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REVIEW ARTICLE

Insight into the Pathogenic Mechanism of *Mycoplasma pneumoniae*

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Abstract

Mycoplasma pneumoniae, an obligate parasitic pathogen without cell wall, can cause severe upper and lower respiratory tract symptoms. It is the pathogen of human bronchitis and *walking pneumonia*, and named community-acquired pneumonia. In addition to severe respiratory symptoms, there are clinical extrapulmonary manifestations in the skin, brain, kidney, musculoskeletal, digestive system, and even blood system after *M. pneumoniae* infection. Hereby, we comprehensively summarized and reviewed the intrapulmonary and extrapulmonary pathogenesis of *M. pneumoniae* infection. The pathogenesis of related respiratory symptoms caused by *M. pneumoniae* is mainly adhesion damage, direct damage including nutrient predation, invasion and toxin, cytokine induced inflammation damage and immune evasion effect. The pathogenesis of extrapulmonary manifestations includes direct damage mediated by invasion and inflammatory factors, indirect damage caused by host immune response, and vascular occlusion. The intrapulmonary and extrapulmonary pathogenic mechanisms of *M. pneumoniae* infection are independent and interrelated, and have certain commonalities. In fact, the pathogenic mechanisms of *M. pneumoniae* are complicated, and the specific content is still not completely clear, further researches are necessary for determining the detailed pathogenesis of *M. pneumoniae*. This review can provide certain guidance for the effective prevention and treatment of *M. pneumoniae* infection.

Abbreviations

CARDS	Community-acquired respiratory distress syndrome toxin
GlpO	L-α-glycerophosphate oxidase
KATP channels	ATP-sensitive K^+ channels
ROS	Reactive oxygen species
LAMPS	Lipid-associated membrane proteins
HDAC 5	Histone deacetylase 5
IbpM	Immunoglobulin binding protein of *Mycoplasma*
NET	Neutrophil extracellular traps
EF-Tu	Elongation factor thermo unstable
MpEPDs	*Mycoplasma pneumoniae*-related extrapulmonary diseases

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Introduction

Mycoplasmas are the smallest free-living, wall-less and self-replicating prokaryotes having an extremely small genome size of 580–2200 kb [1, 2]. More than 200 species of *mycoplasma* have been identified in humans, animals, plants and arthropods, but only a few of them have been proven to cause human diseases. The main pathogenic mycoplasmas include *Mycoplasma pneumoniae*, *M. genitalium*, *M. fermentation*, *M. hominis*, *M. penetrans*, *M. pirum* and *Ureaplasma urealyticum*, which are identified to be responsible for humans and animal diseases in the respiratory tract and urogenital system.

Among pathogenic *Mycoplasma*, *M. pneumoniae* is the most predominant and intensely studied species. *Mycoplasma pneumoniae* is one of the main pathogens that cause chronic human respiratory tract disease and pneumonia, especially children and adolescents are most susceptible. *Mycoplasma pneumoniae* infection is generally self-limiting and mild. However, it may develop into a severe or life-threatening disease in some patients. This atypical pathogen was identified to be responsible for up to 40% community-acquired pneumoniae (CAP) among children over five years of age, and lower respiratory tract infections are considered a common cause of morbidity and mortality in children [1,

3]. *Mycoplasma pneumoniae* infection are also thought to be associated with chronic lung disease and bronchial asthma [4]. In addition to causing severe lower respiratory tract disease and milder upper respiratory tract symptoms, *M. pneumoniae* also produces other extrapulmonary diseases and post-infection events. Extrapulmonary complications occur in the skin, kidneys, stomach, intestines, heart, musculoskeletal, brain and blood system, etc., resulting in some unusual clinical symptoms. Central nervous system manifestations are the most common extrapulmonary complications of *M. pneumoniae* infection, at times be life-threatening. Many studies have addressed that due to the atypical symptoms, no obvious clinical and imaging features of *M. pneumoniae* infection, the early stage of infection is easily underestimated [5]. The main laboratory diagnosis methods are rapid culture based on throat swabs, PCR and serological test, as well as other laboratory diagnostic methods such as rapid antigen test.

