Mast cell TNF receptors regulate responses to *Mycoplasma pneumoniae* in surfactant protein A (SP-A)−/− mice

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Abstract

Background—*Mycoplasma pneumoniae* (Mp) frequently colonizes the airways of patients with chronic asthma and likely contributes to asthma exacerbations. We previously reported that mice lacking surfactant protein A (SP-A) have increased airway hyperresponsiveness (AHR) during *M pneumoniae* infection versus wild-type mice mediated by TNF-α. Mast cells (MCs) have been implicated in AHR in asthma models and produce and respond to TNF-α.

Objective—Determine the contribution of MC/TNF interactions to AHR in airways lacking functional SP-A during Mp infection. Methods: Bronchoalveolar lavage fluid was collected from healthy and asthmatic subjects to examine TNF-α levels and *M pneumoniae* positivity. To determine how SP-A interactions with MCs regulate airway homeostasis, we generated mice lacking both SP-A and MCs (SP-A−/−Kit^{W-sh/W-sh}) and infected them with *M pneumoniae*.

Results—Our findings indicate that high TNF-α levels correlate with *M pneumoniae* positivity in human asthmatic patients and that human SP-A inhibits *M pneumoniae*–stimulated transcription and release of TNF-α by MCs, implicating a protective role for SP-A. MC numbers increase in *M pneumoniae*–infected lungs, and airway reactivity is dramatically attenuated when MCs are absent. Using SP-A−/−Kit^{W-sh/W-sh} mice engrafted with TNF-α−/− or TNF receptor (TNF-R)−/− MCs, we found that TNF-α activation of MCs through the TNF-R, but not MC-derived TNF-α, leads to augmented AHR during *M pneumoniae* infection when SP-A is absent. Additionally, *M pneumoniae*– infected SP-A−/−Kit^{W-sh/W-sh} mice engrafted with TNF-α−/− or TNF-R−/− MCs have decreased mucus production compared with that seen in mice engrafted with wild-type MCs, whereas burden was unaffected.

Conclusion—Our data highlight a previously unappreciated but vital role for MCs as secondary responders to TNF-α during the host response to pathogen infection.

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Keywords

Mast cells; TNF; Mycoplasma species; airway hyperres-ponsiveness; mucus

Mycoplasma pneumoniae is well established as a human pathogen, adheres tenaciously to the airway epithelia,[1,2] and infects approximately 2 million persons annually with *M pneumoniae*–induced respiratory tract infections. Although asthma can be caused by a myriad of triggering stimuli, such as air pollutants and allergens, symptoms are often exacerbated by colonization with *M pneumoniae*. Several studies have examined the relationship between *M pneumoniae* and asthma and suggest as many as 50% of patients with stable asthma might be colonized with *M pneumoniae*.[3-5] Bronchoscopic biopsy samples from asthmatic patients with positive test results for *M pneumoniae* airway colonization show significant increases in mast cell (MC) numbers compared with those seen in patients with negative test results for *M pneumoniae* burden.[5]

MCs are highly granular immune cells that reside in all tissues exposed to the environment, are intricately involved in allergic responses, and more recently have been described as sentinels of the innate immune system during bacterial infections.[6] Depending on the type and strength of stimulus, MCs are able to discharge very specific mediators, including a variety of cytokines and chemokines, only minutes after the initial encounter. In addition to being newly synthesized in the ensuing hours after stimulation, some specific mediators, such as TNF-α, are stored in a preformed manner and can be immediately released from granular stores on activation of MCs. MCs also express receptors for a variety of cytokines and chemokines and can therefore respond to intrinsic signals during infection.[7] Engagement of these receptors leads to the release of inflammatory mediators commonly associated with asthma, such as histamine, tryptases, and leukotrienes, and can also alter the response of MCs to degranulation stimuli.[8-11]

Primary pulmonary defense is supplied by the pulmonary innate immune system, which is comprised of phagocytes and natural killer cells, as well as soluble mediators, such as surfactant proteins. A major protein constituent of surfactant is surfactant protein A (SP-A). SP-A has well-established roles in modulating innate immunity in the lung.[12-14] SP-A binding moieties have been identified on *M pneumoniae*, and SP-A inhibits *M pneumoniae* growth *in vitro*.[15,16] We have previously shown that *M pneumoniae*–infected SP-A$^{-/-}$ mice have significantly augmented airway hyperresponsiveness (AHR), mucus production, and cellular inflammation compared with wild-type (WT) mice.[17] Inhibition of TNF-α by a transcriptional inhibitor protected SP-A$^{-/-}$ mice from *M pneumoniae*–induced phenotypes, but the cell type responsible for the TNF-α–mediated response during *M pneumoniae* infection was not elucidated.

Previously, we demonstrated that SP-A binds to MCs,[18] which led to our current hypothesis that MCs might be critical to SP-A–mediated regulation of airway reactivity and inflammation. Here we report novel information on a key role played by airway MCs in modulation of AHR in SP-A$^{-/-}$ mice infected with *M pneumoniae*. Using mice deficient in SP-A and MCs (SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice) engrafted with either TNF-α$^{-/-}$ or TNF receptor (TNF-R)$^{-/-}$ MCs, we found that augmented airway responses during *M pneumoniae* infection is a result of TNF-α interactions with MCs through the TNF-R and not MC-derived TNF-α. Therefore we describe a previously overlooked contribution of MCs as secondary responders to TNF-α during *M pneumoniae* infection.

