Exploring the pathogenetic mechanisms of *Mycoplasma pneumoniae* (Review)

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Received December 17, 2023; Accepted April 17, 2024

DOI: 10.3892/etm.2024.12559

Abstract. Mycoplasmas, the smallest self-replicating prokaryotes without a cell wall, are the most prevalent and extensively studied species in humans. They significantly contribute to chronic respiratory tract illnesses and pneumonia, with children and adolescents being particularly vulnerable. *Mycoplasma pneumoniae* (*M. pneumoniae*) infections typically tend to be self-limiting and mild but can progress to severe or even life-threatening conditions in certain individuals. Extrapulmonary effects often occur without pneumonia, and both intrapulmonary and extrapulmonary complications operate through separate pathological mechanisms. The indirect immune-mediated damage of the immune system, vascular blockages brought on by vasculitis or thrombosis and direct harm from invasion or locally induced inflammatory cytokines are potential causes of extrapulmonary manifestations due to *M. pneumoniae*. Proteins associated with adhesion serve as the primary factor crucial for the pathogenicity of *M. pneumoniae*, relying on a specialized polarized terminal attachment organelle. The type and density of these host receptors significantly impact the adhesion and movement of *M. pneumoniae*, subsequently influencing the pathogenic mechanism and infection outcomes. Adjacent proteins are crucial for the proper assembly of the attachment organelle, with variations in the genetic domains of P1, P40 and P90 surfaces contributing to the variability of clinical symptoms and offering new avenues for developing vaccines against *M. pneumoniae* infections. *M. pneumoniae* causes oxidative stress within respiratory tract epithelial cells by adhering to host cells and releasing hydrogen peroxide and superoxide radicals. This oxidative stress enhances the vulnerability of host cells to harm induced by oxygen molecules. The lack of superoxide dismutase and catalase of bacteria allows it to hinder the catalase activity of the host cell, leading to the reduced breakdown of peroxides. Lung macrophages play a significant role in managing *M. pneumoniae* infection, identifying it via Toll-like receptor 2 and initiating the myeloid differentiation primary response gene 88-nuclear factor κB signaling cascade. However, the precise mechanisms enabling *M. pneumoniae* to evade intracellular host defenses remain unknown, necessitating further exploration of the pathways involved in intracellular survival. The present comprehensive review delves into the pathogenesis of *M. pneumoniae* infection within the pulmonary system and into extrapulmonary areas, outlining its impact.

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Key words: Mycoplasma pneumoniae, pneumonia, pathogenesis, damage, immune, adhesion

Contents

1. Introduction

Mycoplasmas, the tiniest self-replicating prokaryotes devoid of a cell wall, possess an extremely small genome ranging from 580-2,200 kb (1,2). While >200 species of this pathogen have been recognized in animals, arthropods, humans and plants, only a select few have been confirmed to induce diseases in humans. Of note, *Mycoplasma pneumoniae* (*M. pneumoniae*), *Mycoplasma pirum*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma penetrans* and *Ureaplasma urealyticum* stand out as prominent pathogenic *Mycoplasmas* linked to diseases affecting the respiratory and urogenital systems in both humans and animals (3).

Of these pathogenic strains, *M. pneumoniae* stands out as the most prevalent and extensively studied species. It significantly contributes to chronic respiratory tract illnesses

and pneumonia in humans, with children and adolescents being particularly vulnerable. Although *M. pneumoniae* infections typically tend to be self-limiting and mild, they can progress to severe or even life-threatening conditions in certain individuals. *M. pneumoniae* has been attributed to up to 40% of community-acquired pneumonia cases among children >5 years old, where lower respiratory tract infections pose a significant risk of morbidity and mortality in this demographic (1,4).

It is considered that *M. pneumoniae* infections are associated with bronchial asthma and chronic lung diseases (5). Apart from triggering severe conditions in the lower respiratory tract and less severe in the upper respiratory tract, *M. pneumoniae* can lead to various other conditions and events after infection. These complications manifest in diverse areas, such as the skin, gastrointestinal system, kidneys, heart, musculoskeletal, central nervous and circulatory system, leading to atypical clinical manifestations (Fig. 1). Notably, central nervous system complications are the most frequent extrapulmonary issues arising from *M. pneumoniae* infection, occasionally posing life-threatening risks. It has been highlighted that due to the unusual symptoms and that lack of clear clinical and imaging indications in the early stages of *M. pneumoniae* infection, it is often underestimated (6). Rapid culture techniques utilizing throat swabs, polymerase chain reaction (PCR), serology and other laboratory diagnostic methods, including rapid antigen tests, constitute the primary means of laboratory diagnosis (7).

Extrapulmonary effects frequently occur without pneumonia, and both intrapulmonary and extrapulmonary complications operate through separate pathological mechanisms (8). The indirect immune-mediated damage of the immune system, vascular blockages brought on by vasculitis or thrombosis and direct harm from invasion or locally induced inflammatory cytokines are the potential causes of extrapulmonary manifestations due to *M. pneumoniae*. On the other hand, intrapulmonary infection mechanisms involve adhesion, invasion, nutrient depletion, immune and inflammatory response and toxin release (9). It is important to note that these mechanisms are not mutually exclusive but can co-exist concurrently in the body of the patient. Considering the severity of *M. pneumoniae* infections, in the present review, a summary of both intrapulmonary and extrapulmonary pathogenesis is provided, aiming to offer valuable insight for further pathogenesis research and treatment strategies concerning *M. pneumoniae* infections. The present review provides an update of all the pathogenetic mechanisms that have been previously mentioned in the literature (6,9).

2. Mechanisms of intrapulmonary infection

The process by which *M. pneumoniae* causes infection is intricate. In its initial phase, *M. pneumoniae* attaches to the bronchial epithelium using specialized terminal structures, triggering alterations in the metabolism and structure of the infected cells. Simultaneously, it invades these host cells, depletes essential nutrients and releases various components, such as community-acquired respiratory distress syndrome (CARDS) toxin, hydrogen peroxide and superoxide radicals, resulting in direct damage. Alongside these actions, *M. pneumoniae* components, including hydrogen sulfide (H_2S), alanine and pyruvate-producing enzymes (HapE), lipids, lipoproteins and glycolipids, stimulate the production of cytokines, initiating inflammation and causing indirect damage. Furthermore, *M. pneumoniae* employs mechanisms to evade the host immune system, potentially prolonging its survival within the body and leading to more severe clinical manifestations (10). A summary of the mechanisms is illustrated in Fig. 2.