Extrapulmonary manifestations often occur in the absence of pneumonia, and both involve independent pathological mechanisms [6]. The extrapulmonary manifestations caused by *M. pneumoniae* can be explained by three possible mechanisms: direct damage caused by invasion or locally induced inflammatory cytokines, Immune-mediated indirect damage, and vascular occlusion caused by vasculitis or thrombosis. The intrapulmonary infection mechanism includes adhesion, nutrient depletion, invasion, toxin, immune and inflammatory damage [7]. It should be noted that these mechanisms are not considered mutually exclusive, but can act in the patient's body at the same time. Based on the severe infection of *M. pneumoniae*, we summarized the intrapulmonary and extrapulmonary pathogenesis of *M. pneumoniae*, and this review will provide a certain reference for the pathogenesis research and treatment strategy of *M. pneumoniae* infection.

Intrapulmonary Infection Mechanism

The pathogenesis of *M. pneumoniae* is complicated. At the initial stage, *M. pneumoniae* adheres to the host bronchial epithelium through the terminal structure, induces intracellular metabolism and ultrastructural alters in the infected cells. At the same time, *M. pneumoniae* invades host cells, depletes nutrients and releases CARDS toxin, hydrogen peroxide and superoxide radicals, leading to direct damage. Combined with *M. pneumoniae* Hape enzymes, lipids, lipoproteins, glycolipids and other components to induce cytokines production, the occurrence of inflammation ultimately causes indirect damage. Furthermore, *M. pneumoniae* evades the host immune system through its immune evasion mechanism, which may help surviving in the body for a long time and thus cause more serious clinical manifestations (Fig. 1).

Adhesion Related Proteins

Adhesion is the primary factor and prerequisite for the pathogenicity of *M. pneumoniae*, this ability depends on a special polarized terminal attachment organelle, some pathogenic factors, such as toxic effects, are based on the adhesion step. *Mycoplasma pneumoniae* interacts with the host respiratory epithelium by attaching to the surface of the bronchial ciliary epithelium, which induces intracellular metabolism and ultrastructural alters in the infected cells, rearranges the cytoskeleton, and causes nutrition depletion of host cells [5]. Changes in intracellular metabolism such as parallel decrease of the uptake rate of host cells orotic acid and amino acid, and the evident inhibition of ribonucleic acid and protein synthesis. The ultrastructural changes such as an accompanying deterioration in the integrity of the airway lumenal surface membranes and subsequent loss of the epithelial cell cytosol. In addition, the disorder of host cells carbohydrate metabolism, amino acid uptake and protein synthesis, eventually promotes the transmission of pathogens in cells, leading to cilia stagnation, cell death, and coordinating other factors to produce human respiratory symptoms[5]. *Mycoplasma pneumoniae* tightly binds to the host epithelial cells through its unique attachment organelle, which are considered to mediate cell division, cytoadherence, and cell motility at host cell surface [8]. The main receptors for *M. pneumoniae* to recognize are sialylated and sulfated oligosaccharide receptors [9] (Fig. 1a). The nature and density of the host receptors can profoundly affect the adhesion and sliding of *M. pneumoniae*, which in turn affects the pathogenic mechanism and infection outcome [10].

The attachment organelle at a cell polar is a membrane protrusion composed of some nap-like surface structures and an internal core (Fig. 2). It realizes a complex and multi-factor adhesion process through the interaction between the internal network-like cytoskeletal system and the surface adhesion protein [11]. The nap-like surface structure is mainly composed of P1 adhesin, P30, P40 and P90. The internal core structure can be divided into three parts, including a terminal button, paired plates, and a bowl (wheel) complex from the front end, and that it is essential for the formation of an attachment organelle. The main proteins include high-molecular-weight proteins (HMW1, HMW2, HMW3), proteins P65, P200, CpsG, *mpn387*, Lon protease, P41 and P24, etc. [12] (Fig. 2).

