until 7 days and IgA and IgG class levels until 14 days p.i. (9,14). Based on these data, nonspecific intrapulmonary killing of *M. pulmonis* occurs and is most likely mediated by rapidly activated resident AMs. However, it must be remembered that in the absence of specific antibody, clearance of *M. pulmonis* from the respiratory tract is greatly influenced by the strain of mycoplasma (15), mouse (13,16) or rat (17) studied. Thus differences in disease severity reflect differences in a wide range of organism and host factors.

3. CELLULAR RESPONSES

There is strong indirect evidence that innate immunity involving AMs but not PMNs is of major importance in antimycoplasmal defense of the lungs. The clearance of *M. pulmonis* from the peritoneal cavity of CBA mice was enhanced by increased macrophage numbers but not by increased PMNs (18), and the ability of different strains of *M. pulmonis* to survive and produce disease within the respiratory tract of CBA mice was correlated to the ability of these mycoplasmas to avoid phagocytosis by AMs (19). Likewise, in a bovine mastitis model, induction of a neutrophilia in response to endotoxin failed to control a secondary challenge with *M. dispar* or ureaplasmas (20). C57BL/6 mice exposed to nitrogen dioxide and infected with *M. pulmonis* demonstrated a decreased clearance of respiratory mycoplasmas that could not be correlated with damage to the respiratory epithelium, to mechanical clearance, or to the induction of an acute inflammatory response (21). Subsequently, it was demonstrated that exposure to nitrogen dioxide damages AMs, and that intrapulmonary killing of mycoplasmas decreased as AM viability decreased and increased as AM viability was restored (23). Finally, studies designed to determine the role of PMNs and antibody in the clearance of *M. pneumoniae*, *M. salivarium*, *M. hominis* and *U. urealyticum* suggest that human PMNs play no part in anti-mycoplasma defense as mycoplasmas were rapidly ingested regardless of the presence or absence of antibody but remained viable within the cells. Through this mechanism PMNs were hypothesized to contribute to the dissemination of mycoplasmas to other organs (23).

Macrophage depletion has been used to investigate the protective roles of AMs in the lung (24) and the resident macrophages in the liver and spleen (25). To further delineate the role of AMs in early clearance of mycoplasmas from the lung, intratracheal insufflation of liposome encapsulated dichloromethylene bisphosphonate (L-Cl$_2$MBP) was used to selectively deplete AMs in mice (26). Cl$_2$MBP is a bisphosphonate compound used clinically for the treatment of osteolytic bone diseases. The drug itself is not toxic, does not easily cross cell membranes and has an extremely short half life in circulation (27). When encapsulated into multi-lamellar

liposomes, Cl_2MBP is highly specific for phagocytic cells. Phagocytes ingest the L-Cl_2MBP which are degraded in the lysosome releasing free Cl_2MBP into the cytoplasm and causing cell death. However, PMNs appear to be functionally and morphologically unaffected by L-Cl_2MBP both *in vivo* and *in vitro* presumably because of their low liposome ingestion (28). The precise mechanism of Cl_2MBP cytotoxicity for macrophages is unknown, but it may be due to depletion of iron or other metal complexes in the cell, or a direct effect on ATP metabolism (27). L-Cl_2MBP has little effect on alveolar epithelium or interstitial macrophages (29), although free Cl_2MBP was shown by electron microscopy to cause edema of the alveolar epithelium (24). AM depletion prior to infection with *M. pulmonis* reduced mycoplasma killing in resistant C57BL/6 mice to a level comparable to that in susceptible C3H/He mice without AM depletion. In contrast, AM depletion did not alter killing of mycoplasmas in the lungs of infected C3H/He mice (26). These *in vivo* results directly identify the AM as the primary phagocytic cell important for early mycoplasmal resistance of C57BL/6 mice.