Proteins associated with adhesion. Adhesion serves as the primary factor crucial for the pathogenicity of *M. pneumoniae*, relying on a specialized polarized terminal attachment organelle. Several pathogenic effects hinge on this initial step. The pathogen engages with the host respiratory epithelium, binding to the bronchial ciliary epithelium and triggering metabolic and structural alterations within the affected cells. This interaction rearranges the cytoskeleton, leading to the depletion of nutrients within the host cells (6). These alterations in intracellular metabolism are characterized by a simultaneous decrease in the uptake rate of certain host cell components, such as orotic acid and amino acids, along with a noticeable inhibition of ribonucleic acid and protein synthesis. These metabolic alterations contribute to the proliferation of the pathogen within cells, culminating in ciliary stagnation, cell death and the orchestration of various factors resulting in respiratory symptoms in humans (6). *M. pneumoniae* firmly attaches to host epithelial cells using its unique attachment organelle, purportedly aiding in cell division, cytoadherence and movement across the host cell surface (11). Sialylated and sulfated oligosaccharides are the primary receptors that *M. pneumoniae* recognizes (12).

The type and density of these host receptors significantly impact the adhesion and movement of *M. pneumoniae*, subsequently influencing the pathogenic mechanism and infection outcomes (13). The attachment organelle, situated at a cellular extremity, encompasses nap-like surface formations and an internal core. It coordinates a sophisticated adhesion process through interactions with the intricate network of the cytoskeleton and surface adhesion proteins (14).

The nap-like structure primarily comprises P1 adhesin, P30, P40 and P90. The internal core, crucial for forming the attachment organelle, is subdivided into three components: A terminal button, paired plates and a bowl (wheel) complex positioned at the leading edge. Key proteins within this core include high-molecular-weight proteins (HMW1, HMW2, HMW3), P65, P200, phosphomannomutase, mpn387, Lon protease, P41 and P24, among others (15).

The membrane protein P1 serves as the primary cellular adhesin and is localized on the cell surface, exhibiting sensitivity to trypsin (16). Upon the contact of *M. pneumoniae* with a target cell, the scattered P1 precursor proteins within cell membranes swiftly migrate to the terminal organelle. Here, the leader peptide on the amino terminal undergoes hydrolysis, transforming into a mature P1 protein that binds to the host receptor (9). It is crucial to note that for adhesion, the P1 adhesin must be correctly positioned on the terminal organelle. A previous study confirmed that, apart from its role in mediating adhesion between *M. pneumoniae* and host receptors, the P1 adhesin contributes to the gliding movement on host cell surfaces (16). Additionally, according to the same study, the significance of P1 adhesin in the cytokine response that

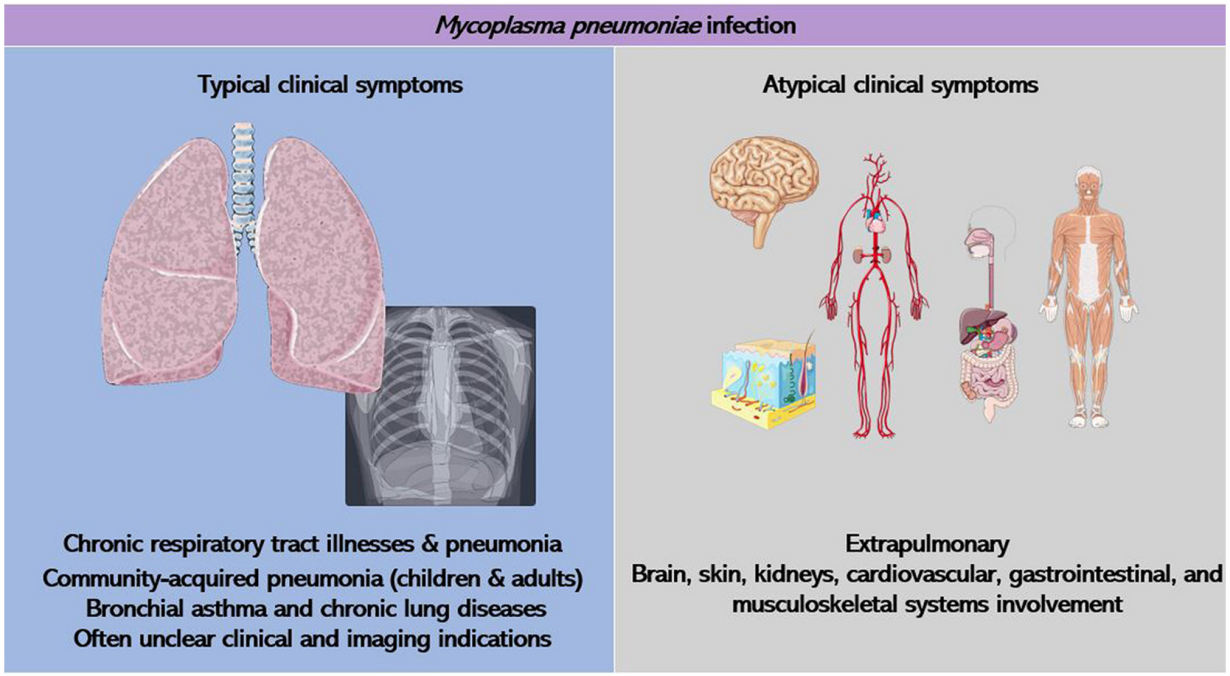

Figure 1. A summary of the clinical outcomes of *M. pneumoniae* infection. Parts of this image derived from the free medical site http://smart.servier.com/(accessed on December 15, 2023) by Servier, licenced under a Creative Commons Attribution 3.0 unported licence. *M. pneumoniae*, *Mycoplasma pneumoniae*.