METHODS

M pneumonia preparation

M pneumoniae from American Type Culture Collection (catalog no. 15531) was prepared as described previously.[17]

Mice

An inbred strain of SP-A$^{-/-}$ mice was generated, as previously described.[19] Please see the Methods section in this article's Online Repository at www.jacionline.org for additional information. All protocols were approved by the Institutional Animal Use and Care Committee at Duke University.

M pneumoniae inoculations

Mice 8 to 12 weeks of age were anesthetized by means of intraperitoneal injection of 10 μL/g body weight of a 12% ketamine (100 mg/mL) and 5% xylazine (20 mg/mL) mix and were infected with either 50 μL of sterile saline or 50 μL of 1×10^8 *M pneumoniae* units in sterile saline by means of intranasal instillation.

Airway physiology

Direct measurements of respiratory mechanics in response to methacholine were made by using the Flexivent system (SCIREQ, Montreal, Quebec, Canada) and reported as total pulmonary resistance (R_T) in centimeters of H_2O per milliliter per second, as described previously.[17] See the Methods section in this article's Online Repository for more information.

Isolation and analysis of bronchoalveolar lavage fluid from mice

Mice were euthanized and the lungs lavaged either 12 or 72 hours post Mp infection. For more information on isolation and analysis of bronchoalveolar lavage (BAL) fluid from mice, please see the Methods section in this article's Online Repository.

Isolation of BAL fluid from human subjects

Fiberoptic bronchoscopy was performed on subjects after achievement of conscious sedation, as previously described.[20] Asthma subjects met the National Asthma Education and Prevention Program criteria for asthma.[21] Please see the Methods section in this article's Online Repository for complete descriptions.

MC culture and reconstitution

Bone marrow was collected from C57BL/6, TNF-α$^{-/-}$, or TNF-R$^{-/-}$ mice and cultured in the presence of 5 ng/mL recombinant IL-3 and 5 ng/mL recombinant stem cell factor (R&D Systems, Minneapolis, Minn), as previously described.[22] Please see the Methods section in this article's Online Repository for complete descriptions.

M pneumoniae quantitation

RT-PCR was performed with primers for the *M pneumoniae*–specific P-1 adhesion gene, as previously described,[17] to assess the *M pneumoniae* burden. Please see the Methods section in this article's Online Repository for complete descriptions.

Gravity fixation and immunohistochemistry methods

For more information on gravity fixation and immunohistochemistry methods, please see the Methods section in this article's Online Repository.

In vitro MC assays

WT MCs were plated on a 24-well plate, and human SP-A, isolated as previously described,[12] was added at a concentration of 10 to 40 μg/mL for 1 hour at 37°C. *M pneumoniae* was added at a multiplicity of infection of 100:1. Please see the Methods section in this article's Online Repository for complete descriptions.

RESULTS

Association between asthmatic patients displaying high TNF-α levels in BAL fluid and *M pneumoniae* infection

Our previous observations showed that *M pneumoniae*–infected SP-A$^{-/-}$ mice have enhanced *M pneumoniae* colonization, increased levels of TNF-α, and significantly increased AHR compared with WT mice.[17] Because some asthmatic patients have positive test results for *M pneumoniae* in the airway, we determined whether *M pneumoniae* positivity associated with increased TNF-α levels in BAL samples from human asthmatic patients. Lavage samples were assessed for TNF-α levels, and based on the results, before assessing *M pneumoniae* positivity, the samples from asthmatic subjects were grouped into a low TNF-α–producing group (defined as less than 15-fold over the average TNF-α levels in healthy subjects) and a high TNF-α–producing group (defined as greater than 45-fold over the average TNF-α levels in healthy subjects). There were significantly higher TNF-α levels in both the "low asthmatic" and the "high asthmatic" groups compared with those seen in the healthy subjects (Fig 1, *A*), and there was also a significant difference between the 2 groups of asthmatic subjects. Cells from the lavage samples of each subject were then analyzed for *M pneumoniae* positivity; the investigator was blinded to the TNF-α levels. None of the healthy subjects had positive results for *M pneumoniae*, whereas approximately 10% of asthmatic subjects with low TNF-α levels versus about 50% of asthmatic subjects with high TNF-α levels had positive test results for *M pneumoniae* (Fig 1, B). In addition, we performed the reverse analysis comparing TNF-α levels in *M pneumoniae*–positive versus *M pneumoniae*–negative BAL samples and found that the presence of *M pneumoniae* was associated with significantly increased TNF-α levels (Fig 1, *C*). Although previous studies have shown that a subset of asthmatic patients are colonized with *M pneumoniae* and that certain asthmatic subjects have increased TNF-α levels in BAL fluid, ours is the first to establish a temporal link between these 2 phenotypes.[3,4,23,24]