P1

Membrane protein P1 is considered as the major cellular adhesin, which is surface localized and trypsin sensitive

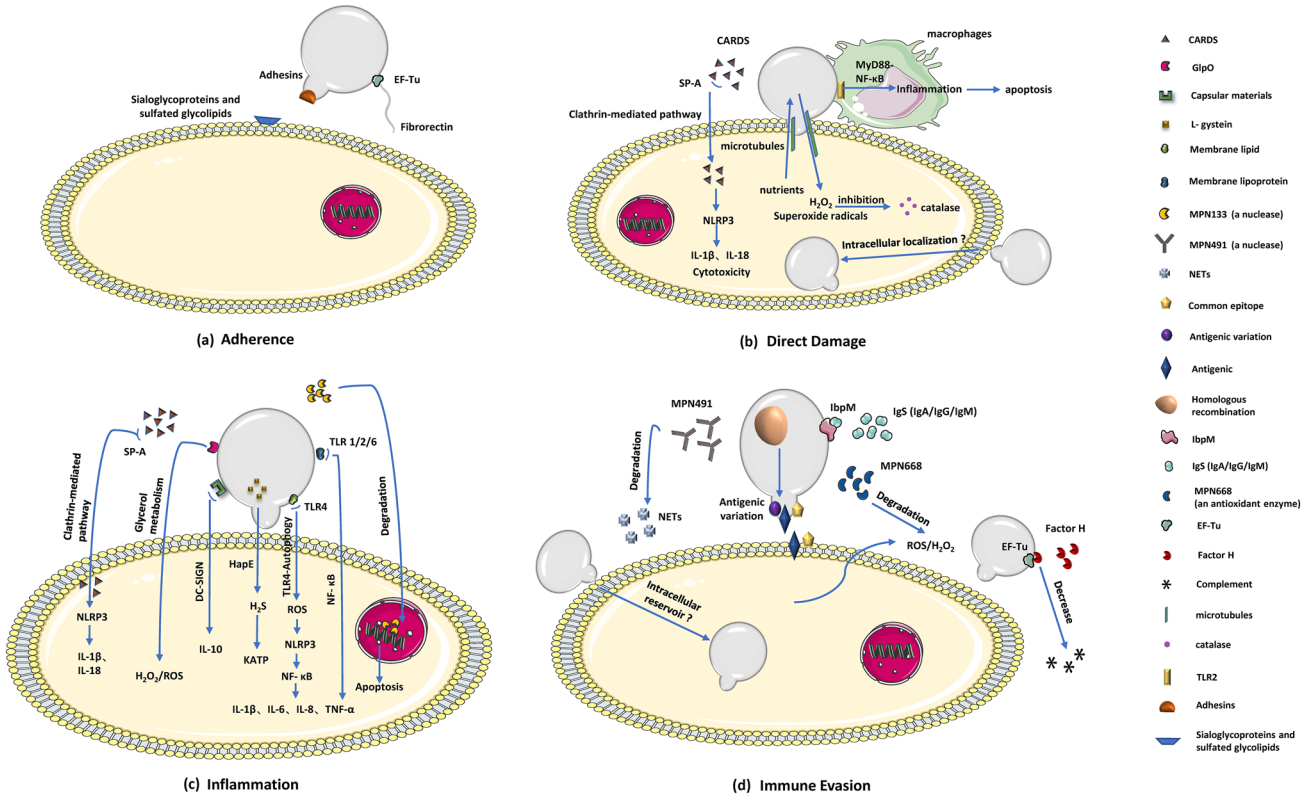

Fig. 1 Pathogenic mechanisms of *M. pneumoniae* intrapulmonary infection. **a** *M. pneumoniae* adhesion causes cell damage. Adhesins binds to sialoglycoproteins and sulfated glycolipids receptors on host cell surface to obtain nutrition for *M. pneumoniae*, which induces intracellular metabolism and ultrastructural changes in the infected cells. Additionally, EF-Tu can bind to a range of host molecules (such as fibronectin), increase *mycoplasmas* attach to tracheal epithelial cells. **b** *M. pneumoniae* releases CARD toxin, H_2O_2 and superoxide radicals into host cell to produce host cytotoxicity. **c** Inflammation-inducing factors (membrane lipid, lipoprotein, HapE enzyme, nuclease, oxidase GlpO, capsular materials) activate host inflammatory pathways to produce inflammatory damage. **d** *M. pneumoniae* produces a nuclease (encoded by MPN491) and an antioxidant enzyme (encoded by MPN668) to degrade NETs and peroxides, respectively. Homologous DNA recombination leads to antigenic variation. Furthermore, IbpM and EF-Tu enable *M. pneumoniae* evade the host's immune response