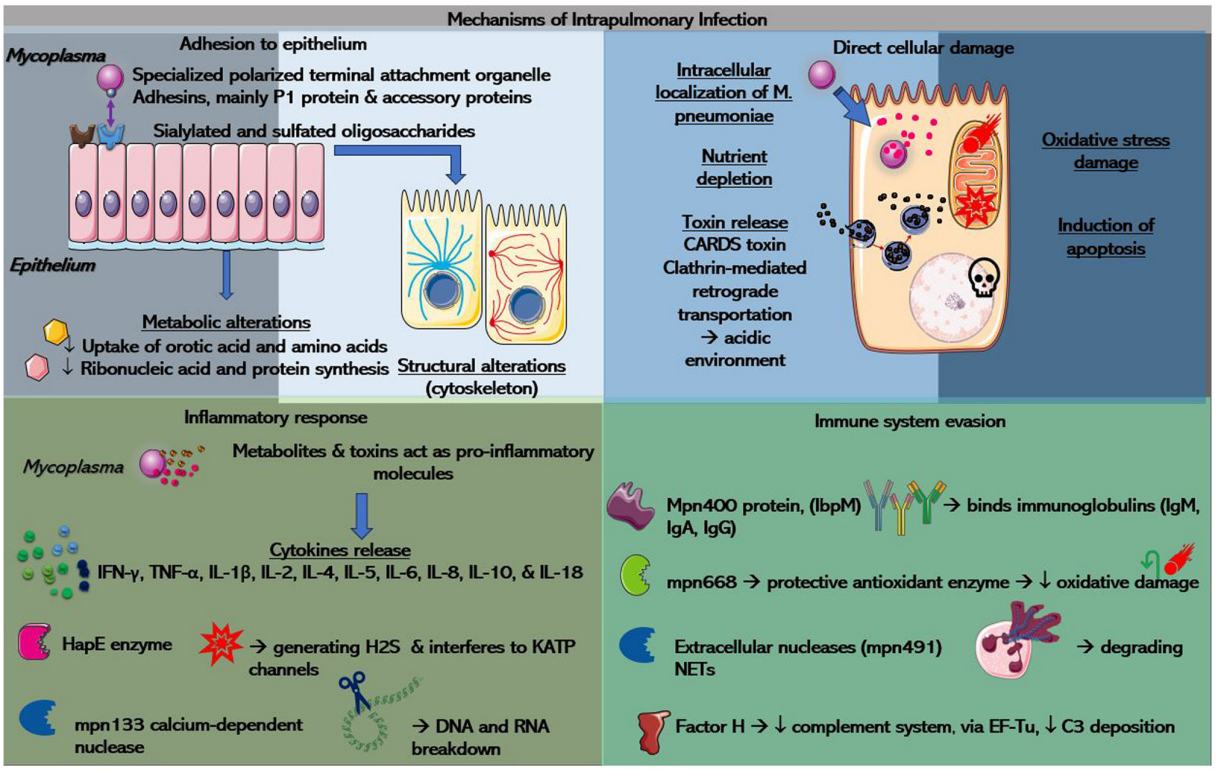

Figure 2. A summary of the various pathophysiological mechanisms of intrapulmonary *Mycoplasma* infection. Please refer to main text for more details. Parts of this image derived from the free medical site http://smart.servier.com/(accessed on December 15, 2023) by Servier, licenced under a Creative Commons Attribution 3.0 unported licence. CARDS, community-acquired respiratory distress syndrome; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL, interleukin; HapE, hydrogen sulfide, alanine and pyruvate producing enzymes; H_2S, hydrogen sulfide; KATP, ATP-sensitive K^+; IbpM, immunoglobulin-binding protein of *Mycoplasma*; Ig, immunoglobulin; NETs, neutrophil extracellular traps; EF-Tu, elongation factor thermal unstable Tu.

M. pneumoniae causes in mast cells. The interaction between *M. pneumoniae* and sialylated residues on mast cell surfaces triggers mast cell activation, leading to inflammatory damage. Antibodies targeting the highly immunogenic carboxyl terminus of P1 are believed to diminish *M. pneumoniae*'s adherence to non-biological and host cells (16).

The P30 protein exhibits a specific level of sequence similarity with distinct domains of the P1 protein, both serving as primary proteins governing adherence. Positioned at the tip of the attachment organelle, the P30 adhesin holds a significant role in transmitting cellular signals from the interior to the exterior. These signals trigger crucial processes in cytoadherence and movement, orchestrating the assembly of the P1 adhesin complex and fostering binding interactions between P1 and host receptors (15). A previous study conducted by Romero-Arroyo *et al* (17) confirmed the pivotal role of P30 in cellular development. The absence of P30 results in irregular *Mycoplasma* morphology, characterized by oval or leaf-like cells with ill-defined apical structures. Nevertheless, the introduction of P30 mutants and the wild-type P30 alleles can restore their typical morphology.

Protein surface exposure verification confirms P116 as a crucial cell adhesin. Anti-P116 antibodies prevent *M. pneumoniae* attachment to HEp-2 cells independently of P1. Moreover, P116 stands out as a significant immunogenic antigen within *M. pneumoniae* (6). The complete P116-C-terminal protein level has been instrumental in the serological diagnosis of *M. pneumoniae*. Furthermore, the study by Tabassum *et al* (18) suggested that a 27-kDa N-terminal segment of the P116 protein exhibits potential for the diagnosis of *M. pneumoniae* infection serologically (18).

Protein P65 showcases an intimate spatial and functional correlation with P30. The analysis of the fluorescent fusion proteins, P65 and P30, expressed in growing *Mycoplasma* cultures, indicates their concurrent positioning in developing terminal organelles. The suggested role of P65 involves facilitating a close attachment between the terminal button and the front side of the membrane by potentially interacting with the internal structural domain of P30 (15). Adhesins P40 and P90, derived from mpn142 cleavage, collectively form a transmembrane adhesion complex along with protein P1 (16).

