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Review

Elucidating the Role of Nrf2 Signaling Pathway in *Mycoplasma* Infections

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Abstract

Mycoplasmas are the smallest cell-wall-less self-replicating prokaryotes. *Mycoplasma* species can be found within and outside cells as "silent parasites" that live intracellularly and as membrane surface parasites. The pathogen's impact on respiratory health seems primarily caused by its capacity to alter immune responses, cause airway inflammation, and damage epithelial barriers. Much progress has been made in understanding *Mycoplasma*-induced inflammation and oxidative stress. However, there are still issues in therapeutic management, such as the development of strains that are resistant to antibiotics, the shortcomings of the available diagnostic techniques, and possible long-term respiratory consequences. On the other hand, to combat oxidative stress, inflammation, and metabolic abnormalities, activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is becoming a more appealing therapeutic strategy. Nrf2 activation coordinates a thorough defense through its transcriptional targets, enabling adaptability and survival under a variety of cellular stressors. Nrf2 is regarded as a therapeutic target, and pharmacological Nrf2 activators have demonstrated protective effects in multiple pathological consequences and advantages in clinical trials. In this review, we discussed the rationale for targeting Nrf2 in a series of inflammatory responses caused by *Mycoplasma* species.

Keywords: *Mycoplasma*; HO-1; Nrf2; gene expression

1. Introduction

Mycoplasma pneumoniae plays a major role in the onset and aggravation of chronic lung pathology. Human tracheobronchitis, pharyngitis, asthma, and primary atypical pneumonia are all brought on by *Mycoplasma pneumoniae* [1]. The smallest self-replicating prokaryotes without a cell wall, *Mycoplasmas*, have a genome that is incredibly tiny, ranging from 580 to 2200 kb [2]. Only a small number of the more than 200 species of this pathogen known to affect humans, animals, arthropods, and plants. Particularly noteworthy are the pathogenic bacteria *Mycoplasma pneumoniae* (*M. pneumoniae*), *M. genitalium, M. pirum, M. hominis, M. fermentans*, and *M. penetrans* impact both human and animal systems [3]. Among these harmful strains, *M. pneumoniae* is the most common and well-researched species. Pneumonia due to *M. pneumoniae* sufferers is more common in school-age children and young adults than in newborns and elderly people [4]. Lower respiratory tract infections are thought to be a common source of morbidity and mortality in children, and this atypical pathogen is the cause of up to 40% of community-acquired pneumonia (CAP) in children older than five [5,6]. Compared to healthy controls, respiratory samples from adults and children experiencing asthma attacks or exacerbations have greater incidences of *M. pneumoniae* [7]. The association between *M. pneumoniae* and asthma is further supported by the high rates of the bacteria in the airways of chronic stable asthmatics [8].

Chronic infections are typically caused by *Mycoplasmas*, and it is unclear what the majority of their pathogenic factors are and how they work [9]. *Mycoplasmas* have a propensity to penetrate, harm, and colonize deep tissues due to tissue necrosis, local trauma, surgery, disturbance of the mucosal surface, and compromised sterile site clearance [9]. *Mycoplasmas* are indeed thought to be the cause of these localized infections in a lot of cases, because it is difficult to isolate and identify them using laboratory techniques [10–13]. A diagnosis based solely on serology may not be accurate since IgM antibodies might not be detectable early in the course of infection. It has been discovered that the seroprevalence of *M. pneumoniae* in persons with pneumoniae varies greatly, ranging from 1.9% to more than 30% [14]. Nucleic acid amplification tests (NAATs), sequencing, and proteomic investigations are examples of molecu-

lar diagnostic advancements that have helped us learn more about the pathophysiology of this organism [15,16].

The development of protein sequence techniques has made it possible to identify potential factors that influence the pathogenicity of *Mycoplasmas* in both humans and animals [17]. Thus, several findings suggest that the lipoproteins on *Mycoplasmas*' membrane interact with monocytes and macrophages and occasionally lead to immune system evasion [18–20]. Confocal micrographs have recently shown that, depending on the kind of cell, *M. pneumonia* can bind and internalize [21,22].

The pathophysiology of respiratory infections caused by *M. pneumoniae* is complex and includes both direct cytotoxic effects on host cells and indirect processes that cause the host's immune responses to become dysregulated [23]. *M. pneumoniae* effectively invades and adheres to pulmonary epithelial cells, resulting in impaired mucociliary clearance, significant cell damage, and disruption of the epithelial barrier [24]. *M. pneumoniae* can use its apical structure to attach to the surface of host cells during infection, after which it can absorb nutrients from the cells [25]. Apical structural attachment and gliding have the potential to physically harm host cells [25]. Furthermore, upon attachment to host cells, modifications in *M. pneumoniae*'s pathogenicity have been documented [26]. Increased production of superoxide and hydrogen peroxide radicals, the development of the toxin associated with community-acquired respiratory distress syndrome (CARDS), and modifications to *M. pneumoniae*'s lipid proteins are among the virulence-related regulators [26]. When exposed to noxious stimuli, neutrophils are usually the first cells to extravasate into tissues. They are extremely sensitive to a wide range of stimuli, especially infections and molecular patterns linked to injury [27]. By promoting the recruitment of more granulocytes and phagocytic monocytes, these prodigious leukocytes serve as the first line of defense against microbial threats. They also effectively control infection and preserve mucosal integrity through processes like degranulation reactions, oxidative bursts, and the release of neutrophil extracellular traps (NETs) [27]. Significant neutrophil recruitment and infiltration in the lungs 24 hours after *M. pneumoniae* infection, a notable rise in the neutrophil ratio in bronchoalveolar lavage fluid (BALF), and a sizable neutrophil infiltrate visible in lung histopathology are further indications from animal studies that Mycoplasma pneumoniae (MP) infection causes a strong early neutrophil response. Numerous signaling pathways are involved in neutrophil recruitment and migration following *M. pneumoniae* infection [28,29]. Pro-inflammatory cytokine production is linked to the symptoms of pneumonia brought on by *M. pneumoniae*, which imply that *M. pneumoniae*-induced exaggerated immune responses are a significant contributor to pneumonia development [30]. Although *M. pneumoniae* infections usually resolve on their own in healthy people, they can have more serious effects in other groups, such as those with weakened immune systems or underlying respiratory disorders [31].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor made up of 605 amino acids and features conserved domains ranging from Neh1 to Neh7, which play a crucial role in its function. The N-terminal domain interacts with (Kelch-like ECH-associated protein 1) Keap1 through binding motifs like ETGE and DLG to stabilize and ubiquitinate Nrf2 [32]. DNA binding through the basic-region leucine zipper (bZIP) domain is carried out by the Neh1 domain [33]. The interaction of Nrf2 with other coactivators is then mediated by the Neh3 to Neh5 domains [34]. Ubiquitination through β-transducin repeat-containing protein is carried out by another domain, Neh6 [35]. Lastly, through the retinoic X receptor, Neh7 suppresses the antioxidant response element (ARE) signaling pathway. Keap1 is a 624 amino acid protein that is high in cysteines [36]. The Keap1-cullin 3 (Cul3)-Ring box protein-1 (RBX1) E3 ligase complex is formed when the (Broad-complex, Tramtrack, and Bric-a-brac) BTB domain in Keap1 homo-dimerizes to bind to the E3 ligase [37]. Likewise, Keap1 binds to Nrf2 and p62 as a result of the Kelch repeats [38]. The DLG motif from Keap1 may uncouple due to oxidative stress and electrophile assault, releasing Nrf2 from the Keap1-Cul3-RBX1 complex. Following this process, Nrf2 translocates into the nucleus, where it hetero-dimerizes with small Maf proteins (sMaf) and binds to the ARE further. This further stimulates several antioxidant enzymes, including glutathione-S-transferase (GST) and heme oxygenase-1 (HO-1) [39]. It is now widely known that thiol changes of C151 in the BTB domain activate Nrf2 after inhibiting the Keap1-Cul3 interactions [40].