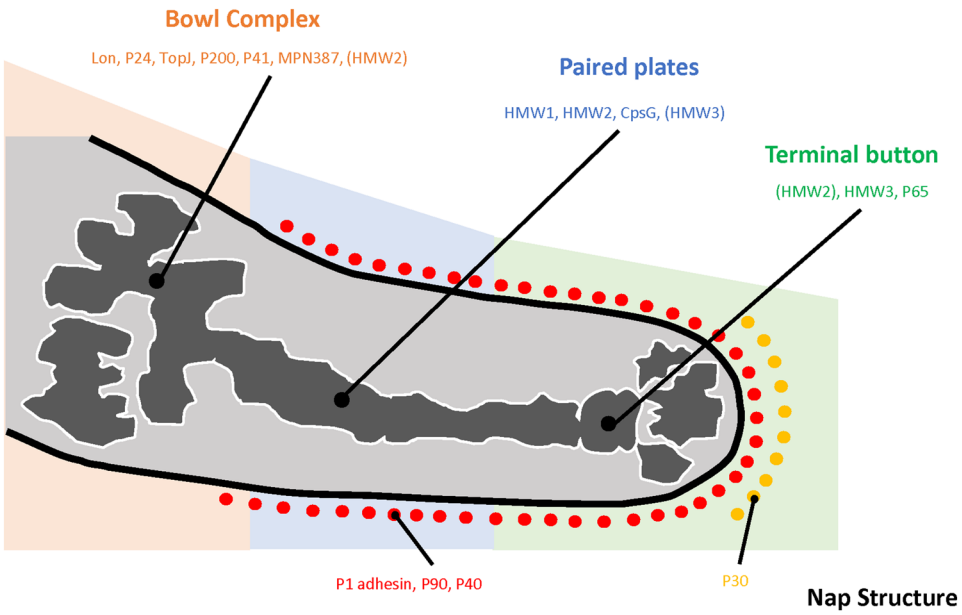

Fig. 2 Component proteins of the internal structure and the surface adhesion complex of attachment organelle. The nap-like surface structure composed of the main adhesins (P1 and P30) and accessory proteins (P40 and P90) surrounding the cell membrane. The internal structure is made up of terminal button (HMW2, HMW3, P65), paired plates (HMW1, HMW2, CpsG, HMW3), and a bowl complex (Lon, P24, TopJ, P200, P41, MPN387, HMW2). HMW1, HMW2, HMW3 refer to three high molecular weight (HMW) proteins

[13]. When *M. pneumoniae* contacts the target cell, the P1 precursor proteins, which are scattered in the cell membranes, rapidly traffics to the terminal organelle, and the leader peptide on the amino terminal is hydrolyzed to become a mature P1 protein that binds to the host receptor [7]. It should be noted that P1 adhesin can mediate adhesion only if its correct positioned on the terminal organelle. Researches have confirmed that P1 adhesin not only participates in the binding between *M. pneumoniae* and host receptors to play an adhesion role, but also in gliding on the surface of host cells. Studies have also shown that P1 adhesin plays an important role in the mast cells cytokine response induced by *M. pneumoniae*, the mast cells are activated to cause inflammation damage by the direct contact between *M. pneumoniae* and the sialylated residues on the surface of mast cells. The antibodies against the highly immunogenic carboxyl terminus of P1 produced by the humoral immunity are considered to reduce *M. pneumoniae* adhering to non-biological and host cells [13].

P30

Studies have found that there is a degree of sequence homology between some specific domains of the P30 protein and the P1 protein, both representing the dominant proteins responsible for adherence. P30 adhesin located at the tip of attachment organelle, plays a crucial role in conveying signals from the cell interior to the exterior to activate key steps in cytoadherence and motility, such as the arrangement of P1 adhesin complex and the binding between P1 and host receptors [12]. Romero-Arroyo et al. have proved that P30 is instrumental to cell development, the loss of P30 could lead to abnormal *mycoplasma* morphology, including oval or leafy cells with poorly defined apical structures, while transformation of P30 mutants and wild-type P30 alleles can restore their normal morphology.