Several studies have identified the NK cell as being important in the innate immune response of the host to mycoplasmas. Mitogen isolated from *Mycoplasma arthritidis* augmented human NK cell activity in a dose dependent manner either directly by increasing their lytic capacity or indirectly as a consequence of cytokine production by T cells (30). Intraperitoneal infection of C57BL/6J mice with *M. pulmonis* transiently increased splenic NK cell tumoricidal activity with peak activation occurring on day 3 p.i. (31). Likewise, it was demonstrated that intraperitoneal infection of C57BL/6J mice with *M. pulmonis* also increased pulmonary NK cell activity and that this activity was abrogated by pretreatment of these mice with the NK cell blocking antibody anti-asialo GM1. Depletion of NK cells from the lungs and spleens of these mice prior to challenge with *M. pulmonis* effectively blocked killing of this pathogen *in vitro* (32). NK cells isolated from SCID mice and infected with mycoplasmas secrete interferon (IFN)-gamma and pretreatment of SCID mice with anti-asialo GM1 or anti-IFN-gamma prior to intratracheal infection with *M. pulmonis* decreased intrapulmonary clearance of mycoplasmas (32). Secretion of IFN-gamma by NK cells has been shown to activate AMs, and IFN-gamma mRNA has been demonstrated in the lungs of C57BL/6N and C3H/HeN mice infected with *M. pulmonis* as early as 24 hours p.i. (33).

AM activation has been demonstrated to be essential for killing of *M. pulmonis in vitro* (34). Several species of mycoplasma induce production of the proinflammatory cytokines. Mycoplasma infection of mouse or human macrophages stimulate the production of tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-6 *in vitro* (35-38) via a mechanism distinct from that of lipopolysaccharide (LPS) (39). Likewise, studies utilizing peritoneal macrophages from LPS-resistant C3H/HeJ mice identified in *M. fermentans* a 2 kD macrophage-activating lipopeptide (MALP-2) capable of activating macrophages to produce NO• (40). Recent studies indicate that MALP-2 has endotoxin-like capabilities but does not utilize the Toll-like receptor 4 signaling pathway (41). *In vivo* infection studies utilizing *M. pulmonis* and *M. pneumoniae* indicate that during primary infection, transcription of cytokine genes for TNF-alpha, IFN-gamma, IL-1beta and IL-6 is induced within the first 24 hours p.i. (34, 43). These data indicate that mycoplasmas activate host cells very early during the infection process thereby triggering an inflammatory response that may aid in the colonization of the host or conversely may prime the host innate immune response for mycoplasma killing.

4. PHAGOCYTOSIS

4.1. Nonspecific-opsonins

In vitro, studies of host defense against mycoplasmas have concentrated on the role of phagocytes and their ability to phagocytose mycoplasmas. Most mycoplasmas *in vitro* resist phagocytosis in the absence of some type of opsonin [reviewed in (10)]. In the absence of specific antibody, mycoplasmas can attach to the surface of phagocytes but are not ingested (43). The mechanisms by which most mycoplasmas resist ingestion are unknown, although resistance of *M. pulmonis* has been shown to be trypsin sensitive, suggesting the presence of an antiphagocytic surface protein (44). In the presence of specific antibody, however, mycoplasmas are rapidly ingested and 90 to 99% of cell associated mycoplasmas are killed within 4 hours (18). Previous studies also have shown that the concentrated noncellular portion of lavages from *M. pulmonis*-infected C57BL/6 mice, although unable to kill mycoplasmas alone, could initiate killing of mycoplasmas when introduced into AM cultures (45). The requirement of opsonins for *in vitro* mycoplasmacidal activity coupled with the *in vivo* data showing significant clearance of organisms by resistant animals within hours of infection suggests the presence of some nonspecific opsonin. Nonspecific opsonization may involve the deposition of complement breakdown products, C-reactive protein, fibronectin, or surfactant proteins. There are surprisingly little data concerning the role of any of these nonspecific opsonins on mycoplasma clearance. Early studies performed with *M. pulmonis*-infected mouse peritoneal macrophages *in vitro* indicated that complement was not as effective as specific antibody in enhancing attachment and ingestion of mycoplasmas by macrophages (46). Subsequently, the ability of mycoplasmas to avoid killing by the alternative complement pathway was identified as a possible virulence factor (47). Studies involving *M. pneumoniae*, *M. salivarium*, *M. hominis* and *U. urealyticum* showed that these mycoplasmas did indeed activate the classical complement pathway but were not killed by complement alone (23). However, a number of studies have identified a role for complement, specifically C3a and C3b, in the sterilization of mycoplasma infected cell lines (48,49).