The Nrf2 and nuclear factor kappa B (NF-κB) signaling pathways are thought to work together to control the cellular response to stress and inflammation as well as to preserve the physiological equilibrium of cellular redox status. Nevertheless, it is still unclear what molecular mechanisms underlie this functional connection, which seems to be tissue and cell-type specific. At the transcriptional level, the Nrf2 pathway can be directly inhibited by the NF-κB p65 subunit. The Nrf2 pathway is inactivated as a result of the NF-κB p65 subunit's competition with Nrf2 for the CH1-KIX domain of the transcriptional co-activator CREB-binding protein (CBP). However, by encouraging histone deacetylase 3 (HDAC3) to bind with CBP or musculoaponeurotic fibrosarcoma oncogene homolog K (MafK), NF-κB recruits HDAC3, resulting in local hypoacetylation and blocking Nrf2 signaling [41]. It's interesting to note that several anti-inflammatory drugs can interfere with NF-κB activation and hence activate the Nrf2 pathway [42,43]. The *Nrf2* gene features an NF-κB binding site where the p65/p50 heterodimer is recruited, according to the data. IκK-β is essential for NF-κB signaling and possesses an ETGE motif that allows it to attach to Keap1 [44]. Un-

derstanding the role of these structural components in *Mycoplasma* infections can provide valuable insights into the mechanisms of Nrf2 and its significance as a potential therapeutic target.

2. Inflammation and Oxidative Stress in *M. pneumoniae*

According to reports [45], several *Mycoplasma* species stimulate different immune cells and produce pro-inflammatory cytokines. It has long been unclear what causes inflammatory reactions in *Mycoplasma* species, as they lack immune cell stimulators such as peptidoglycan and lipopolysaccharide (LPS). Regardless of whether they are affixed to the surface of eukaryotic cells or invade them, certain *Mycoplasmas* disrupt and modify the host cell's biological pathways at the functional and/or regulatory level [45]. After infection, the host organism initiates a series of reactions involving multiple signaling pathways to protect itself from such harmful outcomes. These reactions ultimately lead to the activation of the immune response, which triggers processes that promote inflammation. In response, *Mycoplasmas* evolved defense mechanisms that allow them to evade immune regulation and infiltrate many bodily regions, including mucosal surfaces. However, *Mycoplasmas* are frequently able to adapt because of the lag between initial triggering and the emergence of a full-scale reaction [45,46].

As previously stated, *Mycoplasma* adheres to the outer cellular membrane, which causes specific bacterial proteins (lipoproteins (LPs) or lipopeptides) to interact with particular cellular receptors on the target cells' surface. In this context, various investigations have discovered a variety of *Mycoplasmas* LPs that can communicate with the host organism's leukocytes and epithelial cells [47,48]. The first lipopeptide expressed in *Mycoplasmas* that was shown to bind toll-like receptors (TLRs) was *M. fermentans*' macrophage-activating lipopeptide-2 (MALP-2) [49]. Heterodimers of TLR 1/2 or TLR 2/6 were then demonstrated to bind to triacylated or diacylated lipopeptides, respectively [50,51]. There are no *Mycoplasma* genomes that have the *Lnt* gene, which codes for the enzyme responsible for N-acylation [52]. However, according to a study on the proportion of N-amide to O-ester linkages in *M. gallisepticum* and *M. mycoides*, triacylated lipoproteins may be present [53]. Additionally, *M. mycoides* proteins' resistance to Edoman degradation suggested the presence of N-acylation [54]. The diacylated lipoprotein subunit b of the F0F1 ATP synthase (MPN602) in *M. pneumoniae* is known to trigger inflammatory reactions via TLR2 [55]. Interestingly, leukocyte infiltration in the respiratory tract can be promoted by specific lipopeptides that have been identified and purified from *Mycoplasmas*, suggesting that these factors may potentially have an effect when the entire *Mycoplasma* organism is absent [56].

The presence of lipoproteins in non-pathogenic *Mycoplasma* species, however, points to the possibility of an additional mechanism by which *M. pneumoniae* triggers inflammatory reactions. It was already been reported that *M. pneumoniae* causes significant inflammatory reactions, even in macrophages generated from mice with TLR2 deletion [57].

Certain *Mycoplasma* species, notably *M. pneumoniae*, generate the cytotoxic CARDs toxin. Another report demonstrated that NLRP3 inflammasome activity is regulated by CARDS toxin (MPN372) (Fig. 1) [58]. This work showed that CARD toxin increases the production of interleukin (IL)-1β and activates inflammasomes by ADP-ribosylating NLRP3. According to Shimizu *et al.* [30], *M. pneumoniae* causes host cells to release ATP, which activates inflammasomes and releases IL-1β. Sugiyama *et al.* [59] showed that *M. pneumoniae* causes IL-1β in dendritic cells via the NLRP3 inflammasome.

With its ability to produce H_2S, which damages blood cells, the HapE found in *M. pneumoniae* serves as a possible virulence factor. HapE's generation of H_2S causes phagocytes to produce pro-inflammatory substances, which in turn intensifies inflammatory reactions [60]. Additionally, HapE uses ATP-sensitive K^+ (KATP) channels to contribute to inflammatory reactions [61]. When cysteine is broken down within the KATP channel complex, more H_2S is produced, which exacerbates inflammation.

The immunoglobulin-binding protein of *Mycoplasma* (IbpM), sometimes referred to as mpn400, binds firmly to a variety of immunoglobulins generated by the host, including IgM, IgA, and IgG. The importance of IbpM as a virulence factor was demonstrated by a prior investigation that revealed strains of *M. pneumoniae* lacking IbpM show a minor decrease in cytotoxicity [62].

When *M. pneumoniae* adheres to host cells, it enters the cells and releases superoxide and hydrogen peroxide radicals, leading to oxidative stress in the respiratory tract epithelial cells. Furthermore, *M. pneumoniae* does not have catalase or superoxide dismutase, thus the radicals it generates can prevent the host cell's catalase action. As a result, there is less peroxide breakdown, making host cells more susceptible to damage from oxygen molecules [63]. It has been documented that *M. pneumoniae*'s hydrogen peroxide can control the detachment of infected cells, promoting the bacterial infection's persistence [64]. L-α-glycerophosphate oxidase is essential for the metabolism of glycerol, which produces hydrogen peroxide and affects the pathophysiology of *M. pneumoniae* [65]. Furthermore, the *M. pneumoniae* genome encodes histidine phosphocarrier protein kinase, an essential regulator of carbon metabolism, which causes the production of peroxide and ultimately oxidative stress [66].

Reactive oxygen species (ROS) are generated by nicotinamide adenine dinucleotide phosphate oxidase and are a component of the host's non-specific immunologi-