Recruitment of MCs into the lungs of *M pneumoniae*–infected mice

The above data show that TNF-α levels increase during *M pneumoniae* infection, and an association between *M pneumoniae* infection and the number of MCs in airway biopsy specimens from human asthmatic patients has been shown.[4] Because MCs are potent reservoirs of TNF-α, we sought to determine the contribution of MCs in our model of *M pneumoniae* infection. MCs were localized to the subepithelial layer of the large airways and trachea in uninfected WT mice, as previously reported,[6,25] and were comparable between uninfected lungs of WT and SP-A$^{-/-}$ mice (Fig 2, *A* and *F*). The number of MCs per lung section increased significantly in WT and SP-A$^{-/-}$ mice 72 hours after *M pneumoniae* infection (Fig 2, *B* and *F*). MCs are often adjacent to the basement membrane of the airway epithelium in *M pneumoniae*–infected mice (Fig 2, *C*), and positively stained granules, which are consistent with MC degranulation, were detected in *M pneumoniae*–infected lungs (Fig 2, *D*); no granules were detected in saline-treated lungs. MCs were detected in the lung parenchyma in close proximity to alveolar type II cells, which are known to produce and secrete SP-A (Fig 2, *E*). We found no significant effect of the lack of SP-A on the number or localization of MCs during *M pneumoniae* infection.

MCs promote increased AHR and inflammation in *M pneumoniae*–infected SP-A$^{-/-}$ mice

Our previous findings suggested that during *M pneumoniae* infection, SP-A plays an important role in maintaining airway homeostasis by limiting an overzealous TNF-α response. Because MCs play key roles in asthma, we sought to determine the role of MCs in airway reactivity during *M pneumoniae* infection. Similar to published reports in other models, Kit$^{W-sh/W-sh}$ mice had reduced AHR compared with that seen in WT mice, although it was significantly increased over that seen in saline-treated Kit$^{W-sh/W-sh}$ control animals (Fig 3, *A*). To determine whether MCs play a role in increased AHR when SP-A is absent, we developed a mouse deficient in both MCs and SP-A (SP-A$^{-/-}$Kit$^{W-sh/W-sh}$). As we previously reported, SP-A$^{-/-}$ mice treated with *M pneumoniae* have significantly greater AHR than WT mice.[17] We found that by removing the MCs from the SP-A$^{-/-}$ mice (SP-A$^{-/-}$Kit$^{W-sh/W-sh}$), AHR was reduced to baseline levels (Fig 3, *B*). These data indicate that MCs play a key role in the enhanced AHR observed in SP-A$^{-/-}$ mice.

Examination of BAL fluid cells from *M pneumoniae*–infected mice revealed an increase in the number of polymorphonuclear leukocytes-neutrophils (PMNs), the main responder to *M pneumoniae* infection,[23] in SP-A$^{-/-}$ and SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice compared with that seen in WT mice 12 hours after infection (Fig 3, *C*). By 72 hours, the majority of BAL fluid cells were macrophages. SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice had significantly fewer macrophages compared with SP-A$^{-/-}$ mice but similar levels of neutrophils (Fig 3, *D*).

As a general indicator of lung injury, we measured total protein levels in the BAL fluid of the mice 72 hours after *M pneumoniae* infection. There were no differences between the groups, indicating that the changes in AHR are not associated with changes in total protein levels in the BAL fluid (Fig 3, *E*).

Recovery of AHR in SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice reconstituted with WT MCs

To assess further the role of MCs in the increased AHR observed in *M pneumoniae*–infected SP-A$^{-/-}$ mice, we performed a series of reconstitution experiments. Retro-orbital injection of bone marrow–derived MCs results in engraftment of the lung with significant numbers of MCs.[22] We repeated the *M pneumoniae* infection in MC-engrafted mice and assessed AHR. Engraftment of SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice with MCs was verified by means of immunohistochemistry throughout the lung tissue (Fig 4, *A* and *B*). Engraftment of SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice with WT MCs restored high levels of AHR to methacholine challenge, as expected in *M pneumoniae*–infected mice (Fig 4, *C*). These data indicate that the loss of MCs contributed to the decrease in AHR observed in SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice compared with that seen in mice lacking only SP-A. TNF-α levels in BAL fluid were measured 12 hours after infection, and as previously reported, SP-A$^{-/-}$ mice had a significant increase in TNF-α levels compared with levels seen in WT mice.[17] However, there was no difference in TNF-α levels observed in the SP-A$^{-/-}$ mice with MCs (SP-A$^{-/-}$ and SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice engrafted with WT MCs) or without MCs (SP-A$^{-/-}$Kit$^{W-sh/W-sh}$), and all were significantly increased over levels seen in WT infected mice (Fig 4, *D*).

SP-A negatively regulates MC TNF-α responses to *M pneumoniae*

Purified bone marrow–derived MCs were stimulated with *M pneumoniae* to determine whether SP-A plays a role in limiting *M pneumoniae*–induced MC release of TNF-α. TNF-α released by the MCs stimulated with *M pneumoniae* peaked 12 to 14 hours after stimulation, and addition of exogenous human SP-A significantly inhibited this release (Fig 5, *A*). Analysis of MC RNA extracted after 1 hour of *M pneumoniae* stimulation indicated that exogenous SP-A added at a physiologic concentration (10 μg/mL) inhibited *M*

pneumoniae–stimulated transcription of TNF-α (Fig 5, *B*). These findings suggest that SP-A can regulate the release and production of TNF-α in response to *M pneumoniae* infection.