4.2. Surfactant proteins

Recently, a number of *in vivo* and *in vitro* studies have concentrated on the role of surfactant proteins in mycoplasma binding and clearance. Surfactant proteins

provide the alveolar lining fluid with a first line of defense against infection that can act quickly before specific immunity is acquired. Surfactant proteins (SP)-A and SP-D are hybrid molecules termed collectins that belong to the Ca^{2+}-dependent animal lectin superfamily. Collectins are carbohydrate-binding proteins (other than antibodies and enzymes) that share the common structural characteristics of an amino-terminal collagen-like domain connected to a Ca^{2+}-dependent carbohydrate-recognition domain (CRD). SP-A and SP-D are expressed in the lungs of all mammals, while humans and rodents also express the serum collectin mannose-binding protein (MBP), and cattle express three serum lectins: conglutinin, CL-43 and CL-1 (50). Collectins are assembled as oligimers of trimers with SP-A or MBP assembling into hexamers of trimers. SP-A and MBP resemble the first complement protein component Clq in their arrangement of eighteen CRDs with kinked collagen stalks. SP-D and conglutinin arrange their globular heads around collagen spokes as cruciform structures, and CL-43 has the simplest arrangement of a single unit consisting of three polypeptides. The collectins are ideally suited to the role of first line defense in that they are widely distributed, capable of antigen recognition and can discern self versus non-self (51). They recognize bacteria, fungi and viruses by binding mannose and N-acetylglucosamine residues on microbial cell walls (52). The collectins bind a wide range of pathogens *in vitro*; however, it is likely that regulatory and microbial specificity exists for collectin binding *in vivo*.

SP-A and SP-D are thought to participate in two major physiologic processes: (i) the regulation of surfactant homeostasis associated with tubular myelin formation and (ii) nonspecific innate immune responses of the lung (50,53). With the creation of SP-A deficient mice, there has been a greater emphasis placed on the role of SP-A following infectious or toxic insult. SP-A knockout mice have an increased susceptibility to infection with *M. pulmonis* as compared to immunocompetent controls although there are no apparent breeding or survival abnormalities when these mice are maintained free of all other pathogens (54). The presence of SP-D in SP-A knockout mice may account for the lack of gross abnormalities in respiratory function. Although SP-A and SP-D probably have discrete functions in the normal animal, similar anatomic distribution and structure may allow SP-A and SP-D to be functionally interchangeable under certain conditions.

SP-A interacts with AMs in a highly specific manner through a cell surface SP-A receptor (55). SP-A has been shown to: (i) effect release of reactive oxygen species from AMs (56); (ii) stimulate chemotaxis of monocytes (57); (iii) enhance phagocytosis and killing of bacterial, viral and fungal pathogens by AMs (58-61); and (iv) enhance FcgammaR- and ClqR-mediated phagocytosis *in vitro* (62). It has been demonstrated that SP-A is capable of stimulating phagocytosis of bacteria to which it binds (59). SP-A was shown to bind to *M. pulmonis* in a concentration- and partially Ca^{2+}-dependent manner. While significant binding to mycoplasmas was seen in the absence of Ca^{2+}, binding increased by as much as 70% in the presence of Ca^{2+}. SP-A did not bind to mycoplasmas at low concentrations (0 to 5 µg/ml) of SP-A (34); however, this is not surprising considering that mycoplasmas lack cell walls and are the smallest of the self-replicating organisms (5) which may allow mycoplasmas to effectively avoid interaction with SP-A except at higher concentrations. Recently, Voelker et al. have developed a number of point and domain mutants of SP-A and SP-D (63-68) in order to determine the structure and function of these proteins in interactions with mycoplasma membrane components. Direct comparison of lipopolysaccharide and *M. pneumoniae* binding to SP-A and SP-D variants have demonstrated that these proteins can be engineered to display selective binding to this pathogen, a fact that may be useful for manipulating host-pathogen interactions and controlling infection (69).

The role of SP-A in early antimycoplasmal defense has been studied extensively utilizing C57BL/6 AMs and the *M. pulmonis* strain UAB CT. To determine the capability of SP-A to stimulate phagocytosis and killing of mycoplasmas, isolated AMs were activated with IFN-gamma, incubated with SP-A and infected with *M. pulmonis*. SP-A significantly enhanced the killing of mycoplasmas with a maximal decrease of 83% in total recoverable organisms by 6 hours p.i. Mycoplasma killing was SP-A- and time-dependent, with the SP-A-mediated mycoplasmacidal effect being lost by 8 hours p.i. when SP-A became depleted in the media (34). SP-A-mediated mycoplasma killing by AMs is thought to involve modulation of AM function by SP-A rather than SP-A working as a nonspecific opsonin for several reasons: (i) M. pulmonis preincubated with SP-A prior to the addition to IFN-gamma-activated AMs does not result in significant killing; (ii) killing occurs when SP-A is adhered to AMs and the excess SP-A removed prior to infection with M. pulmonis and (iii) mycoplasmas do not preferentially bind to SP-A-treated AMs (34). SP-A is known to effect the release of reactive nitrogen species (70,71), another mechanism that could account for the SP-A mediated mycoplasmacidal activity of AMs.

