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T LYMPHOCYTE RESPONSES ARE CRITICAL DETERMINANTS IN THE PATHOGENESIS AND RESISTANCE TO MYCOPLASMA RESPIRATORY DISEASE

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1. ABSTRACT

Mycoplasmas are responsible for many human and animal respiratory diseases and have a tremendous economic and health impact worldwide. Currently, there is no vaccine against any animal or human mycoplasma species. Despite the vast amount of research, the mechanisms that control hosts' resistance and susceptibility to mycoplasma infection remains unclear. Immune responses, particularly T lymphocyte responses, are believed to be critical players in the pathogenesis of mycoplasma disease. In this review, we will highlight the potential roles of T lymphocytes as influential mediators in animal and human mycoplasma pathogenesis and resistance infection.

2. INTRODUCTION

2.1. Mycoplasma disease in human and animals

As an infectious disease, mycoplasmas are probably the most under recognized pathogens known today. Often misconceived as a common cross-contaminate (1), mycoplasmas are in fact the etiological agent of a wide range of diseases in both animals and humans (2, 3). Because of their small size, mycoplasmas were first believed to be of viral origin. First isolated in 1898, *Mycoplasma mycoides* subsp. *mycoides*, was reported as the agent of contagious bovine pleuropneumonia in cattle (4). Since, mycoplasma species have been isolated from almost every domestic and laboratory animal.

Importantly, mycoplasmas have a significant impact in human and animal disease. In humans for example, 8 to 15 million cases of *Mycoplasma pneumoniae* infection were reported during an epidemic year (3, 5, 6), and it remains the leading cause of respiratory related illness worldwide. Furthermore, there is increasing evidence suggesting that mycoplasmas contribute to other diseases. In particular, mycoplasmas have been associated with exacerbating pre-existing diseases such as asthma, periodontal disease and cardiovascular related disease among others (7-10). Although currently controversial, it has been recently proposed that mycoplasmas may also be associated with the progression of AIDS, tumor formation, gulf war syndrome, Crohn's disease and rheumatoid arthritis (11). Because of their prevalence, mycoplasmas have a profound economic impact on not only health care and biomedical research but also equally as an infectious agent of cattle, swine, sheep and other agricultural animals (2). It is likely that future studies will reveal mycoplasmas alone, or in conjunction with other agents or conditions, to be very important contributors in an increasing number of human and animal diseases.

Immune responses in mycoplasma disease are critical components to disease pathogenesis. Although immunity is known to control and prevent infections, immune responses can also promote the inflammatory lesions associated with mycoplasma disease. T cells are critical in the regulation of immunity and have an impact on mycoplasma disease. In this review, we will highlight the potential roles of T lymphocytes in pathogenesis and resistance to animal and human mycoplasma disease.

2.2. The complexity of immune interactions with mycoplasma

Disease pathogenesis of mycoplasmas is influenced by a multitude of factors, providing a complex picture of subtle host-parasite interactions that result in either control of infection or disease development. As with

other pathogens, the virulence of the organism can determine the chronicity and severity of disease resulting from mycoplasma infection (12-19). For a wide variety of mycoplasma diseases, a major determinant in disease progression is the host genetic background (2, 20, 21). For example, inbred strains of mice display a continuum from resistant to severe disease after infection with the murine pathogen *Mycoplasma pulmonis* (22). These studies further demonstrated that host susceptibility or resistance is under multigenic control. Most likely many of these genetic differences influence components of the immune system, as differences in disease susceptibility in laboratory rodents are linked to the types and intensity of immunity generated (23-28). In addition, gender of the host can also contribute to the extent of disease. Our recent studies demonstrate that male mice develop more severe alveolar pneumonia after *M. pulmonis* infection (29), which is consistent with disease due to the human mycoplasma, *M. pneumonia* (5, 6, 30). In addition to host factors, environmental factors, such as the levels of ammonia and pollutants, also impact on mycoplasma respiratory diseases (31, 32). However, the effects of the environmental factors are most likely on the host, particularly defense mechanisms. In support, pollutants were demonstrated to alter the capacity of alveolar macrophages to control mycoplasma infection (32). The list of factors modulating the susceptibility and resistance to mycoplasma disease continues to expand, and despite the vast amount of information regarding mycoplasmas, there is still much to understand about the mechanisms involved in mycoplasma disease pathogenesis, particularly the impact of host responses on disease.

Immunologic responses probably have the greatest impact on the progression of mycoplasma respiratory disease. As expected, immunity does play a role in protection from mycoplasma diseases. Vaccination against mycoplasma disease is possible (33-41). Mucosal IgA responses are likely important in resistance to disease associated with mycoplasma infection of mucosal surfaces (42, 43). In addition, patients with impaired humoral immunity can suffer from chronic sinopulmonary disease due to mycoplasma (44), suggesting a role for antibody in recovery from disease. However, immunity is often short-lived, as humans and animals are susceptible to repeated infections (45, 46). Furthermore, asymptomatic carriers are common in animals (2) and humans (6). Similarly, immunization with *M. bovis*, *Mycoplasma hyopneumoniae* and *M. pulmonis* confers only partial protection from disease, as organisms are easily isolated from challenged animals (18, 39, 47-49). Indeed, development of adaptive immunity apparently has limited effect on clearance of an established mycoplasma infection (25, 28). One intriguing hypothesis is that the mycoplasma infection promotes the development of immune responses that are unable to resolve infection and avoids immune mechanisms effective against continuing mycoplasma infection/disease. *In vitro* and *in vivo* studies demonstrate that mycoplasma species and their components can not only stimulate antigen-specific immune responses, but can also activate lymphocytes irrespective of antigen specificity, as well as macrophages and other cell types (23, 50-55). This ability to nonspecifically activate cells of the immune system may be one possible mechanism to modulate immunity and avoid clearance. In any case, immunity can be effective in prevention from mycoplasma disease, but immune responses that develop after infection often fail to completely eliminate the mycoplasma and/or disease.