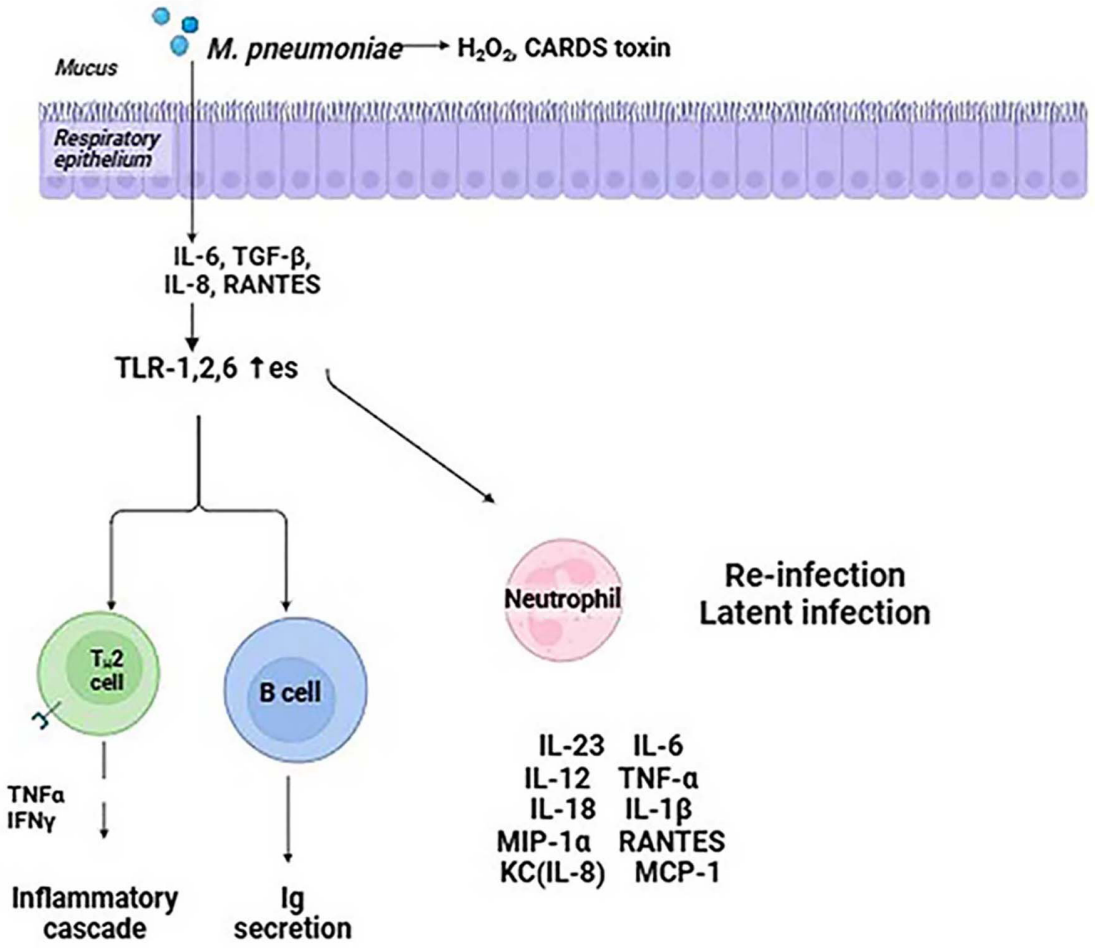

Fig. 1. Cellular pathways implicated in inflammation and cellular transformation are impacted by *Mycoplasma pneumoniae*. H_2O_2, hydrogen peroxide; CARDS, community-acquired respiratory distress syndrome; TLR, Toll-like receptor; IL, interleukin; TGF-β, transforming growth factor β; TNF, tumor necrosis factor; INF, interferon; MCP-1, monocyte chemoattractant protein-1; IG, Immunoglobulin; IFNγ, Interferon-γ; MIP-1α, macrophage inflammatory protein-1α. $\uparrow$, increase or upregulation. Created with Biorender.com.

cal defense against invasive pathogens [67]. ROS have two roles: they target proteins, lipids, carbohydrates, and nucleic acids in *M. pneumoniae* cells, acting as direct antibacterial agents and significantly impairing these biological components. At the same time, ROS are crucial signals for innate immunity signaling, which activates the immune system to fight off infections. A recent investigation found that mpn668 encodes a protective antioxidant enzyme in *M. pneumoniae* [68]. This enzyme breaks down hydroperoxide, which may lessen the oxidative damage the host generates. Furthermore, neutrophils quickly gather at the infection site after an *M. pneumoniae* infection due to chemokine chemotaxis. As a result of their increased phagocytic activity, infections are successfully eradicated by neutrophil extracellular traps (NETs) and the release of several bactericidal chemicals. Extracellular nucleases produced by *M. pneumoniae* can break down NETs. Interestingly, the magnesium-dependent nuclease produced by *M. pneumoniae* mpn491 is an important extracellular nuclease that increases the pathogen's survival rate and helps it evade the host's immune response by breaking down NETs, which further damages the host [68].

3. Role of Nrf2 Signaling Pathway

The lipoprotein components of the *M. pneumoniae* membrane, which are produced on the surface as lipid-associated membrane proteins (LAMPs), are essential for the host immunological inflammatory response [69]. It has been discovered that LAMPs attach to monocytes and macrophages' TLRs 1, 2, and 6 as well as CD-14. These cells then activate downstream IL-1R-associated signal molecules, which activate NF-κB and activating protein 1 (AP-1) (Fig. 2) [55]. Ultimately, this mechanism sets off the production of inflammatory mediators like prostaglandin E2 (PGE2), ROS, nitric oxide (NO), and other bioactive compounds, as well as proinflammatory cytokines [70]. LAMPs have been demonstrated to impact Nrf2 location, and LAMP-stimulated Nrf2-silenced THP-1 cells exhib-

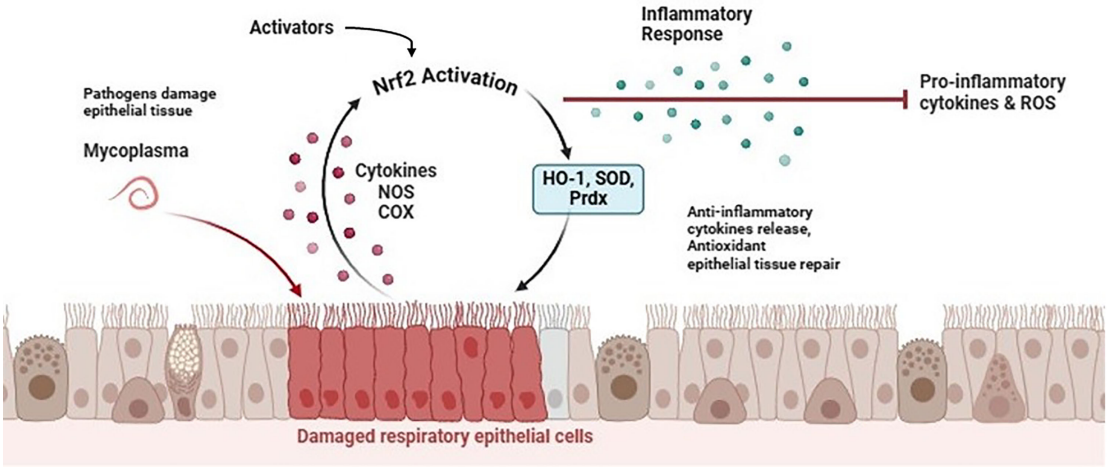

Fig. 2. Progression of *Mycoplasma*-induced infections and role of Nrf2 signaling pathway. Nrf2, erythroid 2-related factor 2; HO-1, heme oxygenase-1; SOD, superoxide dismutase; Prdx, peroxiredoxin; NOS, nitric oxide synthase; ROS, reactive oxygen species; COX, cyclooxygenase. Created with Biorender.com.

ited significantly higher amounts of ROS and inflammatory reactants, such as NO, PGE2, and cytokines (IL-6, IL-8) [71]. Additionally, LAMPs increased HO-1 expression and mRNA levels.

Macrophage-activating lipopeptide-2 (MALP-2), derived from the NH_2 terminal of the mycoplasmal lipoprotein, is commonly used as the pathogen-associated molecular pattern (PAMP) for *Mycoplasma* studies because it shares many pro-inflammatory properties with membrane lipoproteins in nearly all *Mycoplasmas* [72]. In an earlier report, it was shown that MALP-2 might activate HO-1 monocyte expression to control the overexpression of cyclooxygenase 2 [73]. In THP-1 cells, MALP-2 reportedly increased HO-1 enzyme activity and stimulated HO-1 mRNA and protein expression [73]. However, HO-1 expression was dramatically reduced by mitogen-activated protein kinase (MAPK) inhibitors SB203580, PD98059, and SP600125. This implies that MAPK pathways might be an upstream signal required for the production of HO-1 that is dependent on MALP-2.

Furthermore, Nrf2 translocation was also triggered by MALP-2 and the levels of MALP-2-mediated HO-1 expression were reduced in THP-1 cells when Nrf2 expression was silenced. Additionally, THP-1 cells' levels of cyclooxygenase 2 (COX-2) protein expression were elevated in response to MALP-2, and transfected with HO-1 siRNAs markedly raised COX-2 accumulation. According to another study, phosphoinositide 3-kinases (PI3K)/Akt, ROS, and Nrf2-dependent HO-1 expression influence the inhibitory action of sulforaphane on the release of several pro-inflammatory mediators in MALP-2-stimulated monocytes [74]. Sulforaphane induced Nrf2 to move from the cytoplasm to the nucleus, however, HO-1 expression was markedly suppressed by short interfering RNA-mediated Nrf2 knockdown. The pharmacological inhibitors LY294002 and N-acetylcysteine (NAC) also demonstrated that PI3K/Akt and ROS were implicated in Sulforaphane-induced Nrf2 activation and HO-1 expression. Additionally, animals treated with sulforaphane showed reduced lung inflammation and pro-inflammatory cytokine release, as well as NF-κB activation driven by MALP-2. Additionally, the inhibitory effects of Sulforaphane were reversed by Sn-protoporphyrin IX (SnPP), a selective inhibitor of HO-1, and the cytokine secretion generated by MALP-2 was significantly reduced by carbon monoxide releasing molecule 2 (CORM-2), a chemical that releases carbon monoxide.