5. REACTIVE OXYGEN-NITROGEN SPECIES

NO• production following IFN-gamma stimulation of macrophages has been shown to play an important role in the control of intracellular and extracellular pathogens (72). The addition of the inducible nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA), to AM cultures prior to treatment with SP-A and infection with *M. pulmonis* abrogated the SP-A mediated mycoplasmacidal activity, indicating that NO• is involved in mycoplasma killing. Nitrate and nitrite, the decomposition products of NO•, were significantly increased in cultures containing SP-A and decreased in cultures containing L-NMMA, further implicating NO• as a factor involved in this SP-A mediated effect (34). Likewise, pretreatment of these cultures with superoxide dismutase (SOD) abrogated SP-A-mediated mycoplasma killing indicating that superoxide is also important for mycoplasma killing (54). Peroxynitrite is a strong oxidizing or nitrating agent formed by AMs or PMNs as a

reaction product of superoxide and NO• and is highly bactericidal (73). In the absence of AMs, NO• and superoxide alone are not toxic to *M. pulmonis*, however, peroxynitrite killed mycoplasmas in a time and concentration dependent manner (54).

In vivo, C57BL/6 mice lacking iNOS [C57BL/6 iNOS(-/-)] infected with *M. pulmonis* had higher lung lesion scores and higher lung bacterial loads starting at 24 hours p.i. (54). Nitrotyrosine formation in tissues has been utilized as an indicator of reactive nitrogen species production either by AMs via iNOS or by PMNs via interactions of myeloperoxidase with nitrite (74). C57BL/6 iNOS(-/-) mice infected with mycoplasmas showed significant levels of tissue nitration mainly in areas containing high levels of PMNs. This finding indicates that indiscriminant production of toxic nitrogen radicals is not sufficient to kill mycoplasmas and that iNOS-mediated production of NO• by AMs is crucial for mycoplasmal killing *in vivo* (54). Depletion of PMNs with cyclophosphamide in C57BL/6 iNOS(-/-) mice prior to infection with *M. pulmonis* had no effect on mycoplasma numbers recovered from the lung i.e., the already high number of recoverable mycoplasmas did not increase, but lesion severity and nitrotyrosine staining were decreased indicating that PMNs contribute significantly to nitration of tissues and lesion formation during mycoplasma infection (75). Interestingly, treatment of C57BL/6 iNOS(+/+) control mice with cyclophosphamide prior to infection with mycoplasmas significantly decreased mycoplasma clearance from the lung, an effect which correlated with decreased NO• production by iNOS (75). In other studies, cyclophosphamide treatment has also been linked to NK cell dysfunction with decreased mycoplasma killing (32).

While NO• is a well recognized molecule of microbicidal macrophages, the mechanism(s) by which NO• aids in host defense remain undefined. NO• may have a direct microbicidal effect through: (i) the reaction with iron or thiol groups on proteins forming iron-nitrosyl complexes that inactivate enzymes important in DNA replication or mitochondrial respiration (72), or (ii) the formation of such reactive oxidant species as peroxynitrite discussed above. Despite the detection of iron within mycoplasma membranes (76), the ability of NO• to form iron-nitrosyl complexes is an unlikely mechanism for mycoplasma killing because mycoplasma species lack complete electron transport chains and cytochromes (1).