Recovery of AHR in SP-A^{−/−}Kit[W-sh/W-sh] mice reconstituted with TNF-α^{−/−} but not TNF-R^{−/−} bone marrow–derived MCs

The above data revealed a key role for MCs in the increased AHR seen in SP-A^{−/−} mice, and as previously published, inhibition of TNF-α can attenuate this response.[17] High levels of TNF-α were measured in mice lacking MCs, suggesting that although MCs might be important in the augmented responses, MC-derived TNF-α might not be the key contributor to the increased levels of TNF-α. To better understand the contribution of TNF-α during *M pneumoniae* infection, we engrafted SP-A^{−/−}Kit[W-sh/W-sh] mice with TNF-α^{−/−} and TNF-R^{−/−} MCs. Mice reconstituted with TNF-α^{−/−} MCs and infected with *M pneumoniae* demonstrated an enhancement in reactivity to methacholine that was comparable with the responsiveness observed in mice reconstituted with WT MCs (Fig 6, *A*). In contrast, SP-A–sufficient mice (Kit[W-sh/W-sh]) engrafted with TNF-α^{−/−} MCs continued to have attenuated AHR during *M pneumoniae* infection compared with that seen in mice engrafted with WT MCs (see Fig E1 in this article's Online Repository at www.jacionline.org). However, *M pneumoniae*–infected SP-A^{−/−}Kit[W-sh/W-sh] mice receiving TNF-R^{−/−} MCs had a significantly reduced response to methacholine compared with that seen in mice reconstituted with either WT or TNF-α^{−/−} MCs. These data indicate that MC-derived TNF-α is a contributing factor in SP-A–sufficient mice but not the factor contributing to the high AHR in SP-A^{−/−} mice infected with *M pneumoniae*. TNF-α acting on MCs through the TNF-R is critical in regulating AHR during *M pneumoniae* infection in SP-A^{−/−} mice.

Mucus production in *M pneumoniae*–infected SP-A^{−/−} lungs is dependent on the MC TNF-α axis

We sought to determine the effect of MCs on production of mucus in SP-A^{−/−} mice. As has been previously reported,[17] WT infected mice have little mucus production in their large airways, whereas SP-A^{−/−} mice demonstrate increases in airway periodic acid–Schiff staining (PAS; Fig 6, *B*). In SP-A^{−/−}Kit[W-sh/W-sh] mice PAS staining was barely detectable in the large airways, mimicking what was seen in the WT mice and suggesting a role for MCs in mucus production during *M pneumoniae* infection in SP-A^{−/−} mice. Engraftment with WT MCs resulted in dramatic mucus production, although there was less detectable airway staining for PAS in mice that had been engrafted with either TNF-α^{−/−} or TNF-R^{−/−} MCs (Fig 6, *C*).

MCs contribute to the reduction of *M pneumoniae* burden in lung tissue

To assess the role of MCs in controlling *M pneumoniae* burden in the lung, we used RT-PCR to measure the levels of *M pneumoniae* P1 adhesin in the lung tissue 72 hours after *M pneumoniae* infection. As we reported previously, SP-A^{−/−} mice have a significant increase in *M pneumoniae* burden compared with that seen in WT mice.[17] In SP-A^{−/−}Kit[W-sh/W-sh] mice we found an even greater increase in *M pneumoniae* burden in the lungs. When these mice were engrafted with MCs, the *M pneumoniae* burden levels were reduced to those of the SP-A^{−/−} mice (Fig 7), indicating that MCs are essential in controlling the *M pneumoniae* burden within the lung tissue.

DISCUSSION

Not only do the findings presented here further the understanding of how the lung responds to *M pneumoniae* infection, they also suggest a novel role of the TNF-R on MCs in mediating responses to pathogens in the absence of SP-A or when SP-A is dysfunctional. In support of clinical data, we found increased MC numbers in the lung during *M pneumoniae*

infection in mice.[4] In addition, MCs are required for the enhanced AHR and mucus production observed in the SP-A$^{-/-}$ *M pneumoniae*–infected mice. SP-A$^{-/-}$Kit$^{W\text{-sh}/W\text{-sh}}$ mice continue to have high levels of TNF-α in their BAL fluid yet have lower AHR compared with SP-A$^{-/-}$ mice, indicating a pivotal role for MC products in mediating airway reactivity that is not dependent on MC TNF-α. When these mice (SP-A$^{-/-}$Kit$^{W\text{-sh}/W\text{-sh}}$) are reconstituted with either WT or TNF-α$^{-/-}$ MCs, AHR levels are increased during methacholine challenge; however, an AHR phenotype is not re-established when TNF-R$^{-/-}$ MCs are engrafted. This strongly suggests that the *M pneumoniae*–induced increase in AHR observed in the SP-A$^{-/-}$ mouse is critically and specifically dependent on stimulation of MC TNF-α receptors.

Our data support a model in which SP-A has a profound effect on TNF-α and show that in the absence of SP-A, TNF-α acts on MCs through the TNF-R to induce increased mucus production and AHR (Fig 8). It has been previously reported by our laboratory and others that SP-A directly affects the production of TNF-α by macrophages stimulated with Toll-like receptor 4 agonists, and we show here that SP-A inhibits *M pneumoniae*–induced TNF-α production by MCs *in vitro*.[14,26,27] It is also possible that SP-A affects TNF-α production by airway epithelial cells or PMNs. We speculate that the increased TNF-α that occurs in the absence of SP-A interacts in the submucosa with the TNF-R on MCs, causing release of a myriad of prestored and newly synthesized mediators. Many mediators from degranulating MCs, including cytokines, chemokines, lipid mediators, and histamine, might play a key role in the pathophysiology of the asthmatic lung (reviewed by Reuter et al[28]). Additionally, MC proteases stimulate tissue remodeling and enhance mucus secretion.[29] We hypothesize that it is one of these mediators, or several working in concert, that causes AHR and mucus production in the presence of a robust TNF-α response during *M pneumoniae* infection.