An intact immune system does prevent the dissemination of mycoplasma infection from a mucosal site to other tissues of the body. Immunodeficient animals and humans have an increased incidence of disseminated disease. Hypogammaglobulinemic humans are more susceptible to complications (e.g., polyarthritis and meningitis) due to *M. pneumoniae* (56, 57). T-cell deficient mice (58), infected with *M. pulmonis*, develop more persistent and more severe arthritis than immunocompetent mice. Similar observations were made in studies using *s*evere *c*ombined *i*mmuno*d*eficient (SCID) mice that lack functional B or T cells (59, 60). We have also demonstrated that passive transfer of antibody prevents arthritis from developing in *M. pulmonis*-infected immunodeficient mice (60). Thus, lymphoid, particularly antibody, responses are important in localizing mycoplasma infections to mucosal sites of infection, preventing the spread to other tissues and development of arthritis.

Many mycoplasma respiratory diseases are clearly immunopathologic. One of the most consistent characteristics of most mycoplasma respiratory diseases, including *M. pulmonis* and *M. pneumoniae* disease is the large accumulation of lymphoid cells along the respiratory tract (2, 3, 61). Similarly, both T and B cells accumulate in the lung of *M. bovis* respiratory disease in calves (62). Lymphoid infiltration suggests that lymphocyte activation and recruitment are key events in the progression mycoplasma inflammatory disease. The role of lymphoid responses in the development of other chronic inflammatory diseases is well documented, and lymphocyte activation is similarly important in mycoplasma respiratory disease.

Probably the best evidence for an immunopathologic response in mycoplasma respiratory diseases is from studies using animals that are immunocompromised. T cell-depleted hamsters develop less severe *M. pneumoniae* disease despite an impaired ability to clear the infection (63). Similarly, *M. pulmonis* infection of immunodeficient SCID mice or T cell deficient mice results in milder lung lesions (58, 59). We also found that SCID mice infected with *M. pulmonis* develop less severe lung disease than immunocompetent mice do, despite similar numbers of mycoplasma recovered from lungs (60). We have further shown that reconstitution of SCID mice with lymphocytes increases the severity of mycoplasma disease to that of immunocompetent mice. Thus, animal studies demonstrated that the severity in two different mycoplasma respiratory diseases is increased by an intact lymphocyte response.

Overall, the hallmark of mycoplasma disease pathogenesis is the persistence of the organism, and the provocation of frustrated and ineffective immune responses against the infection results in the development of chronic

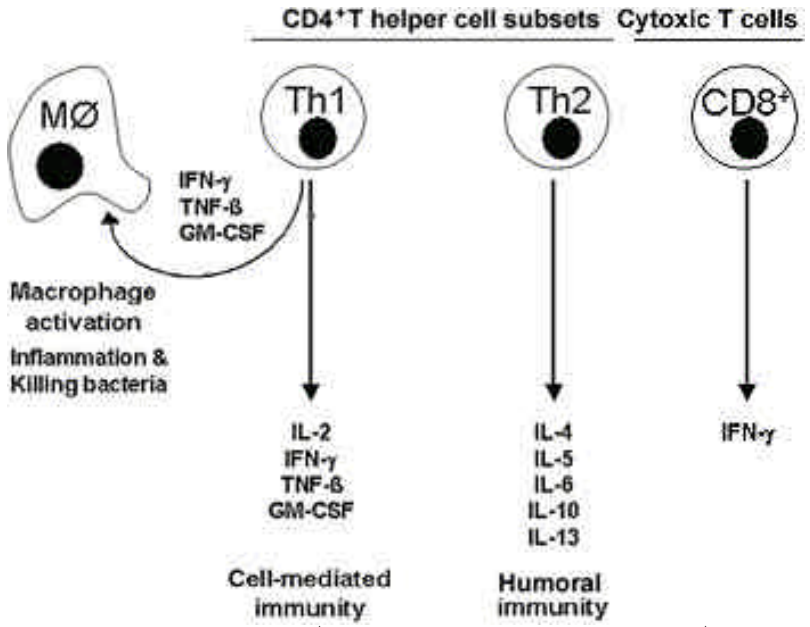

Figure 1. T cell populations are comprised of $CD4^+$ T helper (Th) cell subsets and $CD8^+$ cytotoxic T cells. Th cells are further divided into two cell subsets, Th1 and Th2 cell subsets. The Th1 subset of T helper (Th) cells secretes cytokines primarily associated with inflammatory reactions and activation of macrophages. The Th2 subset produces the cytokines that preferentially provide help for B cells to proliferate and differentiate into antibody-secreting plasma cells. The major function of $CD8^+$ T lymphocytes is in killing intracellular pathogens. Furthermore, $CD8^+$ T cells can also modulate immune and inflammatory responses against infectious agents through the production of IFN-gamma and other cytokines.

inflammation. Immune interactions with mycoplasma are complex and a double-edged sword cutting both ways. Some immune responses are beneficial in controlling or preventing infection, while other responses contribute to the severity of disease. Because of this complexity, the delineation of immune mechanisms involved in mycoplasma disease can be a daunting task. One approach to elucidate these mechanisms is to determine the influence of the central regulatory cell of the immune system, the T lymphocyte. Once understood, the interactions of T cells with the other components of the host response should begin the unraveling of the complexity of the interactions between mycoplasma and the immune system.