Bacteria have developed three levels of defense against peroxynitrite-mediated toxicity, which include prevention, catabolism and repair (69). Mycoplasmas consume oxygen and therefore, the hypothesis that these organisms contain some form of SOD that would act to prevent damage is very appealing. However, while SOD has been identified in certain species of mycoplasma and ureaplasma (77), *M. pneumoniae* has been reported to lack this enzyme (78). Likewise, hydrogen peroxide, the end product of respiration in mycoplasmas, may act as a virulence factor, but the loss of virulence for *M. pneumoniae* does not correlate to decreased hydrogen peroxide production (79). Catalase deficient mice infected

with *M. pulmonis* developed more severe pneumonia earlier but managed to clear the infection faster than immunocompetent controls (80). These data suggest that while hydrogen peroxide production may be important for the establishment of infection, host catalase ultimately controls oxygen radical production and prevents damage to the mycoplasmas. In the absence of AMs, high concentrations of hydrogen peroxide are not toxic to M. pulmonis (54); however, *In vivo*, in the absence of catalase, high levels of hydrogen peroxide may promote the oxidation of nitrite to form peroxynitrite by myeloperoxidase from PMNs (74). This alternative formation of peroxynitrite in catalase deficient mice may provide PMNs with a mechanism to kill mycoplasmas in the absence of specific antibody. Finally, mycoplasmas may prevent reactive oxygen-nitrogen species from being produced by inhibiting NO• production. NO• is produced by the NO-synthase through the five-electron oxidation of L-arginine to NO• and L-citrulline. Some species of mycoplasma contain arginine deiminase that may act to deplete arginine available for NO• production by the host (81).

Mycoplasmas may also avoid injury from reactive oxygen-nitrogen species through their ability to tolerate and repair DNA damage. DNA repair mechanisms serve to limit the amount of mutation that occurs within a species. The number of DNA lesions required to inactivate the genome increases with genome complexity (82), therefore, as the simplest of the self-replicating organisms, mycoplasmas might be expected to be very sensitive to DNA damage. However, mycoplasmas are believed to have arisen by degenerative evolution and have maintained the process of induced error-prone DNA repair. Mycoplasmas utilize error-prone DNA repair in order to accumulate base changes at a more rapid rate than other prokaryotes and evolve to utilize unique environmental niches (83). Environmental stresses such as host production of reactive oxygen-nitrogen species might therefore be essential for mycoplasma adaptation.

6. PERSPECTIVE

Several conclusions can be made based on the current research involving the host innate immune response to respiratory mycoplasma infections. First, activated AMs are essential for mycoplasma killing in the lung. Although pulmonary infection with mycoplasmas causes a suppurative pneumonia, PMNs appear to contribute more to the pathogenesis than to the resolution of infection. Activation of AMs occurs via interaction between the mycoplasma and the macrophage directly or through activation of NK cells with subsequent production of IFN-gamma. Second, the presence of opsonins is important for phagocytosis and killing of mycoplasmas. Although studies on the role of complement in early mycoplasma clearance have been inconclusive, complement components are dramatically increased in animal models following infection with *M. pneumoniae* (84), and complement components in serum have been used successfully to sterilize mycoplasma infected cell cultures. Surfactant proteins can modify a variety of innate immune responses

either by pathogen binding or by direct interaction with activated immune cells. SP-A and SP-D bind mycoplasmas specifically and SP-A mediates mycoplasmal killing by AMs *in vitro* and is important for mycoplasma clearance *in vivo*. Finally, free radical production appears to be an essential element of both mycoplasma colonization and pathogenesis as well as of mycoplasma killing by the host. Mycoplasmas are relatively resistant to damage by reactive oxygen species and NO• alone and indiscriminant production of reactive oxygen-nitrogen species during mycoplasma infection results in significant tissue damage to the host. However, mycoplasmas are extremely susceptible to killing by peroxynitrite *in vitro* and combinations of superoxide and NO• are essential for mycoplasma killing by AMs *in vitro* and *in vivo*. These data confirm the nature of reactive species as a double edged sword capable of protection or mass destruction.

In general, the understanding of pulmonary host defense mechanisms against mycoplasmas lags behind that of other systems because of the poor accessibility and complexity of the lung environment. If the innate mechanisms of the lung are of primary importance in defense against mycoplasma and other bacterial infections, it may be possible to develop practical therapies or to increase the lung's protective capacity through a better understanding of the early immune response.

7. ACKNOWLEDGMENTS

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Send correspondence to: Judy M. Hickman-Davis, University of Alabama at Birmingham, Department of Anesthesiology, University of Alabama at Birmingham, 940 Tinsley Harrison Tower, 1900 University Blvd., Birmingham, AL, 35294-0006, Tel: 205-934-7010, Fax:: 205-934-7437, E-mail: Judy.Hickman-Davis@ccc.uab.edu