Although the link between MC degranulation and mucus production has been shown (reviewed by Hart[29]), the direct contribution of MC-derived TNF-α in the regulation of mucus hyper-secretion remains incompletely defined. Here we show novel findings that although engraftment of WT MCs results in dramatic increases in PAS-positive airway cells, engraftment of either TNF-α$^{-/-}$ MCs or with TNF-R$^{-/-}$ MCs resulted in significantly fewer PAS-positive cells. These findings suggest a critical role for the TNF-mediated pathway in MCs for the increased presence of mucopolysaccharides in airway epithelium during *M pneumoniae* infection, which can contribute to the exacerbations experienced by a subset of asthmatic patients who become colonized with *M pneumoniae*.

The importance of MC-derived TNF-α in lung pathophysiology has been described by several laboratories. Using the well-established ovalbumin model of asthma, Nakae et al[30] found that engraftment of Kit$^{W\text{-sh}/W\text{-sh}}$ mice with TNF-α$^{-/-}$ MCs resulted in decreased lymphocyte recruitment and T$_H$2 cytokine production compared with that seen in Kit$^{W\text{-sh}/W\text{-sh}}$ mice engrafted with WT MCs.[30,31] In a footpad model of *Escherichia coli* infection, MC-derived TNF-α was shown to be important for lymph node hypertrophy.[32] On the basis of these studies, we anticipated that TNF-α produced by MCs in the lung would be pivotal in mediating the host response to *M pneumoniae*. Indeed, SP-A–sufficient Kit$^{W\text{-sh}/W\text{-sh}}$ mice engrafted with TNF-α$^{-/-}$ MCs had attenuated AHR compared with those engrafted with WT MCs. However, SP-A$^{-/-}$Kit$^{W\text{-sh}/W\text{-sh}}$ mice engrafted with TNF-α$^{-/-}$ MCs maintained a high level of increased AHR. The differing role of MC-derived TNF-α in SP-A–sufficient versus SP-A–deficient lung environments is likely attributed to a compensatory mechanism that might be partially explained by increased PMN recruitment in the TNF-α$^{-/-}$ MC–engrafted SP-A$^{-/-}$Kit$^{W\text{-sh}/W\text{-sh}}$ mice compared with that seen in WT MC–engrafted SP-A$^{-/-}$Kit$^{W\text{-sh}/W\text{-sh}}$ mice. This additional increase in PMN recruitment could lead to heightened TNF-α production, therefore masking the importance of MC-derived TNF-α. Our data are interesting in that they show that TNF-α acting through the

TNF-R on MCs is critical in modulating the host response to *M pneumoniae* when SP-A is absent.

Previous murine and clinical studies indicated a key role for MCs in *Mycoplasma* species infections.[33,34] We tested the ability of MCs to kill *M pneumoniae* directly using *in vitro* techniques, and although we found that MCs were unable to kill *M pneumoniae* in samples directly (data not shown), our findings *in vivo* further strongly support the involvement of MCs in the resolution of *M pneumoniae* infection, albeit through indirect mechanisms. When MCs were reconstituted into the MC-deficient mice, the *M pneumoniae* burden was decreased nearly to the level detected in MC-sufficient mice. Interestingly, we found no difference in burden between mice engrafted with WT, TNF-$\alpha^{-/-}$, or TNF-R$^{-/-}$ MCs, indicating that neither signaling through the MC TNF-R nor MC-derived TNF-α is necessary for *M pneumoniae* resolution.

The current understanding of MCs and TNF-α interactions in asthmatic patients is not complete, in part because of the complexity of the multiple phenotypes that encompass human asthma. Researchers have delved into the aspect of how MC-derived TNF-α affects the lung and asthmatic phenotypes, although to the best of our knowledge, no studies have examined the effect of TNF-α acting on MCs to initiate degranulation and activation of newly synthesized mediators *in vivo*. In addition, a recent report by Wang et al[35] concluded that asthmatic SP-A binds less to *M pneumoniae* membranes and is unable to protect airway epithelial cells from *M pneumoniae* stimulation *in vitro*. Although anti-TNF treatment has met with mixed results,[24,36-39] our results suggest that targeting MCs might prove to be a safer and more effective way of reducing symptoms in asthmatic patients, who might have dysfunctional surfactant, with *M pneumoniae* infections. Historically, during microbial infections and asthma, the contributions of MCs to the inflammatory response have been primarily attributed to their ability to directly interact with allergens, pathogens, or both and respond by releasing a panoply of prestored and recently synthesized mediators. Our findings provide evidence that MCs can also respond to locally produced cytokines, such as TNF-α, and therefore serve as secondary responders, bolstering responses initiated by activated epithelial cells, other inflammatory cells, or both.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations used

AHR   Airway hyperresponsiveness

BAL   Bronchoalveolar lavage

| MC | Mast cell |
PAS	Periodic acid–Schiff
PMN	Polymorphonuclear leukocyte-neutrophil
R_T	Pulmonary resistance
SP-A	Surfactant protein A
TNF-R	TNF receptor
WT	Wild-type

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Key messages

- High TNF-α levels associate with *M pneumoniae* positivity in asthmatic patients, and human SP-A inhibits *M pneumoniae*–stimulated transcription and release of TNF-α by MCs.