The p1 gene, which encodes the P1 protein, serves as a target for detecting *M. pneumoniae* through reverse transcription-quantitative PCR and for conducting genotyping (19,20). Although the impact of genotype-specific antibodies on re-infections by different *M. pneumoniae* genotypes remains uncertain, genotyping is essential for molecular epidemiological studies and vaccine development (21). The P1 protein exhibits high immunogenicity and antigenic specificity (22,23) distinguishing its epitopes from those of other bacterial species. Inoculating Bagg albino (BALB/c) mice with a DNA vaccine encoding amino acids 1125-1359 of the *M. pneumoniae* P1 protein C-terminal region (P1C) via intramuscular or intranasal routes led to observable protection against *M. pneumoniae* infection. This protection was associated with elevated levels of IgG (IgG1, IgG2a, and IgG2b isotypes) and cytokines [(interferon-γ (IFN-γ) and interleukin (IL)-4] (24).

P30 shares similar importance with P1 as an immunogenic factor (25). *M. pneumoniae* mutants lacking the P30-encoding gene are non-infectious and incapable of adhering to host cells (26), suggesting P30 as a promising candidate for a clinical vaccine. Szczepanek *et al* (27) constructed P30 cytadhesin mutant that does not cause virus infection so that they could test how well it works as a live-attenuated vaccine candidate in mice. However, this live-attenuated vaccine caused serious problems in BALB/c mice, which were likely caused by T helper type 17 (Th17) cell responses. Previous studies have highlighted the significant role of Th17 cells in antimicrobial immune responses and autoimmune diseases in mouse models (28,29). On the other hand, a vaccine based on recombinant P30 adhesin proved to provide immune protection. It was established by Hausner *et al* (30), who joined protein P30 (amino acids 17 to 274) with the C-terminal of P1 adhesin (amino acids 1287-1518 of P1). When administered to guinea pigs, this recombinant vaccine caused protective IgA to be released in their respiratory tracts. This suggested that it may be possible to create vaccines that can protect individuals from becoming infected with *M. pneumoniae*.

The P116 protein is a 116-kDA protein that comprises 1,030 amino acids. In the study by Svenstrup *et al* (31), protein P116 was shown to be surface-exposed and an essential protein involved in adhesion, as anti-P116 antibody was found to prevent the attachment of *M. pneumoniae* to the HEp-2 cells independently of P1. Additionally, serum from *M. pneumoniae*-infected patients has been found to contain antibodies specifically reactive with P116 (32).

Other proteins. The role of accessory proteins is crucial for the proper assembly of the attachment organelle. The identification of the sialic acid binding site is attributed to P40/P90 rather than P1. Variations in the genetic domains of P1, P40 and P90 surfaces contribute to the variability of clinical symptoms, offering new avenues for developing vaccines against *M. pneumoniae* infections (33). The functions of HMW1, HMW2 and HMW3 proteins encompass the structure and stability of attachment organelles, adherence, gliding, proper positioning of adhesins and the maintenance of cell morphology. Although protein P200 was initially considered to serve as an additional structural element in cytoadherence, it appears to play a more essential role in motility rather than adherence. It is closely linked to biofilms and cell maturation (34). Additionally, the P41/P24 proteins play a crucial role in anchoring terminal organelles to the cell body, exerting a considerable influence on the assembly and development of the attachment organelle of *M. pneumoniae*.

Direct damage. Unlike inflammatory or immune-related injuries triggered by *M. pneumoniae* infection, direct damage denotes the harm inflicted by *M. pneumoniae* directly onto host cells. This form of damage encompasses nutrient depletion, intracellular positioning, toxin discharge, oxidative harm and the initiation of apoptosis (35).

Nutrition depletion and intracellular localization. *M. pneumoniae* depends on host cells to acquire vital nutrients due to its compact genome and restricted ability to synthesize compounds. Interaction between the *M. pneumoniae* cell membrane and the host cell membrane facilitates the transfer of crucial compounds necessary for its growth and proliferation (1). Moreover, there is a hypothesis suggesting that *M. pneumoniae* obtains nutrients, including glucose, amino acids and cholesterol by inserting microtubules into host cells (36).

The recently decoded genomic makeup of *M. pneumoniae* strongly indicates a unique and limited genetic evolution, resembling that of other intracellular bacteria. This implies

the potential specialization of this pathogen as a highly adapted parasitic bacterium in respiratory tissue cells. It offers initial evidence supporting the idea of the invasion of *M. pneumoniae* in host cells. Some experimental data indicate that *M. pneumoniae* may possess the ability to permeate host cells intracellularly, potentially for acquiring nutrients (8).

Role of CARDS toxin. The CARDS toxin shares substantial sequence similarities with the S1 subunit of pertussis toxin, causing clinical manifestations similar to *Bordetella pertussis*. This toxin, which is a distinctive adenosine diphosphate ribosylating and vacuolating toxin encoded by *M. pneumoniae* mpn372, requires disulfide bonds for maintenance (37). A recent study demonstrated that CARDS toxin swiftly binds to surfactant protein-A receptors on host target cells and enters immediately through a clathrin-mediated pathway in a dose- and time-dependent manner. Once internalized, CARDS toxin undergoes retrograde transportation from the endosome via the Golgi complex to the endoplasmic reticulum. This retrograde transport aids in toxin processing and is crucial for inducing vacuole formation (37). Creating an acidic setting within host cell vesicles is deemed essential for managing the processing, movement and transfer of the CARDS toxin of *M. pneumoniae*. Adjusting the acidic conditions within host cells could present fresh opportunities to shield these cells from vacuolation induced by the *M. pneumoniae* CARDS toxin (38).

Effects of oxidative stress. Upon adhering to host cells, *M. pneumoniae* penetrates these cells with microtubules, releasing hydrogen peroxide and superoxide radicals. These compounds, combined with the endogenously generated toxic oxygen molecules of the host cell, create oxidative stress within the respiratory tract epithelial cells. In addition, *M. pneumoniae* lacks superoxide dismutase and catalase, allowing the radicals it produces to hinder the catalase activity of the host cell. Consequently, there is a reduced breakdown of peroxides, heightening the vulnerability of host cells to oxygen molecule-induced harm (35).