3. ROLE OF T LYMPHOCYTES IN MYCOPLASMA DISEASE

T cell responses, most likely, have a significant influence in almost all human and animal mycoplasma diseases. A role for T cells in resistance has been revealed in almost every other infectious disease documented. Significantly, T cells are not only instrumental in protection from disease, but in many cases play a significant role in host immune-mediated pathogenesis. Because of their central regulatory roles, T cell activity determines the balance between the beneficial and detrimental effects of the immune system in many diseases, including those due to mycoplasma infection. As mentioned earlier, the complexity regarding mycoplasma disease is centered on the ability of the immune system to control infection and its potential for immunopathology. The T cell, in many cases, is likely to be the central determinant in the development of host responses against mycoplasma and the subsequent outcome to infection by these ubiquitous agents. In this section, we will first look at the major features of T cell subsets and their responses, and then we will discuss the current knowledge of T cell responses and their role in mycoplasma respiratory diseases.

3.1. T cell subsets and their mediators

T cells can be regarded as the centerpiece, serving as a link between the innate and adaptive arms of the immune system. Antigen presenting cells (e.g. macrophages, dendritic cells, B cells) pick up pathogens or their products and subsequently process and present them to be recognized by pathogen-specific T cells. Once activated, T cells influence all arms of immunity. T cells release factors that initiate and modulate the activity of inflammatory cell populations, such as NK cells, macrophages, eosinophils, etc., during responses against infectious agents. Importantly, they are in charge of inducing and maintaining acquired immunity and generating memory lymphocyte populations. They determine the class and influence the specificity of antibody responses. Overall, T cells are responsible for maintaining the homeostatic environment of the host, and the outcome of disease is dependant of the T cell responses generated. As a result, their wide range in activity makes delineation of T cells' role in disease very complex, but of fundamental importance.

T cells are comprised of two major subpopulations of lymphocytes referred to as T helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells (figure 1). $CD4^+$ T helper cells are further divided into two cell subsets, Th1 and Th2 cell subsets [Reviewed in (64-66)]. The Th1 subset of T helper (Th) cells secretes cytokines primarily associated with inflammatory reactions. These include gamma-interferon (IFN-gamma? interleukin-2 (IL-2) and tissue necrosis factor-beta (TNF-beta). In contrast, the Th2 subset produces the cytokines interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-10 (IL-10) and interleukin-13 (IL-13). Cytokines produced by Th2 cells preferentially mediate humoral immune responses by providing help for B cells to proliferate and differentiate into antibody-secreting plasma cells. In terms of protection, Th1 effector function is primarily targeted toward intracellular pathogens such as mycobacterial infections. Th2 cell activation aids in protection against extracellular bacterial infections through the stimulation of immunoglobulin production by B cells. One of the best models illustrating the importance of Th cell subset activity in infectious disease is that of experimental *Leishmania major* infection (67-75). Th1 cells were shown to promote resistance to or healing from *L. major* disease while Th2 cells increase the severity of disease. There are other examples of opposing roles of Th cell subsets in disease (72, 76-86). Thus, studies to determine the role of Th cells in protection from or pathogenesis of an infectious disease requires the analysis of Th1 and Th2 cells and their contributions to the response against the pathogen.

$CD8^+$ T lymphocytes also contribute to host immune responses. Their major function is to kill bacterially or virally infected cells as well as tumor cells. However, recent evidence suggests that $CD8^+$ T cells can produce substances, e.g. granulysin, that directly kill intracellular bacteria (87-92), suggesting $CD8^+$ T cells can be very important in killing intracellular pathogens. Furthermore, $CD8^+$ T cells can also modulate immune and inflammatory responses against infectious agents through the production of IFN-gamma and other cytokines, which can affect NK cell or macrophage activity. Thus, $CD8^+$ T cells could play a role in the pathogenesis of infectious disease through direct killing of intracellular organisms or through modulation of immunity.

3.2. T cell populations and their role in mycoplasma respiratory diseases

It is clear that lymphocyte recruitment and activation are important in the pathogenesis of mycoplasma respiratory disease, and T cells are an important component of these responses. As indicated earlier, a common characteristic of most animal and human mycoplasma disease is a slow, progressive increase in the number of organisms, accompanied by an accumulation of lymphoid cells consistent with chronic inflammation (3, 61, 93, 94). In humans for example, *M. pneumoniae* infection is characterized by acute perivascular and peribronchial lymphoid infiltration resulting in destruction of respiratory epithelium (3, 61). At later stages, further destruction of airway parenchyma is obvious as a result of massive mononuclear cell infiltration of which T cells are a major component. Increases in pulmonary T cell numbers were shown in *M. pulmonis* infection of laboratory rats with higher number of T cells recovered from lungs of the rat strain that developed more severe disease (20). *M. pulmonis* infection of mice was not only shown to result in an increase and predominance of T cells in the lung, but also the draining (hilar and mediastinal) lymph nodes of the lower respiratory tract (95). Similarly, both T and B cell accumulation in the lungs of goats were shown after pulmonary infection with mycoplasma (94).