- MC numbers increase in *M pneumoniae*–infected lungs, and airway reactivity is dramatically attenuated when MCs are absent from SP-A$^{-/-}$ mice.

- Using SP-A$^{-/-}$Kit$^{W-sh/W-sh}$ mice engrafted with TNF-α$^{-/-}$ or TNF-R$^{-/-}$ MCs, we find that TNF-α activation of MCs through the TNF-R and not MC-derived TNF-α leads to augmented AHR during *M pneumoniae* infection in SP-A$^{-/-}$ mice.
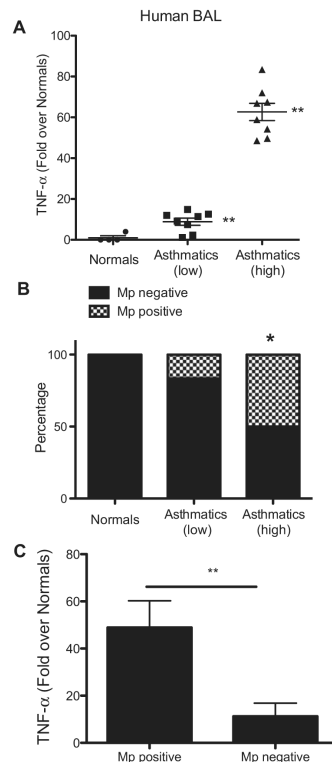
FIG 1.

Mycoplasma pneumoniae (Mp) positivity and TNF-α levels in asthmatic samples. **A**, TNF-α in human BAL fluid (fold over healthy subjects). **P < .01, asthmatic patients *(high)* versus asthmatic patients *(low)* and both asthma groups versus healthy subjects. **B**, RT-PCR for *M pneumoniae*–specific P1 adhesin and dilution of samples into SP-4 broth to verify *M pneumoniae* positivity in subjects. *P < .05, asthmatic patients *(high)* versus healthy subjects and asthmatic patients *(low)*. **C**, TNF values in *M pneumoniae*–positive and *M pneumoniae*–negative samples. **P < .01.
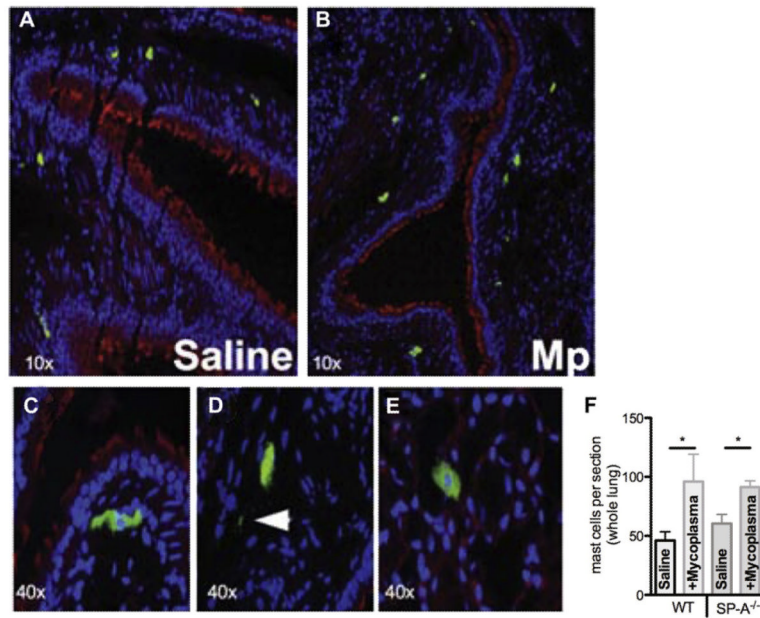
FIG 2.
MCs in *Mycoplasma pneumoniae (Mp)*–infected lung tissue. Mice were instilled with saline (**A**) or *M pneumoniae* (**B-E**) and lungs were analyzed by means of immunohistochemistry 3 days after infection for MCs. Fig 2, C, MCs adjacent to the large airway. Fig 2, *D*, Extracellular MC granules *(arrow)*. Fig 2, E, MCs in the lung parenchyma. **F**, Total number of tissue MCs assessed in WT versus SP-A$^{-/-}$ lungs (n = 5 each). *$P < .05$.
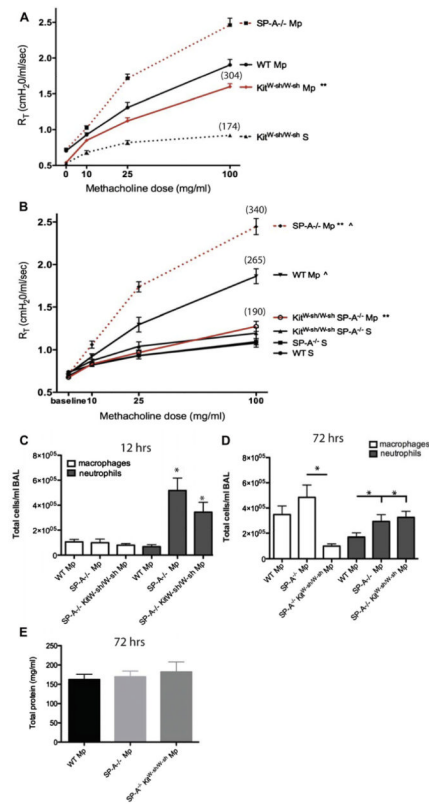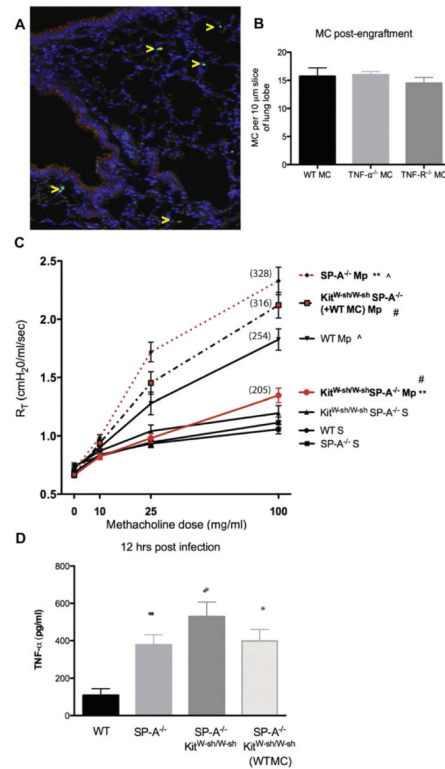
FIG 3.
Contribution of MCs to AHR and inflammation. **A** and **B**, Three days after *Mycoplasma pneumoniae (Mp)*, AHR to methacholine challenge was measured by using the Flexivent system. Mean percentage over baseline for highest methacholine dose is shown in parentheses. **P < .01 and ^*P* < .01, 3 independent experiments per graph. **C** and **D**, Cells in BAL fluid 12 hours (Fig 3, **C**) or 72 hours (Fig 3, **D**) after infection. *P < .05 versus WT *M pneumoniae* unless otherwise noted. **E**, Total protein in BAL fluid, n ≥ 12 mice per group.