It has been reported that hydrogen peroxide produced by *M. pneumoniae* can regulate infected cell detachment, aiding in the persistence of bacterial infection (10). Since it is a wall-less bacterium in the *Mycoplasma* genus, *M. pneumoniae* primarily depends on glycerol derived from animal or human host phospholipids for carbon and energy (39). L-α-glycerophosphate oxidase (GlpO), an enzyme present on the surface, plays a pivotal role in glycerol metabolism, leading to hydrogen peroxide production, thereby influencing the *M. pneumoniae* pathogenesis (40). GlpO, despite its potential as a vaccine antigen, might not incite a protective immune response (25). Additionally, histidine phosphocarrier protein kinase (HPrK), a crucial regulator of carbon metabolism in various Gram-positive bacteria, is among the nine regulatory proteins encoded by the *M. pneumoniae* genome. The activation of HPrK by glycerol results in peroxide production, inducing oxidative stress. This stress leads to changes in respiratory epithelial cells, such as cilia loss, reduced oxygen utilization, vacuolar degeneration, lower glucose intake, amino acid absorption and macromolecular synthesis (41).

Inflammatory response. Lung macrophages hold considerable influence in managing *M. pneumoniae* infection. They identify *M. pneumoniae* via toll-like receptor (TLR)2, initiating the myeloid differentiation primary response gene 88 (MyD88)-nuclear factor κB (NF-κB) signaling cascade and engulfing the bacteria through phagocytosis. MyD88, an essential signaling adapter molecule downstream of TLR, plays a pivotal role in orchestrating the lung macrophage reaction to *M. pneumoniae*. However, the activation of the NF-κB pathway concurrently can provoke intense inflammation, prompting apoptosis in macrophages, monocytes and lymphocytes, consequently compromising immune functionality (42).

Components released by *M. pneumoniae*, including metabolites and toxins, can serve as pro-inflammatory molecules, triggering an inflammatory response. The cytokines released due to *M. pneumoniae* infection, such as IFN-γ, tumor necrosis factor-α (TNF-α) and ILs (IL-β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and IL-18) are linked to the exacerbation of asthma. Variations in cytokines may represent one of the pathogenic mechanisms of *M. pneumoniae* infection (43).

The HapE, present in *M. pneumoniae*, functions as a potential virulence factor by generating H_2S, which harms blood cells (5). This H_2S production by HapE prompts phagocytes to release pro-inflammatory factors, leading to increased expression of several inflammatory mediators and cytokines that intensify inflammatory reactions, ultimately causing tissue damage (44). Moreover, HapE contributes to inflammatory responses through ATP-sensitive K^+ (KATP) channels (6). An enhanced H_2S production is achieved by breaking down cysteine within the KATP channel complex. This increase in H_2S levels alters cellular excitability, affecting ion channel function and intensifying inflammation.

It is hypothesized that lipids bind to TLR4, acting as potential ligands. This interaction triggers macrophage autophagy, activating the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasomes and NF-κB pathway by stimulating reactive oxygen species (ROS) production. Eventually, this process leads to the release of pro-inflammatory cytokines (45). When TLR2 and TLR6/TLR1 bind to lipoproteins, they promote the production of cytokines and mediators by immune cells (45). Another study demonstrated that when lipid-associated membrane proteins (LAMPs) were administered to BALB/c mice, they caused lung lesions that were consistent with the aggravation of the disease. The removal of lipid components from LAMPs prior to vaccination could eradicate these symptoms (46). As regards CARDS toxin, in the natural immune response, CARDS activates NLRP3-related inflammasomes, thereby controlling caspase-1 activation, promoting the release of IL-1β and IL-18, and inducing inflammatory cell death alongside stress-related conditions. Furthermore, CARDS toxin has been shown to increase IL-6 and TNF-α expression in a dose-dependent manner (14,47). In cases of refractory *M. pneumoniae* pneumonia (RMPP), CARDS toxin levels have a positive association with TNF-α, rendering it a potential diagnostic biomarker for distinguishing RMPP from non-RMPP cases (48).

It has been suggested that nucleases in parasitic Mollicutes bacteria have a notable contribution to host pathology by catalyzing reactions that allow *M. pneumoniae* to extract nucleic acids for survival in the host. The lipoprotein

encoded by *M. pneumoniae*, known as mpn133, operates as a calcium-dependent nuclease responsible for the degradation of DNA and RNA. This action leads to programmed cell death, inflammatory cell infiltration and tissue damage (5,6). While glycolipids and capsules are considered possible virulence factors, their specific pathogenetic mechanisms remain ambiguous and need further exploration (6).

Although glycolipid and capsule are potential contributors to virulence, their specific mechanisms for promoting illness remain unclear and require further exploration (6). Histone deacetylase 5, a participant in inflammation control, could potentially boost inflammatory reactions triggered by *M. pneumoniae* in macrophages by activating NF-κB (49).

Immune evasion. The mpn400 protein, also known as the immunoglobulin-binding protein of *Mycoplasma* (IbpM), strongly binds to various host-produced immunoglobulins (IgM, IgA and IgG). A previous study suggested that strains of *M. pneumoniae* lacking IbpM exhibit a slight impairment in cytotoxicity, thereby indicating the significance of IbpM as a virulence factor (26).

M. pneumoniae is presumed to invade cells and tissues, residing within cells to evade immune cell phagocytosis and antibiotic effects, leading to prolonged survival in the host. However, the precise mechanisms enabling *M. pneumoniae* to evade intracellular host defenses remain unknown, necessitating further exploration of the pathways involved in intracellular survival. Intracellular invasion likely contributes to *M. pneumoniae* evasion strategies, prolonged host incubation, and the establishment of chronic infection (36).