In a previous report, the authors examined H_2S's anti-inflammatory properties in an *in vitro* model of proinflammatory *Mycoplasma*-infected macrophage microbe with the ability to initiate the rapid enrolment of a significant number of macrophages particularly in the airways and lungs [19]. Additionally, the research demonstrated that exogenous H_2S can prevent NF-κB from activating and moving to the nucleus, which lowers the transcription of pro-inflammatory genes and their release. A study clarifies the function of Nrf2 and how it reacts to H_2S therapy in a cellular model of acute *M. fermentans* infection [75]. A human monocytic cell line (U937) was infected with *M. fermentans* and treated concurrently with NaHS, a fast-releasing H_2S donor, to test the theory that H_2S activated the Nrf2 pathway. In U937 *Mycoplasma*-infected cells, they noticed a gradual rise in Nrf2 expression, which was exacerbated by the H_2S donor treatment. To support these findings, the expression of the Nrf2-ARE inducible detoxification enzymes, HO-1, peroxiredoxin (Prdx), and superoxide dismutase 1 (SOD1), was measured using a real-time reverse transcription polymerase chain reaction (RT-PCR). However, at separate times, they noticed notable increases in the mRNA expression levels of all three enzymes. The results provided by this study suggest that the Nrf2/HO-1 pathway overex-

pression is at least partially responsible for the H_2S antioxidant effects in infected monocytes. However, one significant unresolved issue concerns the mechanism via which H_2S triggers Nrf2 activation. According to certain theories, H_2S causes Keap1's essential cysteine residues to change, releasing Nrf2 [76]. It will take more research to determine whether H_2S directly or through upstream signaling modifies Keap1 and/or Nrf2, and how this can affect NF-κB activity.

LncRNAs, or long non-coding RNAs, are crucial for controlling respiratory conditions including pneumonia [77]. In many inflammatory disorders, the growth arrest-specific 5 (GAS5) lncRNA plays an essential role. It has been shown that GAS5-silencing reduces the viability and exacerbates the inflammatory damage of chondrocytes produced by LPS [78]. Significantly, children with *M. pneumoniae* pneumonia have increased expression of miR.222.3p [79]. A study examined the expression of GAS5, miR-222-3p, and tissue inhibitor of metalloproteinases-3 (TIMP3) in *M. pneumoniae* pneumonia [80]. GAS5 and TIMP3 expression was found to be downregulated in THP-1 cells stimulated by LAMP. GAS5 was found to interact directly with miR-222-3p, which in turn targets TIMP3. The stimulating effect on cell viability and the suppression of inflammation generated by GAS5-overexpression in LAMP-induced THP-1 cells was reversed by miR-222-3p upregulation or TIMP3-knockdown. There is supportive evidence that showed that blocking miR-222-3p reduces inflammation via activation of Nrf2/HO-1 signaling, which in turn attenuates oxidative stress [81]. However, the specific regulatory association between GAS5, miR.222.3p, and Nrf2 signaling pathway remains to be elucidated in *M. pneumoniae* pneumonia.

M. hyopneumoniae, causes swine enzootic pneumonia, a chronic respiratory disease. A study used *M. hyopneumoniae* strain J to infect swine epithelial NPTr cells to detect mRNAs and miRNAs that were differently expressed [82]. Genes linked to redox homeostasis and antioxidant defense, which are known to be controlled by the transcription factor Nrf2 in similar species, had up-regulated mRNAs. Since they found that miRNAs anticipated to target antioxidant genes were down-regulated and miRNAs targeting ciliary and cytoskeleton genes were up-regulated, the bioinformatic analysis indicated a relationship between changes in miRNA and mRNA levels.

4. Challenges in Pharmacotherapy of *Mycoplasma* Infections

Treating chronic lung disorders linked to *M. pneumoniae* poses several difficulties that need to be resolved to guarantee the best possible outcomes for patients and avoid long-term issues. These difficulties include the necessity for long-term management plans, possible adverse effects, and antibiotic resistance. Since macrolides and fluoroquinolones have historically been the cornerstones of treatment for *M. pneumoniae*, the bacterium has become resistant to several widely used antibiotics [83]. The extensive and frequently careless use of antibiotics, along with *M. pneumoniae*'s capacity to develop resistance through mutations and horizontal gene transfer, have been connected to the rise of antibiotic-resistant strains [84]. High-level resistance to this family of antibiotics can be conferred by mutations in the 23S rRNA gene, which is the primary mechanism of macrolide resistance in *M. pneumoniae* [85]. The genes parC and parE encode DNA topoisomerase IV subunits, and mutations in these genes are frequently linked to fluoroquinolone resistance [86]. The genome of *M. hominis* contains two rRNA operons, the full sequences of which are not yet known, whereas *M. pneumoniae* only has one copy of 23S rRNA [87]. Genetic changes in DNA gyrase (GyrA and GyrB) and/or the topoisomerase IV complex (parC and parE) are linked to bacterial resistance to fluoroquinolones [88]. *M. hominis*'s resistance to 16-membered macrolides may be caused by changes in the ribosomal proteins L4 and L22, as well as in domain II or V of 23S rRNA [88]. Both *M. fermentans*, another erythromycin-resistant *Mycoplasma*, and *M. hominis* had a G2057A transition in their 23S rRNA sequence in comparison to that of *M. pneumoniae* [87]. Antibiotic-resistant *M. pneumoniae* strains are becoming more common, which makes it difficult to effectively treat chronic lung diseases because they reduce the number of available treatments and can result in treatment failures or the need for alternative, possibly less effective, or more toxic agents [89]. Chronic lung disease patients frequently need to take several drugs at the same time to treat their underlying problems, thus it is important to carefully assess the possibility of drug interactions and cumulative toxicity [90]. Long-term management techniques might be necessary in cases with chronic lung disorders made worse by *M. pneumoniae* infection to manage symptoms, stop the disease from getting worse, and reduce the chance of recurring infections or exacerbations [91]. Various supportive therapies, including bronchodilators, corticosteroids, and antibiotics, may be used in these regimens, depending on the severity of the disease and the underlying condition [92].

On the other hand, there is disagreement over the best long-term management strategies and the best duration and regimens for antibiotic therapy in chronic lung disorders linked to *M. pneumoniae* [93]. Long-term or recurrent antibiotic courses may raise the likelihood of antibiotic resistance, side effects, and respiratory microbiome disturbance, all of which could have negative consequences [94]. These elements, along with *M. pneumoniae*'s long-term effects on respiratory health, make treating chronic lung diseases especially difficult due to its antibiotic resistance.

5. Discussion

One essential innate immune response that produces a protective host response is inflammation. Nevertheless,

proinflammatory factors, ROS, NO, and other bioactive molecules may be released following a prolonged infection, which might distort the host's reaction to "hyperinflammation" and cause excessive tissue damage. Therefore, some sort of negative regulatory mechanism must be in place to control an excessive inflammatory response.

Certain transcription factors and cellular signaling pathways influence inflammatory responses [95]. According to Sundaresan *et al*. [96], the traditional NF-κB pathway stimulates the synthesis of proinflammatory cytokines and ROS. On the other hand, Nrf2 is thought to be a crucial nuclear transcription factor that detects oxidizing environments and shields cells from inflammation and oxidative damage [97]. Nrf2 activation suppresses the production of adhesion molecules generated by proinflammatory cytokines in endothelial cells. On the contrary, Nrf2-null macrophages exhibit increased NF-κB activity and TNFα production [96] and maintain a high ROS level, leading to increased mortality and TLR4 signaling amplification [98]. Moreover, Nrf2-deficient mice that receive endotoxin treatment express more TNFα and IL-6 than wild-type mice [95]. In this review, we discussed how Nrf2 translocation from the cytoplasm to the nucleus can be triggered by *M. pneumoniae*, and how Nrf2 silencing can adversely affect the production of pro-inflammatory cytokines and ROS in cell lines. When *Mycoplasmas* are present, there may be an "interplay" between the Nrf2/ARE and NF-κB signaling pathways. The protective effect of drugs such as sulforaphane via the Nrf2 signaling pathway could be a good place to start for several structure-activity studies aimed at maximizing potency in terms of inhibiting the inflammatory response, especially inflammation linked to *Mycoplasma*.