FIG 4.
AHR in WT bone marrow–derived MC–reconstituted infected mice. **A**, Engraftment of MCs (arrows) verified by means of immunohistochemistry in lung tissue 1 month after injection. **B**, Quantification of engraftment. **C**, AHR to methacholine challenge was measured by using the Flexivent system. Mean percentage over baseline for highest methacholine dose is shown in parentheses. **P < .01, #*P* < .01, and ^*P* < 0.05, 3 independent experiments per graph. **D**, TNF-α in BAL fluid 12 hours after infection. *P < .05 versus WT *Mycoplasma pneumoniae* group, n   12 mice per group. *Mp, Mycoplasma pneumoniae.*
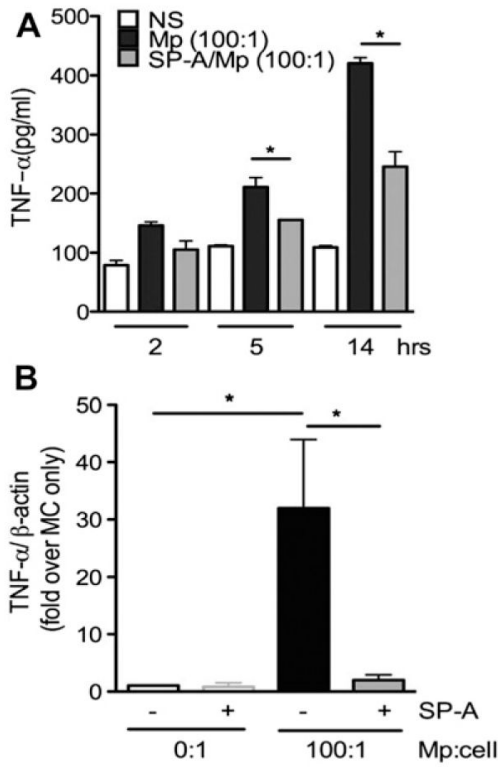
FIG 5.
TNF-α production from *Mycoplasma pneumoniae (Mp)*–stimulated MCs. Nonstimulated *(NS)* or *M pneumoniae*–stimulated (multiplicity of infection, 100:1) bone marrow–derived MCs with and without SP-A (40 μg/mL) were analyzed over 14 hours (**A**) or 1 hour (**B**). Supernatants were analyzed for TNF-α release by means of ELISA; cell pellets were assessed by using RT-PCR for TNF-α transcription. *P < .05. Data are representative of 3 independent experiments.
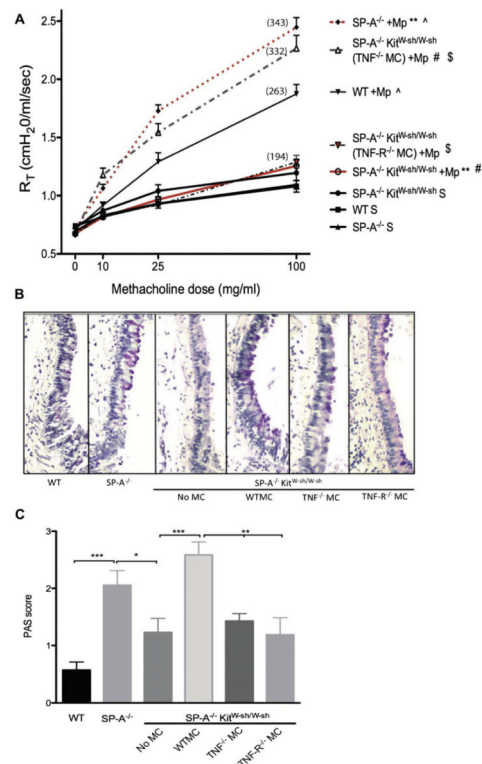
FIG 6.