The association of lymphocyte, particularly T cells, infiltration in mycoplasma respiratory disease suggests their role in disease pathogenesis or control of mycoplasma infection. In fact, a role for T cells in resistance and immunopathogenesis is supported by studies demonstrating T cell deficient (NUDE) mice or hamsters treated with anti-thymocyte antibody have less severe mycoplasma respiratory disease than their immunocompetent counterparts (58). Similarly, SCID mice, lacking functional B and T cells, develop significantly less severe *M. pulmonis* lung disease than immunocompetent mice (59, 60). Importantly, the presence or absence of T cells seems to have little effect on the number of mycoplasmas in the lungs of these animals. This suggest that T cells may not mediate localize resistance, but they are primarily involved in inciting and regulating pro-inflammatory response that are deleterious to the host. In fact, reconstitution of SCID mice with splenic lymphocytes resulted in disease as severe as in normal mice (60), and in unpublished studies, reconstitution of SCID mice with T cells alone promoted development of more severe pulmonary lesions after mycoplasma infection. However, antibody responses were shown to be important in preventing extrapulmonary dissemination of infection that lead to arthritis and the eventual death in SCID mice (60); however, it is not clear whether T cells alone can also prevent dissemination of the mycoplasma infection from the lung. Furthermore, adaptive immunity, also dependent on functional T cells, has been shown to promote resistance and recovery to *M. pneumoniae* and *M. pulmonis* infection (39, 96-99). Thus, these findings demonstrate T cells are active participants in mediating host's responses to mycoplasma infection.

The cells are a major component of the lymphoid responses generated in the lower respiratory tract during the pathogenesis of mycoplasma disease and are likely to contribute to immune mediated inflammatory responses after mycoplasma infection. *M. pneumoniae* infection of laboratory rodents reveal $CD4^+$ Th cells within the inflammatory sites associated with mycoplasma infection (100), and in peripheral blood from patients, mycoplasma specific Th cell responses are found (101). Similarly in goats infected with *Mycoplasma agalactia* or *M. bovis*, T cells were the major cell type associated with lymphocyte hyperplasia, and Th cells were more prominent than $CD8^+$ T cells (94). In mice infected with *M. pulmonis*, our laboratory demonstrated a preferential increase in the numbers of $CD4^+$ Th cells within draining respiratory lymph nodes as early as seven days after infection (95). This is followed by a large increase in the number of $CD4^+$

Th cells within the pulmonary tissues fourteen days following infection; representing chronic stages of mycoplasma disease. This preferential increase has been documented in a number of other animal and human mycoplasma diseases.

Along with the preferential increase in $CD4^+$ Th cells observed in mycoplasma disease, *in vivo* depletion of $CD4^+$ Th cells in a model of experimental mycoplasma disease supports their role in immune mediated pathogenesis (95). Mice were depleted of Th helper cells using injections of monoclonal antibodies specific for CD4 and subsequently infected with *M. pulmonis*. The $CD4^+$ Th cell depleted mice develop less severe pulmonary lesions than their immunocompetent counterparts. Interestingly, depletion of $CD4^+$ Th cells had no effect on the number of mycoplasma recovered from the lungs of these animals. These findings are consistent with previous studies using immunodeficient mice that implicated T cells as prominent players in the modulation of the inflammatory responses in mycoplasma disease (58). Furthermore, the preferential increase in $CD4^+$ Th cell populations is consistent with other proinflammatory disease conditions such as asthma, leishmaniasis, autoimmune disease in which $CD4^+$ Th cells exacerbate disease severity rather than resolve infection or the condition. Thus, a population Th cells promotes the development of immune mediated lesions in lungs due to mycoplasma infection, and further studies are needed to determine whether the Th cell populations involved in immune mediated pathology of mycoplasma disease are different from those that control or prevent of mycoplasma infection.

In comparison to Th cells, $CD8^+$ T cell responses represent a lesser component of the T cell response against a mycoplasma infections. In our studies on T cell responses in *M. pulmonis* respiratory disease (95), the appearance of mycoplasma-specific $CD8^+$ T cells was somewhat of a surprise. We had previously shown that Th, but not $CD8^+$ T cell, responses were elicited after nasal-pulmonary immunization (102). We expected to see a Th cell response because of these studies and due to the preferential processing of exogenous mycoplasma antigen via MHC II restricted antigen processing, which is the common pathway for most extracellular pathogens. However, MHC I restricted antigen presentation is the major pathway for $CD8^+$ T cell activation, and this pathway is most commonly associated with presentation of antigen derived from intracellular pathogens. Although some mycoplasma have been shown to have intracellular phases (103, 104), *M. pulmonis* has not been found to do this, despite studies which have examine this possibility (105). One possibility is suggested by recent studies demonstrating the ability of dendritic cells to present exogenous antigen in the context of MHC I through a novel pathway (106-108), but further studies are needed to support the role of dendritic cells in eliciting mycoplasma immune responses. Thus, both Th and $CD8^+$ T cell responses are induced in the lungs of after mycoplasma infection.