6. Conclusions and Future Directions

Even though there is increasing evidence that *M. pneumoniae* infections are linked to chronic lung disorders, more research is still needed in a few areas to further our knowledge and create efficient therapeutic approaches. Several pathogenic factors contribute to the development of linked diseases. The development of medications that target the common pathogenic mechanism of extrapulmonary and intrapulmonary infections may aid in the prevention of *M. pneumoniae* infections. Further studies are needed to develop a complete treatment plan that incorporates immunomodulators, and antibiotics for problems brought on by *M. pneumoniae*. Furthermore, investigating other approaches to treating macrolide antibiotics may alleviate the clinical signs of therapy failure. Patients who were infected with other prevalent respiratory pathogens also had *M. pneumoniae* infections. The emergence and spread of antibiotic-resistant *M. pneumoniae* strains must be tracked, and ongoing research and surveillance are required to look into the processes behind resistance and possible countermeasures. To tackle the increasing problem of antibiotic resistance, it should also be a top priority to discover new antimicrobial drugs with unique modes of action. In this regard, Nrf2 controls the expression of hundreds of genes that code for proteins with anti-inflammatory, antioxidant, drug-metabolizing, and other homeostatic properties. In this article, we have highlighted the role of the Nrf2 pathway in *Mycoplasma* infections and some of the ongoing challenges in the therapeutic management of *Mycoplasma* that need to be addressed. Thus, the aforementioned research suggests that targeting Nrf2 activation as a therapeutic target is probably going to be beneficial. Additionally, it is currently unknown if long-term pharmacological Nrf2 activation could change the risk of inflammation in situations that are susceptible to *M. pneumoniae* infection; this could be affected by the intervention's timing about the disease's course.

Abbreviations

NAATs, nucleic acid amplification tests; Nrf2, erythroid 2-related factor 2; bZIP, basic-region leucine zipper; RBX1, ring box protein-1; GST, glutathione-S-transferase; ARE, antioxidant response element; HO-1, heme oxygenase-1; LPS, lipopolysaccharide; LPs, lipoproteins; MALP-2, macrophage-activating lipopeptide-2; IbpM, immunoglobulin-binding protein; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; LAMPs, lipid-associated membrane proteins; AP-1, activating protein 1; PGE2, prostaglandin E2; NO, nitric oxide; PAMP, pathogen-associated molecular pattern; COX-2, cyclooxygenase 2; PI3K, phosphoinositide 3-kinases; CORM-2, carbon monoxide releasing molecule 2; lncRNA, long non-coding RNA; NF-κB, nuclear factor kappa B; TIMP3, tissue inhibitor of metalloproteinases-3; GAS5, growth arrest-specific 5; Prdx, peroxiredoxin; SOD1, superoxide dismutase 1; miRNAs, micro RNA.

Author Contributions

SS, NS, OK, EN and NZ, conceptualization, writing, and editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clinical Microbiology Reviews. 2004; 17: 697–728, table of contents. https://doi.org/10.1128/CMR.17.4.697-728.2004.

[2] Zhang L, Lai M, Ai T, Liao H, Huang Y, Zhang Y, *et al*. Analysis of *mycoplasma pneumoniae* infection among children with respiratory tract infections in hospital in Chengdu from 2014 to 2020. Translational Pediatrics. 2021; 10: 990–997. https://doi.org/10.21037/tp-21-139.

[3] Pilianidis G, Tsinari A, Pandis D, Tsolakidou H, Petridis N. Chronic seronegative spondyloarthropathy following acute Mycoplasma pneumoniae infection in a human leukocyte antigen B27-positive patient: a case report. Journal of Medical Case Reports. 2020; 14: 155. https://doi.org/10.1186/s13256-020-02479-6.

[4] Foy HM, Kenny GE, Cooney MK, Allan ID. Long-term epidemiology of infections with Mycoplasma pneumoniae. The Journal of Infectious Diseases. 1979; 139: 681–687. https://doi.org/10.1093/infdis/139.6.681.

[5] Kumar S. *Mycoplasma pneumoniae*: A significant but underrated pathogen in paediatric community-acquired lower respiratory tract infections. The Indian Journal of Medical Research. 2018; 147: 23–31. https://doi.org/10.4103/ijmr.IJMR_1582_16.

[6] Bajantri B, Venkatram S, Diaz-Fuentes G. *Mycoplasma pneumoniae*: A Potentially Severe Infection. Journal of Clinical Medicine Research. 2018; 10: 535–544. https://doi.org/10.14740/jocmr3421w.

[7] Rollins DR, Good JT, Jr, Martin RJ. The role of atypical infections and macrolide therapy in patients with asthma. The Journal of Allergy and Clinical Immunology. in Practice. 2014; 2: 511–517. https://doi.org/10.1016/j.jaip.2014.06.002.

[8] Martin RJ, Kraft M, Chu HW, Berns EA, Cassell GH. A link between chronic asthma and chronic infection. The Journal of Allergy and Clinical Immunology. 2001; 107: 595–601. https://doi.org/10.1067/mai.2001.113563.

[9] Baseman JB, Tully JG. Mycoplasmas: sophisticated, reemerging, and burdened by their notoriety. Emerging Infectious Diseases. 1997; 3: 21–32. https://doi.org/10.3201/eid0301.970103.

[10] Yamaguchi M, Kikuchi A, Ohkusu K, Akashi M, Sasahara J, Takakuwa K, *et al*. Abscess formation due to Mycoplasma hominis infection after cesarean section. The Journal of Obstetrics and Gynaecology Research. 2009; 35: 593–596. https://doi.org/10.1111/j.1447-0756.2008.00993.x.

[11] Mori N, Takigawa A, Kagawa N, Kenri T, Yoshida S, Shibayama K, *et al*. Pelvic abscess due to *Mycoplasma hominis* following caesarean section. JMM Case Reports. 2016; 3: e005059. https://doi.org/10.1099/jmmcr.0.005059.

[12] Koshiba H, Koshiba A, Daimon Y, Noguchi T, Iwasaku K, Kitawaki J. Hematoma and abscess formation caused by Mycoplasma hominis following cesarean section. International Journal of Women's Health. 2011; 3: 15–18. https://doi.org/10.2147/IJWH.S16703.

[13] Kennedy KJ, Prince S, Makeham T. Mycoplasma hominis-associated parapharyngeal abscess following acute Epstein-Barr virus infection in a previously immunocompetent adult. Journal of Clinical Microbiology. 2009; 47: 3050–3052. https://doi.org/10.1128/JCM.02203-08.

[14] Hammerschlag MR. Mycoplasma pneumoniae infections. Current Opinion in Infectious Diseases. 2001; 14: 181–186. https://doi.org/10.1097/00001432-200104000-00012.

[15] Nilsson AC, Björkman P, Persson K. Polymerase chain reaction is superior to serology for the diagnosis of acute Mycoplasma pneumoniae infection and reveals a high rate of persistent infection. BMC Microbiology. 2008; 8: 93. https://doi.org/10.1186/1471-2180-8-93.

[16] Thurman KA, Walter ND, Schwartz SB, Mitchell SL, Dillon MT, Baughman AL, *et al*. Comparison of laboratory diagnostic procedures for detection of Mycoplasma pneumoniae in community outbreaks. Clinical Infectious Diseases. 2009; 48: 1244–1249. https://doi.org/10.1086/597775.

[17] Jaffe JD, Berg HC, Church GM. Proteogenomic mapping as a complementary method to perform genome annotation. Proteomics. 2004; 4: 59–77. https://doi.org/10.1002/pmic.200300511.