Contribution of MC TNF-R to AHR and mucus. Mice were engrafted with TNF-$\alpha^{-/-}$ or TNF-R$^{-/-}$ bone marrow–derived MCs. **A**, AHR to methacholine challenge was measured by using the Flexivent system. Mean percentage over baseline for highest methacholine dose is shown in parentheses. **, ^, #, \$$P$ < .01, 3 independent experiments per graph, n 12 mice per group. **B** and **C**, PAS-stained airways in infected mice (original magnification ×10) were scored. ***P < .001, **P < .01, and *P < .05; n = 7-12 sections per group. *Mp, Mycoplasma pneumoniae.*
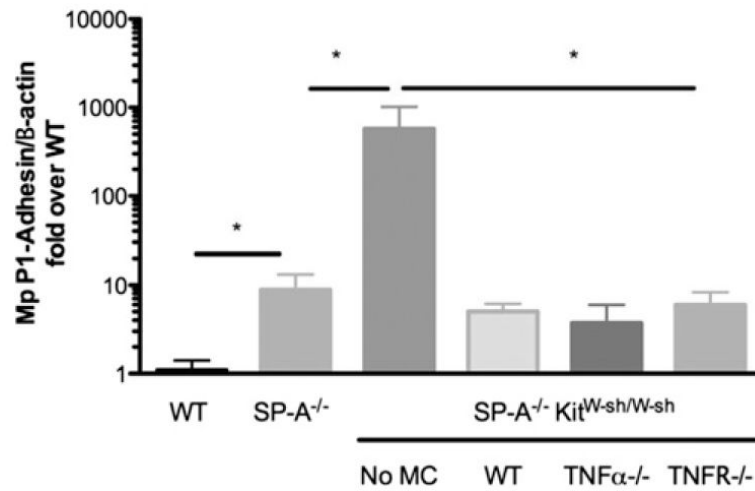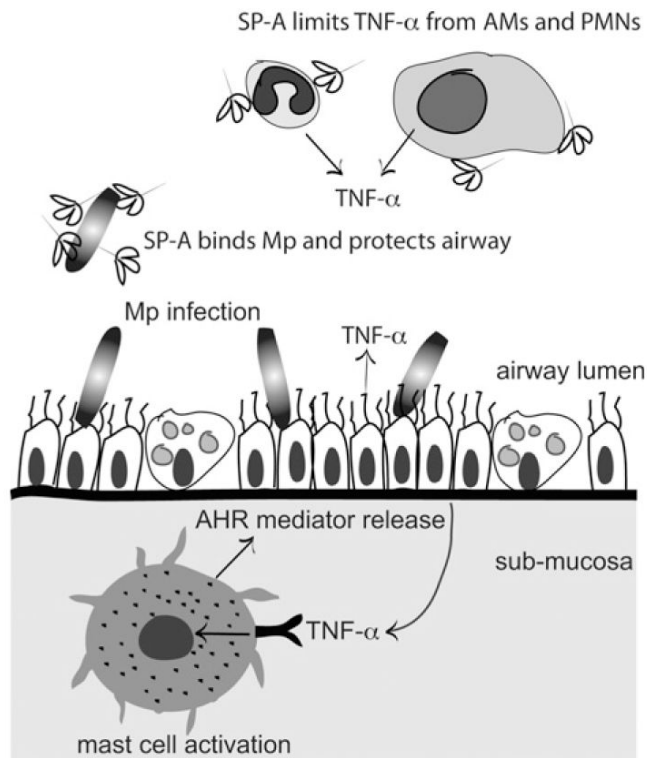
FIG 7.
Contribution of MCs to *Mycoplasma pneumoniae (Mp)* clearance. *M pneumoniae* burden in lung tissue at day 3 was measured by using RT-PCR for the *M pneumoniae*–specific P1 adhesin gene relative to β-actin and is expressed as fold over WT *M pneumoniae*–infected mice. *$P < .05$, n ≥ 12 mice per group.

FIG 8.
Protective role of SP-A against *Mycoplasma pneumoniae (Mp)*–induced AHR. SP-A binds *M pneumoniae* in the large airway and prevents overcolonization. If SP-A is dysfunctional, more *M pneumoniae* burden results in TNF-α overproduction from epithelial cells, alveolar macrophages *(AMs)*, and/or neutrophils *(PMNs)*. TNF-α encounters tissue MCs in the submucosa, which are stimulated through the TNF-R to release mediators that contribute to high levels of AHR.