P116

P116 protein was verified to be surface exposed and considered as a crucial cell adhesin because the anti-P116 antibody has been shown to prevent attachment of *M. pneumoniae* to the HEp-2 cells independently of P1. P116 has also been identified as an important immunogenic antigen of *M. pneumoniae* [5]. The overall level of P116-C-terminal protein has been used for serological diagnosis of *M. pneumoniae,* and Tabassum et. al have shown that an N-terminal 27 kDa fragment of P116 protein also held a promise for serodiagnosis of *M. pneumoniae* infection.

Other Accessory Proteins

The protein P65 have a close spatial and functional relationship with P30. The analysis of the P65 and P30 fluorescent fusion proteins expressed by the growing mycoplasma culture showed that they were situated at the developing terminal organelles almost concurrently. P65 might interact with the internal structural domain of P30 to achieve a close combination between the terminal button and the front side of the membrane [12]. P40 and P90 are adhesins produced by the cleavage of *mpn142*, which form a transmembrane adhesion complex with protein P1 [13]. The presence of accessory proteins is essential for the formation of functional attachment organelle. Vizarraga has reported that the binding site for sialic acid was found in P40/P90 and not in P1, genetic variability of the N-terminal domain surfaces of P1 and P40/P90 results in clinical symptoms variability, these founds provide new strategies in vaccine development against *M. pneumoniae* infections [14]. Studies have shown a function for HMW (1, 2, 3) proteins in the architecture and stability of attachment organelles, also HMW (1, 2, 3) proteins are related to adherence and gliding, participating in the correct positioning of adhesins and the maintenance of cell morphology. Protein P200 was speculated as an accessory structural component in cytoadherence, since it shared several unusual features with the proteins HMW1 and HMW3. However, P200 was considered more essential for motility rather than adherence, and associated with biofilms and cells maturation [15]. P41/P24 are involved in anchoring the terminal organelles on the cell body, both of them play a significant role in the assembly and development of *M. pneumoniae* attachment organelles.

Direct Damage

Direct damage refers to damage to host cells caused by *M. pneumoniae*, instead of inflammatory or immune-mediated injury caused by *M. pneumoniae* infection. The direct damage includes nutrition depletion, intracellular localization, toxin, oxidative damage and induction of apoptosis.

Nutrition Depletion

Mycoplasma pneumoniae depends on host cells to supply necessary nutrients for their survival and development, since their small genome and limited biosynthetic capabilities. The cell membrane of *M. pneumoniae* can closely contact with the host cell membrane, which promotes the exchange of compounds essential for their growth and proliferation [1]. In addition, it is thought to be able to absorb nutrients such as glucose, cholesterol and amino acids by inserting microtubules into host cells (Fig. 1b).

Intracellular Localization

The recently elucidated genomic structure of *M. pneumoniae* strongly suggests that this organism might have undergone a unique and reductive genetic evolution, such as other intracellular bacteria, which indicates this pathogen may be a highly specialized parasitic bacteria in respiratory tissue cells, and provides preliminary evidence for the intracellular invasion of *M. pneumoniae*. In fact, some experimental data and studies have concluded that *M. pneumoniae* may have intracellular permeability and the ability to penetrate the host cell membrane for nutrients acquisition [6] (Fig. 1b).

CARDS Toxin

Community-Acquired Respiratory Distress Syndrome Toxin (CARDS) is a protein shared significant sequence homologies with the S1 subunit of pertussis toxin, which cause clinical symptoms like pertussis. CARDS, encoded by *M. pneumoniae mpn372*, is a unique ADP ribosylating and vacuolating toxin, and the maintenance of these activities requires disulfide bond [16]. Recent study has shown that CARDS toxin binds to SP-A receptors of host target cells (Fig. 1b) and is internalized rapidly in a dose and time-dependent manner by clathrin-mediated pathway. After internalization, CARDS toxin is transported in a retrograde manner from endosome through the golgi complex into the endoplasmic reticulum. Moreover, retrograde transport facilitates toxin clipping and is required to induce vacuole formation [17]. An acidic environment in host cell intracellular vesicle is considered essential for clipping, trafficking and translocation of *M. pneumoniae* CARDS toxin. Regulating the acidic environment in host cells may open new possibilities to protect host target cells against *M. pneumoniae* CARDS toxin-induced vacuolation [17].