A immunomodulatory role for $CD8^+$ T cells mycoplasma respiratory disease was first suggested in studies of resistant and susceptible strains of rats (20, 21, 23). It was found that F344 rats, which develop less severe disease after *M. pulmonis* infection, had a relatively larger proportion of $CD8^+$ T cells to Th cells in their lymphoid tissues and lungs as compared to the susceptible LEW rat strain (23). We suggested that $CD8^+$ T cells may dampen the Th cell responses against mycoplasma, resulting in less severe disease (23, 109). This hypothesis was not addressed until relatively recently when we found that $CD8^+$ T cells do have a regulatory role and dampen mycoplasma associated proinflammatory responses (95). *In vivo* $CD8^+$ T cell depletion of *M. pulmonis* infected mice resulted in a tremendous increase in disease severity, including clinical signs (e.g. a significant loss in body weight) and pulmonary lesion. The increase in disease severity was independent on mycoplasma numbers in lung, supporting a regulatory role for $CD8^+$ T cells. Thus, $CD8^+$ T cells can clearly play a significant role in modulating the inflammatory reactions against mycoplasma pulmonary infection, but further studies are needed to elucidate the mechanisms through which this relatively small population of T cells can have such a dramatic impact on disease severity.

In summary, mycoplasma respiratory disease is characterized by a prominent infiltration of $CD4^+$ Th cells, and populations of Th cells promote the development of the inflammatory lesions in the lungs, as well as regulating the adaptive immune responses in many mycoplasma diseases. However, $CD8^+$ T cells are also responding to mycoplasma infection, and may be involved in minimizing damage due to the Th cell mediated inflammatory responses. This idea is consisting with increasing evidence supporting a role for $CD8^+$ T cell involvement in disease conditions where $CD4^+$ T cells are suspected to the dominate T cell mediated immune responses (110, 111). Thus, it appears that the interactions between Th and CD8+ T cells has a major impact on the outcome of mycoplasma disease. However, further work is needed to understand their role in mycoplasma disease pathogenesis.

3.3. T cell cytokine and T helper subset responses associated with mycoplasma respiratory diseases

In many instances, T cell cytokines determine the outcome of the responses generated. Delineation of which T cells cytokines promotes protective immunity versus those that may be detrimental has yet to be defined in any mycoplasma disease. However, there are studies that examined the production of T cell cytokines. In particular, several studies examined the type of cytokines present during acute and chronic stages of experimental *M. pneumoniae* and *M. pulmonis* infection in mice. Specifically, these studies identified elevated mRNA levels of IFN-gamma, TNF-alpha, IL-4, IL-5, IL-6 and other cytokines (27, 112). The presence of these cytokines corresponded with the presence of gross inflammatory lesions. In studies of human mycoplasma infection, a number of cytokines have also been documented to be present in lungs including: IFN-gamma, IL-5, IL-4, TNF-alpha, and others (113-115). As T cells are known to secrete these cytokines, these findings correspond with the activation at inflammatory sites associated with mycoplasma infection. It is known that IFN-gamma, TNF-

alpha? IL-6 and IL-4 are involved in pro-inflammatory responses and contribute to disease pathogenesis. There is also emerging evidence supporting the role of other cytokines such as IL-10 and IL-13 in augmenting resistance and pathogenesis in other infectious diseases. A particularly intriguing cytokine is IL-10, which through an attempt to prevent damage due to an out of control inflammatory response, is known to promote persistent infection and chronic inflammation in some infectious diseases (116, 117), but this has not been examined in mycoplasma disease. Therefore, although T cell cytokines are produced in response to mycoplasma infection, their role in disease pathogenesis remains to be fully evaluated.

T helper cell subset cytokines likely have a role in modulating immune and inflammatory responses in mycoplasma disease. We demonstrated that both mycoplasma-specific Th1 and Th2 cell responses are generated in the lower respiratory tract (95). Th1 cell responses were characterized by IFN-gamma and IL-2 production while Th2 cell responses consisted of IL-4, IL-6, and IL-13. Historically, IL-4 and IFN-γ are considered the two major cytokines produced by Th cell subsets cells with significant influence on resistance and pathogenesis of many diseases (64, 65). One reason for their notoriety is their ability to counteract each other's regulatory immune function. IL-4 cytokine production by Th2 cells dampens IFN-gamma-mediated Th1 immune responses and vice versa in various models of disease. For example, experimental asthma models demonstrate IFN-gamma reduces IL-4 induced pulmonary hyperresponsiveness in mice (118). Interestingly, our studies demonstrate that there are differences between tissues in the type of T helper cell subset responses. The mycoplasma-specific Th responses in lower respiratory tract lymph nodes were almost exclusively Th2-type, and the Th cell responses in spleen were Th1. In lungs, both Th1 and Th2 cell responses against mycoplasma were found. In addition, $CD8^+$ T cells in lungs produced IFN-gamma in response to mycoplasma antigen, which contributed to the apparent dominance of Th1-type responses in the lung. As earlier studies demonstrated that resident pulmonary Th cells were preferentially of the Th2-type (102), mycoplasma infection results in a shift from the resident Th2 populations to a mixed Th1/Th2 response. Thus, the appearance of Th1 responses corresponds with the development of inflammatory lesions in the lungs and supports a role of Th1 cytokines in the pathogenesis of mycoplasma disease.