Although *M. pneumoniae* genomes are relatively small, they contain a notable portion, ~8%, consisting of dispersed repetitive elements. It has been demonstrated that these elements play a role in generating antigenic variation through homologous recombination among specific repetitive genomic components (50). It has been suggested that these repetitive sequences act as a reservoir for generating antigenic variation, particularly in P1 adhesin genes critical for *M. pneumoniae* adherence and motility (51). Mutations and rearrangements in *M. pneumoniae* surface antigens result in insufficient protective antibodies in hosts post-infection, leading to recurrent *M. pneumoniae* infections.

ROS constitute a part of the non-specific immune defense of the host against invading microorganisms, produced by nicotinamide adenine dinucleotide phosphate oxidase (52). ROS play a dual role: They function as direct antimicrobial agents by targeting nucleic acids, carbohydrates, lipids and proteins within *M. pneumoniae* cells, leading to substantial impairment to these biological components. Simultaneously, ROS serve as essential signals for innate immune signaling, prompting the immune system to combat pathogens. Consequently, *M. pneumoniae* has evolved mechanisms with which to combat this oxidative challenge. In *M. pneumoniae*, mpn668 encodes a protective antioxidant enzyme, according to a recent study (53). Potentially reducing the oxidative damage, the host causes, this enzyme degrades hydroperoxide. Additionally, following *M. pneumoniae* infection, neutrophils accumulate rapidly at the infection site through chemokine chemotaxis. They become highly phagocytic, leading to the formation of neutrophil extracellular traps (NETs) and releasing various bactericidal substances, effectively eliminating pathogens. *M. pneumoniae* derives extracellular nucleases which can degrade NETs. Notably, the magnesium-dependent nuclease encoded by *M. pneumoniae* mpn491 serves as a significant extracellular nuclease, enhancing the survival rate of the pathogen and aiding in the evasion of the immune response of the host by degrading NETs, thereby causing additional harm to the host (53).

Factor H is a negative regulator of the complement system of the host, preventing unintended complement activation. C3 convertase cleaves the complement component C3 into C3b, a key effector molecule that further activates the complement system. A previous study demonstrated that *M. hyopneumoniae* binds Factor H via elongation factor thermal unstable Tu (EF-Tu), reducing C3 deposition on the surface of *M. hyopneumoniae* and effectively halting further complement activation. Several mycoplasmas, including *M. pneumoniae*, exploit EF-Tu to hijack Factor H, mimicking host molecules to evade complement attack. Additionally, EF-Tu reinforces adherence between mycoplasmas and tracheal epithelial cells (54).

Infection by *M. pneumoniae* disrupts both innate and adaptive immunity in the host. Some researchers have observed no significant increases in IgG, IgM and IgA immunoglobulins over a 1-year period in *M. pneumoniae*-infected patients, signaling immune system impairment due to the infection. During the acute stage, the levels of C3 and C4 noticeably increased; however, at a later stage, immune suppression induced by *M. pneumoniae* led to a return of C3 and C4 to normal or even lower levels. This infection prompts the respiratory tract to release pro-inflammatory cytokines and chemokines, activating diverse immune cells. This can lead to the overactivity of T-cells, promoting their apoptosis. Additionally, the adhesins and metabolites of *M. pneumoniae* can damage respiratory epithelial cells and lymphocytes, causing reduced activity and accelerated apoptosis of lymphocytes. $CD4^+$ function reduction contributes to an imbalance in immune function, disrupting antigen presentation, B-cell maturation and relative antibody production. Disruptions in humoral and cellular immunity, and the dysregulation of innate immune system brought on by *M. pneumoniae* exacerbate respiratory system damage (1).

3. Mechanisms of extrapulmonary manifestations

Beyond typical respiratory symptoms, *M. pneumoniae* can induce various extrapulmonary complications. Notably, these manifestations sometimes occur independently of pneumonia or even respiratory symptoms. These complications can potentially affect all bodily systems and organs. Evidence of *M. pneumoniae* infection alongside central nervous system involvement supports the notion that *M. pneumoniae* can disseminate to distant organs through blood transmission, causing disease (55,56). The underlying mechanisms of extrapulmonary effects of *M. pneumoniae* are summarized in Fig. 3.

Direct injury. Early-onset extrapulmonary manifestations may be related to direct damage from *M. pneumoniae* in the bloodstream, whereas late-onset complications may