[18] Rottem S. Interaction of mycoplasmas with host cells. Physiological Reviews. 2003; 83: 417–432. https://doi.org/10.1152/physrev.00030.2002.

[19] Benedetti F, Davinelli S, Krishnan S, Gallo RC, Scapagnini G, Zella D, *et al*. Sulfur compounds block MCP-1 production by Mycoplasma fermentans-infected macrophages through NF-κB inhibition. Journal of Translational Medicine. 2014; 12: 145. https://doi.org/10.1186/1479-5876-12-145.

[20] Seya T, Matsumoto M. A lipoprotein family from Mycoplasma fermentans confers host immune activation through Toll-like receptor 2. The International Journal of Biochemistry & Cell Biology. 2002; 34: 901–906. https://doi.org/10.1016/s1357-2725(01)00164-9.

[21] Meseguer MA, Alvarez A, Rejas MT, Sánchez C, Pérez-Díaz JC, Baquero F. Mycoplasma pneumoniae: a reduced-genome intracellular bacterial pathogen. Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases. 2003; 3: 47–55. https://doi.org/10.1016/s1567-1348(02)00151-x.

[22] Yavlovich A, Tarshis M, Rottem S. Internalization and intracellular survival of Mycoplasma pneumoniae by non-phagocytic cells. FEMS Microbiology Letters. 2004; 233: 241–246. https://doi.org/10.1016/j.femsle.2004.02.016.

[23] Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. FEMS Microbiology Reviews. 2008; 32: 956–973. https://doi.org/10.1111/j.1574-6976.2008.00129.x.

[24] Feberwee A, de Wit S, Dijkman R. Clinical expression, epidemiology, and monitoring of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*: an update. Avian Pathology: Journal of the W.V.P.A. 2022; 51: 2–18. https://doi.org/10.1080/03079457.2021.1944605.

[25] Jiang Z, Li S, Zhu C, Zhou R, Leung PHM. *Mycoplasma pneumoniae* Infections: Pathogenesis and Vaccine Development. Pathogens. 2021; 10: 119. https://doi.org/10.3390/pathogens10020119.

[26] Georgakopoulou VE, Lempesis IG, Sklapani P, Trakas N, Spandidos DA. Exploring the pathogenetic mechanisms of *Mycoplasmapneumoniae* (Review). Experimental and Therapeutic Medicine. 2024; 28: 271. https://doi.org/10.3892/etm.2024.12559.

[27] Fan L, Xu N, Guo Y, Li L. Enhanced insights into the neutrophil-driven immune mechanisms during *Mycoplasma pneumoniae* infection. Heliyon. 2024; 10: e38950. https://doi.org/10.1016/j.heliyon.2024.e38950.

[28] Tamiya S, Yoshikawa E, Ogura M, Kuroda E, Suzuki K, Yoshioka Y. Vaccination using inactivated Mycoplasma pneumoniae induces detrimental infiltration of neutrophils after subsequent infection in mice. Vaccine. 2020; 38: 4979–4987. https://doi.org/10.1016/j.vaccine.2020.05.074.

[29] Wu Q, Martin RJ, Rino JG, Breed R, Torres RM, Chu HW. IL-23-dependent IL-17 production is essential in neutrophil recruit-

ment and activity in mouse lung defense against respiratory Mycoplasma pneumoniae infection. Microbes and Infection. 2007; 9: 78–86. https://doi.org/10.1016/j.micinf.2006.10.012.
[30] Shimizu T, Kida Y, Kuwano K. Cytoadherence-dependent induction of inflammatory responses by Mycoplasma pneumoniae. Immunology. 2011; 133: 51–61. https://doi.org/10.1111/j.1365-2567.2011.03408.x.
[31] Walter ND, Grant GB, Bandy U, Alexander NE, Winchell JM, Jordan HT, *et al*. Community outbreak of Mycoplasma pneumoniae infection: school-based cluster of neurologic disease associated with household transmission of respiratory illness. The Journal of Infectious Diseases. 2008; 198: 1365–1374. https://doi.org/10.1086/592281.
[32] Saha S, Buttari B, Panieri E, Profumo E, Saso L. An Overview of Nrf2 Signaling Pathway and Its Role in Inflammation. Molecules. 2020; 25: 5474. https://doi.org/10.3390/molecules25225474.
[33] Sun Z, Chin YE, Zhang DD. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Molecular and Cellular Biology. 2009; 29: 2658–2672. https://doi.org/10.1128/MCB.01639-08.
[34] Nioi P, Nguyen T, Sherratt PJ, Pickett CB. The carboxy-terminal Neh3 domain of Nrf2 is required for transcriptional activation. Molecular and Cellular Biology. 2005; 25: 10895–10906. https://doi.org/10.1128/MCB.25.24.10895-10906.2005.
[35] Rada P, Rojo AI, Evrard-Todeschi N, Innamorato NG, Cotte A, Jaworski T, *et al*. Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/β-TrCP axis. Molecular and Cellular Biology. 2012; 32: 3486–3499. https://doi.org/10.1128/MCB.00180-12.
[36] Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. Redox Biology. 2013; 1: 45–49. https://doi.org/10.1016/j.redox.2012.10.001.
[37] Zipper LM, Mulcahy RT. The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm. The Journal of Biological Chemistry. 2002; 277: 36544–36552. https://doi.org/10.1074/jbc.M206530200.
[38] Hayes JD, McMahon M. NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. Trends in Biochemical Sciences. 2009; 34: 176–188. https://doi.org/10.1016/j.tibs.2008.12.008.
[39] Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, *et al*. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nature Cell Biology. 2010; 12: 213–223. https://doi.org/10.1038/ncb2021.
[40] Hayes JD, McMahon M, Chowdhry S, Dinkova-Kostova AT. Cancer chemoprevention mechanisms mediated through the Keap1-Nrf2 pathway. Antioxidants & Redox Signaling. 2010; 13: 1713–1748. https://doi.org/10.1089/ars.2010.3221.
[41] Gao W, Guo L, Yang Y, Wang Y, Xia S, Gong H, *et al*. Dissecting the Crosstalk Between Nrf2 and NF-κB Response Pathways in Drug-Induced Toxicity. Frontiers in Cell and Developmental Biology. 2022; 9: 809952. https://doi.org/10.3389/fcell.2021.809952.
[42] Grottelli S, Ferrari I, Pietrini G, Peirce MJ, Minelli A, Bellezza I. The Role of Cyclo(His-Pro) in Neurodegeneration. International Journal of Molecular Sciences. 2016; 17: 1332. https://doi.org/10.3390/ijms17081332.
[43] Kim HR, Kim JC, Kang SY, Kim HO, Park CW, Chung BY. Rapamycin Alleviates 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Induced Aggravated Dermatitis in Mice with Imiquimod-Induced Psoriasis-Like Dermatitis by Inducing Autophagy. International Journal of Molecular Sciences. 2021; 22: 3968. https://doi.org/10.3390/ijms22083968.
[44] Cuadrado A, Martín-Moldes Z, Ye J, Lastres-Becker I. Transcription factors NRF2 and NF-κB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation. The Journal of Biological Chemistry. 2014; 289: 15244–15258. https://doi.org/10.1074/jbc.M113.540633.
[45] Javed MA, Frasca S, Jr, Rood D, Cecchini K, Gladd M, Geary SJ, *et al*. Correlates of immune protection in chickens vaccinated with Mycoplasma gallisepticum strain GT5 following challenge with pathogenic M. gallisepticum strain R(low). Infection and Immunity. 2005; 73: 5410–5419. https://doi.org/10.1128/IAI.73.9.5410-5419.2005.
[46] Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. International Reviews of Immunology. 2011; 30: 16–34. https://doi.org/10.3109/08830185.2010.529976.
[47] Loveless RW, Griffiths S, Fryer PR, Blauth C, Feizi T. Immunoelectron microscopic studies reveal differences in distribution of sialo-oligosaccharide receptors for Mycoplasma pneumoniae on the epithelium of human and hamster bronchi. Infection and Immunity. 1992; 60: 4015–4023. https://doi.org/10.1128/iai.60.10.4015-4023.1992.
[48] Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007; 449: 819–826. https://doi.org/10.1038/nature06246.
[49] Peltier MR, Freeman AJ, Mu HH, Cole BC. Characterization of the macrophage-stimulating activity from Ureaplasma urealyticum. American Journal of Reproductive Immunology. 2007; 57: 186–192. https://doi.org/10.1111/j.1600-0897.2006.00460.x.
[50] Okusawa T, Fujita M, Nakamura JI, Into T, Yasuda M, Yoshimura A, *et al*. Relationship between structures and biological activities of mycoplasmal diacylated lipopeptides and their recognition by toll-like receptors 2 and 6. Infection and Immunity. 2004; 72: 1657–1665. https://doi.org/10.1128/IAI.72.3.1657-1665.2004.
[51] Shimizu T, Kida Y, Kuwano K. Triacylated lipoproteins derived from Mycoplasma pneumoniae activate nuclear factor-kappaB through toll-like receptors 1 and 2. Immunology. 2007; 121: 473–483. https://doi.org/10.1111/j.1365-2567.2007.02594.x.
[52] Sasaki Y, Ishikawa J, Yamashita A, Oshima K, Kenri T, Furuya K, *et al*. The complete genomic sequence of Mycoplasma penetrans, an intracellular bacterial pathogen in humans. Nucleic Acids Research. 2002; 30: 5293–5300. https://doi.org/10.1093/nar/gkf667.
[53] Jan G, Fontenelle C, Verrier F, Le Hénaff M, Wróblewski H. Selective acylation of plasma membrane proteins of Mycoplasma mycoides subsp. mycoides SC, the contagious bovine pleuropneumonia agent. Current Microbiology. 1996; 32: 38–42. https://doi.org/10.1007/s002849900007.
[54] Chambaud I, Wróblewski H, Blanchard A. Interactions between mycoplasma lipoproteins and the host immune system. Trends in Microbiology. 1999; 7: 493–499. https://doi.org/10.1016/s0966-842x(99)01641-8.
[55] Shimizu T, Kida Y, Kuwano K. A dipalmitoylated lipoprotein from Mycoplasma pneumoniae activates NF-kappa B through TLR1, TLR2, and TLR6. Journal of Immunology. 2005; 175: 4641–4646. https://doi.org/10.4049/jimmunol.175.7.4641.
[56] Shimizu T, Kida Y, Kuwano K. A triacylated lipoprotein from Mycoplasma genitalium activates NF-kappaB through Toll-like receptor 1 (TLR1) and TLR2. Infection and Immunity. 2008; 76: 3672–3678. https://doi.org/10.1128/IAI.00257-08.
[57] Shimizu T, Kimura Y, Kida Y, Kuwano K, Tachibana M, Hashino M, *et al*. Cytadherence of Mycoplasma pneumoniae induces inflammatory responses through autophagy and toll-like receptor 4. Infection and Immunity. 2014; 82: 3076–3086. https://doi.org/10.1128/IAI.01961-14.
[58] Bose S, Segovia JA, Somarajan SR, Chang TH, Kannan TR,