Oxidative Damage

After adhering to the host cell, *M. pneumoniae* inserts the microtubule into the host cell and releases hydrogen peroxide and superoxide radicals. These substances and the endogenous toxic oxygen molecules produced by the host cell cause respiratory tract epithelial cells oxidative stress. Moreover, *M. pneumoniae* lacks superoxide dismutase and catalase, and the superoxide free radicals produced by *M. pneumoniae* can also inhibit the activity of catalase in the host cell, both leading to reduced decomposition of peroxides and making host cells more sensitive to the toxic effects of oxygen molecules (Fig. 1b). The study results of Yamamoto suggested that *M. pneumoniae* might develop a mechanism to regulate infected cell detachment by producing hydrogen peroxide, which may contribute to sustaining the bacterial infection [18].

As a wall-less bacteria of the genus *Mycoplasma*, glycerol derived from phospholipids of animal or human hosts is the major source of carbon and energy [19]. L-α-glycerophosphate oxidase (GlpO), a surface-exposed enzyme involved in the metabolism of glycerol, is responsible for the production of hydrogen peroxide during glycerol metabolism and important for the pathogenesis of *M. pneumoniae* (Fig. 1c) [20]. However, Melanie et al. have demonstrated that the GlpO as a candidate vaccine antigen is unlikely to induce a protective immune response [20]. In addition, HPrK, a key regulator of carbon metabolism in many Gram-positive bacteria, is also one of the nine known regulatory proteins encoded by the *M. pneumoniae* genome, the HPrK can be activated by glycerol and induced oxidative stress by the production of peroxides. Oxidative Damage ultimately leads to respiratory epithelial cells alters, such as cilia loss, vacuolar degeneration, reduced oxygen consumption, decreased glucose utilization, amino acid uptake and macromolecular synthesis.

Inducing Apoptosis

Lung macrophages play an important role in controlling *M. pneumoniae* infection. They can recognize *M. pneumoniae* through TLR2, activate MyD88-NF-κB signaling pathway and phagocytose *M. pneumoniae*. MyD88 is the major adaptor molecule for signaling downstream of TLR, MyD88 signaling is essential for macrophage response to *M. pneumoniae* in the lung. At the same time, the activation of the NF-κB pathway can cause severe inflammation, induce the apoptosis of monocytes, macrophages and lymphocytes, and ultimately reduce immune function (Fig. 1b) [21].

Inflammation Injury

The cellular components including metabolites and toxin released by *M. pneumoniae* could act as pro-inflammatory molecules to stimulate the inflammatory response. The release of *M. pneumoniae*-induced cytokines such as gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukins (including interleukins-1β[IL-β], IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-18) are thought to be related to asthma exacerbation. The changes of cytokines may be one of the pathogenic mechanisms of *M. pneumoniae* infection.

HapE

The HapE is an enzyme and potential virulence factor of *M. pneumoniae*, which can produce H_2S to damage blood cells [4] (Fig. 1c). Further, H_2S produced by HapE has been demonstrated to induce phagocytes to secrete pro-inflammatory factors. The up-regulated expression of various inflammatory mediators and cytokines can activate and aggravate

inflammatory reactions, leading to tissue damage [22]. In addition, this enzyme also mediates inflammatory reactions via adenosine triphosphate (ATP)-sensitive K^+ (KATP) channels [5] (Fig. 1c). HapE can degrade cysteine within the KATP channel complex to form H_2S, enhanced production of H_2S alters cellular excitability via modulation of ion channel function and exacerbates inflammation. Studies have shown that mutants with defective HapE genes cannot be isolated, hence suggesting that HapE gene is essential for the development of *M. pneumoniae*.

Lipid and Lipoproteins

Lipid is considered to bind to TLR4 as potential TLR4 ligands, stimulating macrophage autophagy, and then promoting the activation of NLRP3 inflammasomes and NF-κB pathway by inducing ROS production, ultimately leading to the secretion of pro-inflammatory cytokines [23] (Fig. 1c). Lipoproteins are thought to be recognized by TLR2 and TLR6/TLR1 through extracellular leucine repeat sequence region, which induces cytokines production and the expression of mediators in immune cells (Fig. 1c). A recent study by Mara et al. has reported that after inoculating BALB/c mice with *M. pneumonia,* lipid-associated membrane proteins (LAMPs) induce lung lesions consistent with exacerbated disease following challenge, while removing the lipid moieties from LAMP before vaccination could eliminate symptoms. The overall results can be concluded that the lipid moieties of the lipoproteins are the causative factors of *M. pneumoniae* vaccine-enhancing disease (VED) [24].