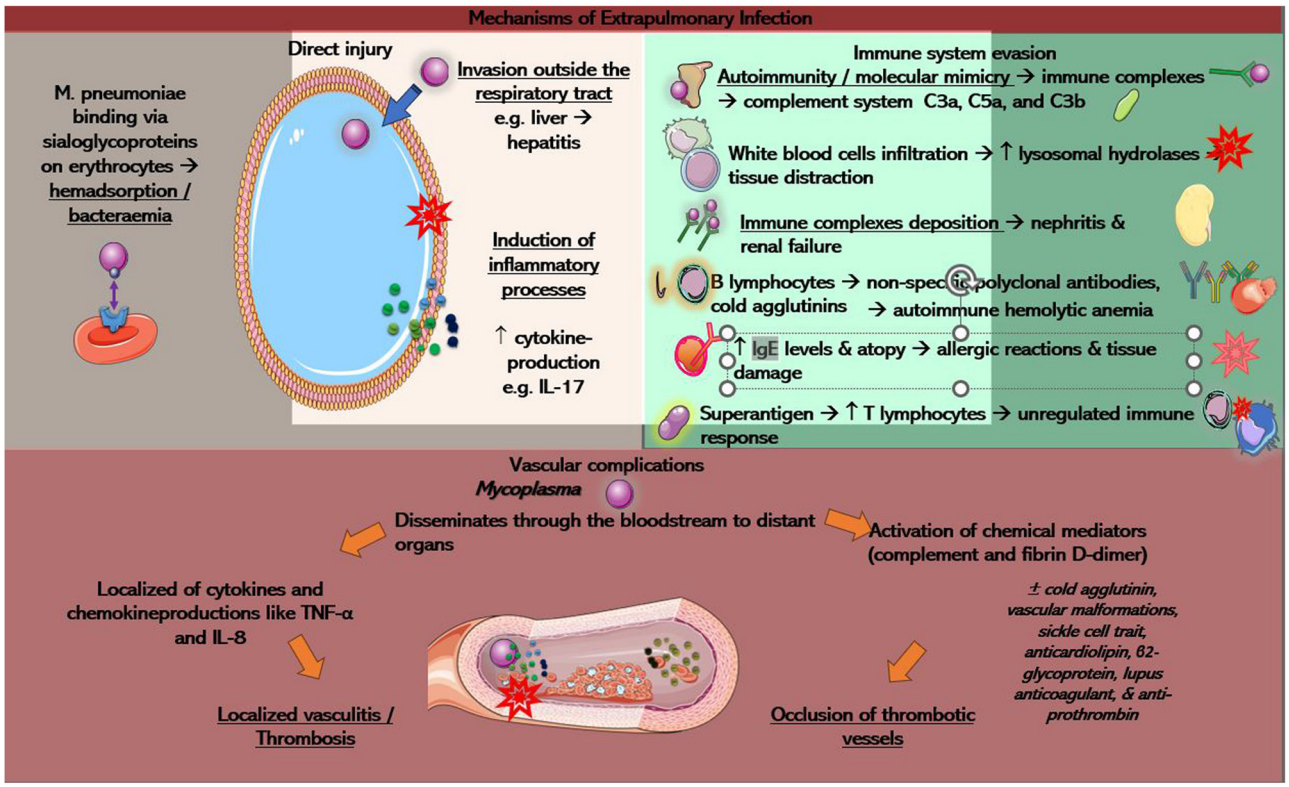

Figure 3. A summary of the various pathophysiological mechanisms of extrapulmonary *Mycoplasma* infection. Please refer to main text for more details. Parts of this image derived from the free medical site http://smart.servier.com/(accessed on December 15, 2023) by Servier, licenced under a Creative Commons Attribution 3.0 unported licence. IL, interleukin; Ig, immunoglobulin; TNF-α, tumor necrosis factor-α.

stem from indirect causes, such as autoimmunity, vascular damage, or drug reactions. *M. pneumoniae* can be found in the blood, skin and pericardial and synovial fluid, detected through PCR and cultures. This suggests the potential for direct damage from the extrapulmonary pathogenesis of *M. pneumoniae* (35).

In cases where the immune barrier of the respiratory tract is immature or impaired, the pathogen may bypass pneumonia development and enter the bloodstream through weakened gaps in damaged lung epithelial cells (57). Given that erythrocytes carry sialoglycoproteins, *M. pneumoniae* can bind to these cells, leading to hemadsorption and possibly causing systemic infection upon infiltrating the bloodstream. The presence of *Mycoplasma* bacteremia stands as a direct extrapulmonary manifestation. Of note, two forms of direct injury are possible: The first one involves *M. pneumoniae* invading outside the respiratory tract. Instances of early-onset hepatitis, unrelated to pneumonia, may be linked to this form of extrapulmonary manifestation (58). While it is hypothesized that *M. pneumoniae* may directly infect liver epithelial cells, this has not been conclusively confirmed. The second form involves inflammatory damage caused by *M. pneumoniae*. In tissues rich in cytokine-producing cells, the membrane lipoprotein of the pathogen can stimulate local cytokine production, resulting in inflammatory tissue damage (59). A previous study emphasized the potential impact of IL-17, a crucial immune mediator, on disease severity and the systemic immune response, potentially influencing extrapulmonary pathogenesis (60).

Immune-driven mechanisms. A number of studies suggested that the recognition of *M. pneumoniae* by innate immune cells and the subsequent cell activation may play a pivotal role in causing severe *M. pneumoniae* complications (61). A previous study conducted by Fink *et al* (62) examining serum IgM, IgA, IgG and cerebrospinal fluid in individuals with sudden neurological manifestations, suggested that nervous system damage may not stem directly from *M. pneumoniae* invasion, but may likely result from an immune response to the infection. Immune-driven mechanisms play a key role in *M. pneumoniae*-associated extrapulmonary diseases (MpEPDs) (9).

Role of autoimmunity. Autoimmunity spurred by molecular mimicry entails *M. pneumoniae* antigens, mimicking host cell components, resulting in alterations in the structure of host cell membrane antigens. This activation induces autoimmune responses, forming immune complexes within the corresponding organs. These complexes activate complements, generating neutrophil chemotaxis factors, including C3a, C5a and C3b. Numerous white blood cells then infiltrate the affected site, releasing lysosomal hydrolases that cause destructive injuries across multiple organs. For example, proteins including P1 and P30 located on the attachment organelles of *M. pneumoniae*, exhibit significant similarity to proteins, such as troponin, keratin, cytoskeletal proteins and fibrinogen within the host. When antibodies are generated due to *M. pneumoniae* infections, they aim at diverse host tissues, resulting in the formation of immune complexes that lead to damage in organs, including the liver, kidneys, brain, smooth

muscle and lungs (6). In summary, autoimmunity significantly contributes to extrapulmonary complications resulting from *M. pneumoniae* infections.

Immune complexes. In instances where *M. pneumoniae* infection results in acute nephritis and renal failure, findings suggest the presence of the *M. pneumoniae* genome and immune complexes containing its antigens within the glomerulus. This discovery may align with increased deposition of immune complexes and activation of the complement system within the tissues. The pathological processes of glomerulonephritis and IgA nephropathy associated with *M. pneumoniae* infections are driven by the circulation of these immune complexes (35).

The *M. pneumoniae* membrane antigen mimics the red blood cell (RBC)-membrane I antigen and shares similar components with *Streptococcus pneumoniae* 23 or 32 and *M. genitalium*. Similar to several plants and bacteria, the membrane glycolipids of *M. pneumoniae* share an antigen present in brain and lung tissues, causing cross-reactivity. The carboxyl end of the P1 and P30 proteins of *M. pneumoniae* in its adhesive organs bears resemblance to eukaryotic cytoskeletal proteins, such as fibrinogen, keratin and troponin. Consequently, infection triggers autoantibody production in the brain, lungs, RBC-membranes, lymphocytes and myocardial cells, forming immune complexes that intensify the autoimmune response, leading to multisystem immune damage (30,63).