Baseman JB. ADP-ribosylation of NLRP3 by Mycoplasma pneumoniae CARDS toxin regulates inflammasome activity. mBio. 2014; 5: e02186–14. https://doi.org/10.1128/mBio.02186-14.

[59] Sugiyama M, Saeki A, Hasebe A, Kamesaki R, Yoshida Y, Kitagawa Y, *et al*. Activation of inflammasomes in dendritic cells and macrophages by Mycoplasma salivarium. Molecular Oral Microbiology. 2016; 31: 259–269. https://doi.org/10.1111/omi.12117.

[60] Li S, Xue G, Zhao H, Feng Y, Yan C, Cui J, *et al*. The *Mycoplasma pneumoniae* HapE alters the cytokine profile and growth of human bronchial epithelial cells. Bioscience Reports. 2019; 39: BSR20182201. https://doi.org/10.1042/BSR20182201.

[61] Dawood A, Algharib SA, Zhao G, Zhu T, Qi M, Delai K, *et al*. Mycoplasmas as Host Pantropic and Specific Pathogens: Clinical Implications, Gene Transfer, Virulence Factors, and Future Perspectives. Frontiers in Cellular and Infection Microbiology. 2022; 12: 855731. https://doi.org/10.3389/fcimb.2022.855731.

[62] Blötz C, Singh N, Dumke R, Stülke J. Characterization of an Immunoglobulin Binding Protein (IbpM) From *Mycoplasma pneumoniae*. Frontiers in Microbiology. 2020; 11: 685. https://doi.org/10.3389/fmicb.2020.00685.

[63] Hu J, Ye Y, Chen X, Xiong L, Xie W, Liu P. Insight into the Pathogenic Mechanism of Mycoplasma pneumoniae. Current Microbiology. 2022; 80: 14. https://doi.org/10.1007/s00284-022-03103-0.

[64] Yamamoto T, Kida Y, Kuwano K. Mycoplasma pneumoniae protects infected epithelial cells from hydrogen peroxide-induced cell detachment. Cellular Microbiology. 2019; 21: e13015. https://doi.org/10.1111/cmi.13015.

[65] Schumacher M, Nicholson P, Stoffel MH, Chandran S, D'Mello A, Ma L, *et al*. Evidence for the Cytoplasmic Localization of the L-α-Glycerophosphate Oxidase in Members of the "*Mycoplasma mycoides* Cluster". Frontiers in Microbiology. 2019; 10: 1344. https://doi.org/10.3389/fmicb.2019.01344.

[66] Waites KB, Balish MF, Atkinson TP. New insights into the pathogenesis and detection of Mycoplasma pneumoniae infections. Future Microbiology. 2008; 3: 635–648. https://doi.org/10.2217/17460913.3.6.635.

[67] Lee D, Lal NK, Lin ZJD, Ma S, Liu J, Castro B, *et al*. Regulation of reactive oxygen species during plant immunity through phosphorylation and ubiquitination of RBOHD. Nature Communications. 2020; 11: 1838. https://doi.org/10.1038/s41467-020-15601-5.

[68] Chen LS, Li C, You XX, Lin YW, Wu YM. The mpn668 gene of Mycoplasma pneumoniae encodes a novel organic hydroperoxide resistance protein. International Journal of Medical Microbiology: IJMM. 2018; 308: 776–783. https://doi.org/10.1016/j.ijmm.2018.04.006.

[69] Chmura K, Bai X, Nakamura M, Kandasamy P, McGibney M, Kuronuma K, *et al*. Induction of IL-8 by Mycoplasma pneumoniae membrane in BEAS-2B cells. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2008; 295: L220–30. https://doi.org/10.1152/ajplung.90204.2008.

[70] Nakao Y, Funami K, Kikkawa S, Taniguchi M, Nishiguchi M, Fukumori Y, *et al*. Surface-expressed TLR6 participates in the recognition of diacylated lipopeptide and peptidoglycan in human cells. Journal of Immunology. 2005; 174: 1566–1573. https://doi.org/10.4049/jimmunol.174.3.1566.

[71] Hu J, Chen C, Ou G, You X, Tan T, Hu X, *et al*. Nrf2 regulates the inflammatory response, including heme oxygenase-1 induction, by mycoplasma pneumoniae lipid-associated membrane proteins in THP-1 cells. Pathogens and Disease. 2017; 75. https://doi.org/10.1093/femspd/ftx044.

[72] Kaufmann A, Mühlradt PF, Gemsa D, Sprenger H. Induction of cytokines and chemokines in human monocytes by Mycoplasma fermentans-derived lipoprotein MALP-2. Infection and Immunity. 1999; 67: 6303–6308. https://doi.org/10.1128/IAI.67.12.6303-6308.1999.

[73] Ma X, You X, Zeng Y, He J, Liu L, Deng Z, *et al*. Mycoplasma fermentans MALP-2 induces heme oxygenase-1 expression via mitogen-activated protein kinases and Nrf2 pathways to modulate cyclooxygenase 2 expression in human monocytes. Clinical and Vaccine Immunology. 2013; 20: 827–834. https://doi.org/10.1128/CVI.00716-12.