In general, the role of T cell cytokines and Th cell subsets responses in mycoplasma respiratory disease remains to be elucidated. Ongoing studies in our laboratory and those of others are beginning to delineate through cytokine gene knockout mice, the roles of the IFN-gamma and IL-4 as they are prominent cytokines produced by T cell populations that are critical in many diseases. However, the network of cytokine interactions combined with their multiplicity of their actions greatly complicates studies on the impact of T cell cytokines on disease. For example, Th1 responses may contribute to the formation of the inflammatory lesions, but Th2 cytokines may impair clearance of organisms through their anti-inflammatory activities on macrophages, a major cell in controlling mycoplasma infection. Furthermore, Th2 responses against mycoplasma could contribute to increased severity of asthma, a Th2 mediated disease (119-121), associated with their infection. Thus, the interactions of T cell cytokines pose an interesting question of which cytokine response(s) may be protective and which may promote disease? Further studies are needed to fully elucidate the cytokine responses and their roles in disease pathogenesis and resistance to infection.

4. MECHANISMS INFLUENCING T CELL RESPONSES

As mycoplasma respiratory diseases are immunopathologic, one strategy is to generate T cell responses that dampen or prevent adverse immune reactions against the pathogen. In fact, we demonstrated that *in vivo* depletion of $CD8^+$ T cells results in significantly more severe disease after mycoplasma infection of mice without affecting the numbers of mycoplasma in the lung (95). This suggests that $CD8^+$ T cells dampen the generation of inflammatory reactions against persistent mycoplasma infection. A similar role of $CD8^+$ T cells has been shown in murine asthma (110). There are also populations of Th cells that are responsible for antigen-specific immune tolerance or T cell homeostasis (122, 123). Secretion of IL-10 and/or TGF-beta is characteristic of these cell populations who are referred to as Th3 or T regulatory cells. Both IL-10 and TGF-beta are anti-inflammatory cytokines in part due to their ability to reduce macrophage activation and secretion of proinflammatory cytokines (124, 125). TGF-beta is also associated with the induction of oral tolerance, resulting in selective unresponsiveness to antigen [Reviewed in (126)]. The role of Th3 or T regulatory cells in pulmonary immunity remains to be elucidated, but aerosolized antigen can reduce IgE responses against subsequent challenge, suggesting a mechanism similar to oral tolerance operates along the respiratory tract (127). Thus by understanding the mechanisms involved in regulating different T cell reactions, novel approaches to prevent or treat mycoplasma and other inflammatory diseases of the respiratory tract can be developed.

As indicated above, the regulation of T cell responses are extremely complex. The remainder of this article will focus on two areas of interest, chemokines and dendritic cells. Chemokines are chemotactic cytokines involved normal cell trafficking and in cell recruitment in disease. Dendritic cells are extremely potent antigen presenting cells that help activate T cell populations. Recent studies suggest that chemokines and dendritic cells are be intimately involved in the induction of regulatory T cells and tolerance, and thus, they are likely to similarly impact on immunity in mycoplasma diseases.

4.1. Chemokines in mycoplasma disease

An important feature of disease pathogenesis associated with mycoplasma infection and other inflammatory diseases is a dramatic infiltration of lymphocytes within and surrounding the site of

mycoplasma infection (2, 3, 61). The findings presented in the above sections, substantiate T cells as a major component of immune cell populations present during chronic mycoplasma disease. Having described T cell's involvement, a logical question would be what mechanisms are directing the type of T cells responding at the site of infection? In this section we will discuss the potential role chemokines, as a mechanism known to support trafficking of T cells that comprise of the inflammatory infiltrate during mycoplasma disease.

In 1996, interest in chemokines surged with the finding that certain chemokine receptors were the elusive co-receptors required, along with the CD4 receptor, for human immunodeficiency virus (HIV) infection. Today, over 40 human chemokines and 17 human chemokine receptors are identified. Chemokines are small proteins involved in numerous biological processes that include hematopoiesis, angiogenesis, and migration of lymphocytes involved in inflammatory responses (128). Recently, studies have emerged, evaluating the influence of chemokine and chemokine receptors involved in the recruitment of lymphocytes responding to infection. The superfamily of chemokines can be divided into three major sub-groups, CXC, CC and C based on the structural positions of the first two cysteines at the amino terminus of the molecule. Chemokines impart their function through the engagement with respective chemokine receptors present on numerous lymphocyte and cellular populations. Because, cellular infiltration is a major hallmark of mycoplasma disease, chemokines are likely to impact on not only the pathology but also the type of immune responses generated.