Non-specific antibodies. *M. pneumoniae* triggers B-lymphocytes, resulting in the production of non-specific polyclonal antibodies not directly targeting the pathogen. The study conducted by Meyer Sauteur *et al* (64) indicated an increase in serum antibodies against both *M. pneumoniae* proteins and glycolipids among infected mice and children. The equivalent recovery of serum antibody levels in Bruton's tyrosine kinase (Btk)-deficient and wild-type mice after *M. pneumoniae* infection suggests that the pulmonary clearance of *M. pneumoniae* is primarily mediated by IgG-reactive *M. pneumoniae* proteins. The presence of *M. pneumoniae* glycolipid-specific IgG or IgM is not crucial (64). Of note, ~50% of *M. pneumoniae*-infected patients develop cold agglutinins, a type of IgM antibody that may persist for weeks. Cold agglutinins serve as proof of clinical suspicions regarding *M. pneumoniae*-caused primary atypical pneumonia. It has been suggested that these agglutinins may emerge due to cross-reactive autoantibodies between the *M. pneumoniae* glycolipid antigen and the I antigen present in human erythrocytes during acute *M. pneumoniae* infections. This particular antibody has the potential to trigger autoimmune hemolytic anemia, commonly known as cold agglutinin disease. This entity stands out as a significant indirect manifestation outside the lungs due to *M. pneumoniae* infection (65).

Increased IgE levels. Increased IgE levels and atopy denote the genetic predisposition of the body to generate IgE antibodies when exposed to small quantities of common environmental factors (60). Poddighe *et al* (66) examined 162 hospitalized children and noted a considerable increase in the overall serum IgE level among children affected by MpEPDs. This level was notably higher than that found in children solely experiencing the typical respiratory issues associated with *M. pneumoniae* infection, suggesting a link between atopy and MpEPDs in children (66). Patients displaying extrapulmonary manifestations exhibit a higher prevalence of atopy compared to those without such manifestations, suggesting a potential association between atopy and MpEPDs (59,66). Elevated IgE levels are indicative of immune irregularities. Self-reactive IgEs exacerbate immune-related entities, leading to clinical symptoms such as allergic reactions (67). For instance, following *M. pneumoniae* infection, the P1 protein may prompt the generation of P1-specific IgE in individuals allergic to *M. pneumoniae*, eventually causing allergic symptoms and tissue damage (68). Some individuals prone to producing IgE may have a predisposition to developing extra-respiratory diseases when infected by *M. pneumoniae* (67).

Vascular complications. Extrapulmonary manifestations are not solely linked to the infection and autoimmunity, but also involve complications from the vascular system. Thrombosis can occur in vessels throughout the body, with the pulmonary vessels being the most commonly affected sites. The primary symptom is chest pain, followed by neurological symptoms and abdominal pain (69). A rare case of pediatric priapism has been reported, suggesting this symptom as an exceptionally rare, yet plausible form of vascular occlusion resulting from infection due to *M. pneumoniae* (70). Extrapulmonary manifestations associated with vascular obstruction arise from a combination of direct and indirect mechanisms. *M. pneumoniae* has the ability to disseminate through the bloodstream to distant organs, provoking localized production of cytokines and chemokines like TNF-α and IL-8. This local response affects the vascular wall, potentially resulting in localized vasculitis or thrombosis without systemic hypercoagulability (69). The alternative form involves the activation of chemical factors, such as complement and d-dimer, which may lead to the occlusion of thrombotic vessels. According to the study by Liu *et al* (69), some factors contributing to thrombosis are transient, while others stem from hereditary thrombophilia in patients experiencing thrombosis due to *M. pneumoniae* infection. Moreover, specific transient elements, such as cold agglutinin, vascular malformations, sickle cell trait and the presence of positive antibodies such as anticardiolipin, β2-glycoprotein, lupus anticoagulant and anti-prothrombin have been proposed to elevate the risk of thrombosis (71,72). These factors contribute to thrombotic vessel occlusion (69).

Superantigen. Additional factors, such as the *M. pneumoniae* superantigen, may also play a role in extrapulmonary manifestations. Superantigens derived from different bacteria have the potential to stimulate excessive production of T-lymphocytes and lipid-related membrane proteins, resulting in an unregulated immune response reminiscent of the pathogenic process observed in Kawasaki disease (73).

Immunosuppression. Infection with *M. pneumoniae* has been observed to trigger immunosuppressive effects in the body, leading to imbalances in T-cell subgroups. Research has shown that this infection severely damages both B-cells and T-cells (74). Between 13- and 18 weeks

following *M. pneumoniae* infection, there is a decrease in serum IgG levels in patients (75). Some children infected with *M. pneumoniae* experience hypogammaglobulinemia, reduced neutrophil chemoattraction, diminished responsiveness to phytohemagglutinin phytolectin and lowered resistance to concurrent infections with other pathogens, including *S. pneumoniae* (74). These alterations suggest that *M. pneumoniae* infection has the potential to induce immunosuppression.

4. Conclusions

Recent advancements have enhanced the understanding of the mechanisms through which *M. pneumoniae* triggers both pulmonary and extrapulmonary manifestations. The underlying mechanisms leading to manifestations beyond pulmonary involvement include direct damage through invasion and inflammatory components, indirect harm from the immune response of the host and vascular blockages. The mechanisms behind intrapulmonary and extrapulmonary pathogenesis in *M. pneumoniae* infection, though distinct, are interconnected and share certain similarities. Despite the complexity of the pathogenic mechanisms of *M. pneumoniae*, the specifics remain incompletely understood, warranting further research for a detailed comprehension of its pathogenesis.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

DAS and VEG conceptualized the study. IGL, VEG, NT, PS and DAS made a substantial contribution to data interpretation and analysis and wrote and prepared the draft of the manuscript. DAS and VEG analyzed the data and provided critical revisions. All authors contributed to manuscript revision, and have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article. The other authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tool Chat GPT was used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the AI tool as necessary, taking full responsibility for the ultimate content of the present manuscript.

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