[74] Haodang L, Lianmei Q, Ranhui L, Liesong C, Jun H, Yihua Z, *et al*. HO-1 mediates the anti-inflammatory actions of Sulforaphane in monocytes stimulated with a mycoplasmal lipopeptide. Chemico-Biological Interactions. 2019; 306: 10–18. https://doi.org/10.1016/j.cbi.2019.04.007.

[75] Benedetti F, Curreli S, Krishnan S, Davinelli S, Cocchi F, Scapagnini G, *et al*. Anti-inflammatory effects of H_2S during acute bacterial infection: a review. Journal of Translational Medicine. 2017; 15: 100. https://doi.org/10.1186/s12967-017-1206-8.

[76] Hourihan JM, Kenna JG, Hayes JD. The gasotransmitter hydrogen sulfide induces nrf2-target genes by inactivating the keap1 ubiquitin ligase substrate adaptor through formation of a disulfide bond between cys-226 and cys-613. Antioxidants & Redox Signaling. 2013; 19: 465–481. https://doi.org/10.1089/ars.2012.4944.

[77] Huang S, Feng C, Chen L, Huang Z, Zhou X, Li B, *et al*. Identification of Potential Key Long Non-Coding RNAs and Target Genes Associated with Pneumonia Using Long Non-Coding RNA Sequencing (lncRNA-Seq): A Preliminary Study. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2016; 22: 3394–3408. https://doi.org/10.12659/msm.900783.

[78] Wang Y, Song Q, Huang X, Chen Z, Zhang F, Wang K, *et al*. Long noncoding RNA GAS5 promotes apoptosis in primary nucleus pulposus cells derived from the human intervertebral disc via Bcl 2 downregulation and caspase 3 upregulation. Molecular Medicine Reports. 2019; 19: 2164–2172. https://doi.org/10.3892/mmr.2019.9883.

[79] Chu C, Lei X, Li Y, Luo Y, Ding Y, Zhou W, *et al*. High expression of miR-222-3p in children with Mycoplasma pneumoniae pneumonia. Italian Journal of Pediatrics. 2019; 45: 163. https://doi.org/10.1186/s13052-019-0750-7.

[80] Yang L, Zhang X, Liu X. Long non coding RNA GAS5 protects against *Mycoplasma pneumoniae* pneumonia by regulating the microRNA 222 3p/TIMP3 axis. Molecular Medicine Reports. 2021; 23: 380. https://doi.org/10.3892/mmr.2021.12019.

[81] Wang XJ, Zhang D, Yang YT, Li XY, Li HN, Zhang XP, *et al*. Suppression of microRNA-222-3p ameliorates ulcerative colitis and colitis-associated colorectal cancer to protect against oxidative stress *via* targeting BRG1 to activate Nrf2/HO-1 signaling pathway. Frontiers in Immunology. 2023; 14: 1089809. https://doi.org/10.3389/fimmu.2023.1089809.

[82] Mucha SG, Ferrarini MG, Moraga C, Di Genova A, Guyon L, Tardy F, *et al*. Mycoplasma hyopneumoniae J elicits an antioxidant response and decreases the expression of ciliary genes in infected swine epithelial cells. Scientific Reports. 2020; 10: 13707. https://doi.org/10.1038/s41598-020-70040-y.

[83] Pereyre S, Goret J, Bébéar C. Mycoplasma pneumoniae: Current Knowledge on Macrolide Resistance and Treatment. Frontiers in Microbiology. 2016; 7: 974. https://doi.org/10.3389/fmicb.2016.00974.

[84] Xin D, Mi Z, Han X, Qin L, Li J, Wei T, *et al*. Molecular mechanisms of macrolide resistance in clinical isolates of Mycoplasma pneumoniae from China. Antimicrobial Agents and Chemotherapy. 2009; 53: 2158–2159. https://doi.org/10.1128/

AAC.01563-08.
[85] Pereyre S, Charron A, Renaudin H, Bébéar C, Bébéar CM. First report of macrolide-resistant strains and description of a novel nucleotide sequence variation in the P1 adhesin gene in Mycoplasma pneumoniae clinical strains isolated in France over 12 years. Journal of Clinical Microbiology. 2007; 45: 3534–3539. https://doi.org/10.1128/JCM.01345-07.
[86] Tagg KA, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and macrolide resistance-associated mutations in Mycoplasma genitalium. Journal of Clinical Microbiology. 2013; 51: 2245–2249. https://doi.org/10.1128/JCM.00495-13.
[87] Pereyre S, Gonzalez P, De Barbeyrac B, Darnige A, Renaudin H, Charron A, *et al*. Mutations in 23S rRNA account for intrinsic resistance to macrolides in Mycoplasma hominis and Mycoplasma fermentans and for acquired resistance to macrolides in M. hominis. Antimicrobial Agents and Chemotherapy. 2002; 46: 3142–3150. https://doi.org/10.1128/AAC.46.10.3142-3150.2002.
[88] Liu W, Yu Y, Wang Y, Yang T, Kong Y, Xie X, *et al*. Deciphering the genetic basis of resistome and virulome diversity among multidrug-resistant Mycoplasma hominis. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. 2024; 72: 101029. https://doi.org/10.1016/j.drup.2023.101029.
[89] Yin YD, Wang R, Zhuo C, Wang H, Wang MG, Xie CM, *et al*. Macrolide-resistant *Mycoplasma pneumoniae* prevalence and clinical aspects in adult patients with community-acquired pneumonia in China: a prospective multicenter surveillance study. Journal of Thoracic Disease. 2017; 9: 3774–3781. https://doi.org/10.21037/jtd.2017.09.75.
[90] Guo ZQ, Gu SY, Tian ZH, Du BY. A comprehensive review of Mycoplasma pneumoniae infection in chronic lung diseases: recent advances in understanding asthma, COPD, and bronchiectasis. Frontiers in Medicine (Lausanne). 2024; 11: 1437731.
[91] Pollock J, Chalmers JD. The immunomodulatory effects of macrolide antibiotics in respiratory disease. Pulmonary Pharmacology & Therapeutics. 2021; 71: 102095. https://doi.org/10.1016/j.pupt.2021.102095.
[92] Shin S, Koo S, Yang YJ, Lim HJ. Characteristics of the *Mycoplasma pneumoniae* Epidemic from 2019 to 2020 in Korea: Macrolide Resistance and Co-Infection Trends. Antibiotics. 2023; 12: 1623. https://doi.org/10.3390/antibiotics12111623.
[93] Kim K, Jung S, Kim M, Park S, Yang HJ, Lee E. Global Trends in the Proportion of Macrolide-Resistant Mycoplasma pneumoniae Infections: A Systematic Review and Meta-analysis. JAMA Network Open. 2022; 5: e2220949. https://doi.org/10.1001/jamanetworkopen.2022.20949.
[94] Chen J, Yin Y, Zhao L, Zhang L, Zhang J, Yuan S. Mycoplasma pneumoniae infection prediction model for hospitalized community-acquired pneumonia children. Pediatric Pulmonology. 2021; 56: 4020–4028. https://doi.org/10.1002/ppul.25665.
[95] Prawan A, Saw CLL, Khor TO, Keum YS, Yu S, Hu L, *et al*. Anti-NF-kappaB and anti-inflammatory activities of synthetic isothiocyanates: effect of chemical structures and cellular signaling. Chemico-Biological Interactions. 2009; 179: 202–211. https://doi.org/10.1016/j.cbi.2008.12.014.
[96] Sundaresan NR, Vasudevan P, Zhong L, Kim G, Samant S, Parekh V, *et al*. The sirtuin SIRT6 blocks IGF-Akt signaling and development of cardiac hypertrophy by targeting c-Jun. Nature Medicine. 2012; 18: 1643–1650. https://doi.org/10.1038/nm.2961.
[97] Yan X, Lee S, Gugiu BG, Koroniak L, Jung ME, Berliner J, *et al*. Fatty acid epoxyisoprostane E2 stimulates an oxidative stress response in endothelial cells. Biochemical and Biophysical Research Communications. 2014; 444: 69–74. https://doi.org/10.1016/j.bbrc.2014.01.016.
[98] Guo RF, Ward PA. Role of oxidants in lung injury during sepsis. Antioxidants & Redox Signaling. 2007; 9: 1991–2002. https://doi.org/10.1089/ars.2007.1785.