The chemokines are likely to play a role in the recruitment of T cells to the site of mycoplasma infection. In support, beta-chemokines are one of the major families of chemokines implicated in controlling inflammatory responses (128). These chemokines include macrophage chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1alpha (MIP-1alpha), macrophage inflammatory protein-1beta (MIP-1beta) and RANTES. In fact, we showed that the beta-chemokines, MIP-1alpha and MIP-1beta and MCP increase during the development of chronic mycoplasma respiratory disease, whereas RANTES does not (129). The role of these chemokines in mycoplasma infection is still under investigation, but they are likely to impact on both the pathology of disease and the immune responses generated. For example, chemokine production is likely to increase the intensity of responses through the recruitment of lymphocytes to the site of infection. In addition, T cells, such as $CD4^+$ T cells, preferentially express chemokine receptors for the beta-chemokines, and there is evidence suggesting that these chemokines may differentially recruit Th cell subsets (130). In fact, Th1 cells preferentially respond to MIP-1beta chemokine via CCR5 chemokine receptor whereas Th2 cells preferentially respond to MIP-1alpha via CCR8 chemokine receptor (130). These findings suggest that differential chemotactic capacity is likely to influence the induction and progression of inflammatory responses that lead to pathogenesis. In support, studies in our laboratory (102) demonstrated that nasal-pulmonary immunization with influenza vaccine antigen alone resulted in a Th2 response and a concomitant increase in MIP-1alpha mRNA expression. In contrast, immunization with influenza vaccine antigen in the presence of the potent mucosal adjuvant cholera toxin (CT) generated both Th2 and Th1 responses along with a corresponding increase in MIP-1alpha and MIP-1beta mRNA expression. The association between MIP-1beta mRNA expression and Th1 responses after nasal-pulmonary immunization (102) is consistent with the previous studies (130) demonstrated the preferential expression of CCR5 chemokine (e.g. MIP-1beta) receptors on Th1 cells. These observations also corresponded with increases in Th cell numbers and the presence of inflammatory responses in the lungs of mice given influenza vaccine antigen plus CT. Thus, there is a link between beta-chemokines, Th cell subset recruitment and modulation of immunity. Future studies however are needed to delineate the role of beta-chemokines in mycoplasma disease, but beta-chemokines and other chemokines are likely to have important roles in orchestrating T cell immune responses in mycoplasma and other infectious diseases.

4.2. Regulation of T cell responses by dendritic cells

Most likely, dendritic cells contribute to the immune-mediated pathology of mycoplasma respiratory disease. There is surprisingly limited information on the role of dendritic cells during generation of immune and inflammatory responses in any respiratory disease. Dendritic cells are extremely potent antigen-presenting cells, which can activate both Th and cytotoxic T cells, and are found in lungs (131-137), as well as other tissues. Importantly, numbers of dendritic cells in lungs can increase in inflammatory disease (138-140), and we have similarly shown this to occur in mycoplasma respiratory disease (Unpublished Results). In support of a role of dendritic cells in inducing immune-mediated inflammatory disease, studies suggest that dendritic cells are critical in the generation of allergic and asthmatic responses (141-144). Presumably, pulmonary dendritic cells during respiratory diseases are capable of driving T cell responses within the lung that are contributing to the pathogenesis of these inflammatory reactions. One possible mechanism to facilitate T cell-DC interactions within the lung is through the production of the ß-chemokines, ABCD-1 and TARC [ABCD-2 is the murine homologue of TARC (145)]. These chemokines are produced by dendritic cells and are chemotactic for activated or primed T cells (146-149). Although these chemokines were thought to play a role primarily in secondary lymphoid tissues (146-149), we have recently shown in preliminary studies that ABCD-1 mRNA expression is increased in the lungs of mycoplasma infected mice and that mycoplasma can induce ABCD-1 mRNA expression in bone marrow derived dendritic cells. Thus, dendritic cells within the lung are likely to play a pivotal role in immune-mediated inflammatory diseases of the respiratory tract, including mycoplasma pneumonia. Furthermore, the role of ABCD-1 or ABCD-2 (TARC) in pulmonary inflammatory disease is unexplored, but the recruitment, activation and retention of effector T cells by antigen presenting cells, e.g. dendritic cells, within lung

tissue will likely have a major impact on the progression of the inflammatory responses due to mycoplasma and other diseases.

Dendritic cells may also be a major factor in determining the type and intensity of immune reactions in mycoplasma respiratory disease, but the role of DCs in pulmonary immune responses is largely unknown. Dendritic cells can clearly promote protective immunity in the lung. For example, intratracheal inoculation of antigen-pulsed dendritic cells confers protection against *Mycobacterium tuberculosis* infection of mice (150). Most importantly, DCs can also modulate the types of immune responses generated. In support, dendritic cells from Peyer's patches preferentially stimulate Th2 responses while splenic dendritic cells promote Th1 type responses (151, 152). Similarly, studies (132) demonstrate that resident dendritic cells from rat lungs preferentially stimulate Th2 responses. We (102) and others (153, 154) have shown that resident Th cells in murine lungs are primarily Th2 like. In addition, recent studies provide insight on the type of dendritic cells populations responsible for the difference in Th cell responses. CD8alpha$^+$ dendritic cells are capable of producing IL-12 and promote differentiation of Th cells to Th1 cell subsets. In contrast, CD8alpha$^-$, CD11b$^+$ dendritic cells stimulate Th2 type responses (155-159). However, studies do suggest that cell surface markers alone cannot be used to predict DC activity or function (160-163). Different factors can influence the ability of dendritic cells to support Th cell subset or cytotoxic T cell responses (160-164). For example, IL-4, histamine or norepinephrine polarizes dendritic cells into Th2 cell-promoting dendritic cells while IL-12, IFN-gamma or LPS treated dendritic cells preferentially support Th1-type responses. It is clear that dendritic cells not only have a central role in the initiation of immunity but they also influence the type of responses that develop. Based on this information, it is reasonable to hypothesize that changes in DC populations are a contributing factor in determining the type of T cell responses associated with mycoplasma respiratory disease, as well as other respiratory diseases. An additional factor to be considered is lung macrophages may preferentially promote Th1 responses within the lung (165). This could lead to a partial compartmentalization of antigen presenting cell activities where for example, mature pulmonary dendritic cells are preferentially supporting activation of effector Th2 cells while Th1-type responses are supported by macrophages.

Dendritic cells may also be involved in preventing the generation of harmful pulmonary immune responses. Studies suggest that the resident dendritic cells in lungs are immature (166) and not as effective in antigen presentation. In support, we have shown that resident CD11c$^+$ dendritic cells in murine lung do not express high levels of co-stimulatory molecules and not fully matured (Unpublished Results). This indicates that the lung is typically not a site where immune responses are initiated. Most likely, dendritic cells normally migrate from the lung to draining lymph nodes of the lung where T cell responses are initiated (167). Recently an intriguing observation was made demonstrating that immature dendritic cells can induce antigen-specific inhibition of effector T cell function in humans (122, 123). This observation may be linked to the induction of T regulatory cells (122). Additional studies have shown that the cytokines, IL-10 and TGF-beta, can be used to maintain the immature phenotype of dendritic cells and depress dendritic cells immunostimulatory activity (168-170). Thus, resident "immature" dendritic cells in lungs may help prevent inflammatory responses due to antigen deposition in lungs, perhaps through their inability to effectively trigger T cell responses or through the activation of T regulatory cells.

Future studies are needed to understand the impact of dendritic cells, and other antigen presenting cells, have on the development of protective immunity against mycoplasma as well as their participation in disease pathogenesis. Importantly, we propose that manipulating dendritic cells and other antigen presenting cells can predictably promote the harmful or beneficial effects of T cell responses in mycoplasma disease.

5. PERSPECTIVE

Because of the chronic nature of these infections, it is likely that almost every component of the host immune system is involved in the responses to mycoplasma disease. T cells are a major component of the immune response against mycoplasma infection. Because T cells modulation most immune responses, the progression of mycoplasma respiratory disease is dependent on the balance between those T cell responses that may promote host resistance, and those that evoke immune-mediated pathogenesis. In this review, we summarized studies that demonstrate the contrasting roles of T cells in host resistance and pathogenesis and discussed the complexity of mechanism that may impact on the outcome of these responses.

T lymphocyte responses are clearly critical determinants in the pathogenesis and resistance to mycoplasma respiratory disease, T cells are a predominant feature of the chronic inflammatory cell infiltrate along mucosal surfaces, which is characteristic in most human and animal mycoplasma infections. This observation seems to support the notion that T cell activation during chronic stages is linked to a frustrated inflammatory response that contributes to disease pathogenesis rather than immune clearance. In particular, CD4$^+$ Th1 cellular responses are believed to play the important role in modulating the immune-mediated pathology partly through the polarizing effect Th1 cells have on the T cell environment. In support, studies presented here demonstrate a shift in the resident Th2 dominant mucosal environment within the respiratory tract following *M. pulmonis* infection. It is also interesting to note that CD8$^+$ T cells were found to dampen CD4$^+$ T cell pro-inflammatory response as well as influence mycoplasma recovery from the lungs of infected mice. Together, these findings strongly suggest there are complex dynamics of immune resistance and disease pathogenesis occurring in many mycoplasma diseases, and that T cells play a significant role in modulating these dynamics.

The contributing factors that influence T cells during mycoplasma disease is still largely unknown. However, we discussed potential mechanisms that we believe are likely to contribute to the regulation of T cell responses within the infected lung tissue. Genetic disposition will undoubtedly be an important factor in determining the nature of hosts' response to mycoplasma. In addition, the innate host defenses will likely contribute to how T cells become activated during mycoplasma disease. Macrophages and perhaps dendritic cells, for example, are capable of modulating downstream T cell responses. The noted inefficiency of activated macrophages to eradicate mycoplasma species could possibly be one of the factors driving a Th1-mediated response, resulting in chronic disease. The release of chemotactic factors, such as beta-chemokines, could be critical factors in the recruitment of different T lymphocytes populations and their subsequent activation in mycoplasma infected lungs. It is also speculated that dendritic cells, as antigen presenting cells, are contributing to the environment that influences the type of T cell-mediated immune response generated. These and other factors are important areas of future research as we begin to dissect the mechanisms involved in the generation of the immune reactions involved in mycoplasma disease.

Clearly, the evidence presented here, and many others not cited in this review, demonstrate the impact immune interactions with mycoplasma in human and animal disease. Importantly, mycoplasmas are emerging as a pathogen that is associated with the onset and development of many human and animal diseases. While there is yet an effective vaccine against mycoplasma species, investigations have begun to unravel key components of host T cell/mycoplasma interactions that participate in surveillance and disease pathogenesis. Future studies utilizing approaches, such as genetically engineered knock out models deficient in T cell cytokine production or adoptive transfer of lymphocytes, should shed more light on the mechanisms that impact on resistance and progression of mycoplasma disease. Hopefully, such studies will elucidate methods to preferentially activate T cell mediated immune responses that confer protection while at the same time minimize immune-mediated lung damage in mycoplasma respiratory disease, and lead to novel vaccines against mycoplasma diseases.

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