CARDS

CARDS activates NLRP3-related inflammasomes to regulate the activation of caspase-1, promotes the maturation and release of IL-1β and IL-18 in the natural immune defense, which results in inflammatory cell death and stress pathological conditions (Fig. 1c). At the same time, CARDS toxin can increase the expression of pro-inflammatory cytokines IL-6 and TNF-α in a dose and activity-dependent manner [11, 25]. Gang et al. has found that CARDS toxin was positively correlated with TNF-α level in refractory *M. pneumoniae* pneumonia (RMPP) cases. Therefore, CARDS provides a good diagnostic biomarker for differentiating children with RMPP and non-RMPP(NRMPP) [26].

Nuclease

Studies have demonstrated the nuclease of parasitic *Mollicutes* bacteria may contribute to host pathology by nuclease catalyzing reactions which help *M. pneumoniae* plunder nucleic acids from host nucleic acid precursors or DNA for survival in the host. The lipoprotein, encoded by *M. pneumoniae mpn133*, participates in the uptake of free glycerol and acts as a calcium-dependent nuclease to degrade DNA and RNA which causes programmed death of host cells, local inflammatory cell infiltration and tissue damage (Fig. 1c) [4, 5].

Others

Glycolipid and capsule are considered as potential virulence factors, but the pathogenic mechanisms are unclear and needs to be further explored [5]. Histone deacetylase 5 (HDAC5) is involved in the regulation of inflammation, which may promote *M. pneumoniae*-induced inflammatory response in macrophages through NF-κB activation [27].

Immune Evasion

IbpM

Mpn400 protein is a surface protein that binds strongly to various immunoglobulins (IgM, IgA and IgG) produced by the host [5]. Therefore, *mpn400* protein was named as immunoglobulin binding protein of *Mycoplasma* (IbpM). Study has demonstrated that *M. pneumoniae* strains lacking IbpM are slightly impaired in terms of cytotoxicity and thus IbpM is considered to be a virulence factor [28] (Fig. 1d).

Intracellular Invasion

Mycoplasma pneumoniae is thought to invade cells and tissues of the body, parasitize inside the cell to escape the phagocytosis of immune cells and the effect of antibiotics, and finally survive in the body for a long time. However, the mechanisms by which *M. pneumoniae* evade host defense while intercellular remain unknown, and the pathways related to intracellular survival remain to be elucidated. Intracellular invasion may be responsible for *M. pneumoniae* evasion, long-term incubation in the host body and establishment of chronic infection (Fig. 1d).

Antigen Polymorphism and Variation

In spite of limited size, the genomes of *M. pneumoniae* consist of a significant portion of repeated elements, which are dispersed throughout the genome and constitute approximately 8% of the genome. It has been demonstrated that these genes are sufficient to generate antigenic variation by homologous recombination between specific repetitive genomic elements [29] (Fig. 1d). Studies have implicated that the repetitive sequences serve as a reservoir for antigenic variation generation of P1 adhesin genes in *M. pneumoniae*, and the production of recombinant P1 adhesin is essential for adherence and motility of *M. pneumoniae* [30].

Due to mutation and rearrangement of *M. pneumoniae* surface antigens, deficiency of protective antibodies in the host after *M. pneumoniae* infection is considered responsible for repeated *M. pneumoniae* infection.

The Mechanism of Oxide Degradation

The production of reactive oxygen species (ROS) is a part of the host cell non-specific immune defense against invading pathogens [31]. ROS is produced by NADPH oxidase in the host. On the one hand, ROS is directly antimicrobial, which targets on nucleic acids, carbohydrates, lipids and proteins in *M. pneumoniae* cells, and causing considerable damage to these biological macromolecules. On the other hand, ROS is also an essential signal for innate immune signaling, which activates the innate immune system to fight against pathogens. Therefore, *M. pneumoniae* needs to evolve a mechanism to deal with this oxidative challenge. Recent studies have found that the protein encoded by *mpn668* is a protective antioxidant enzyme in *M. pneumoniae* (Fig. 1d), which may degrade hydroperoxide and limit the oxidative damage exerted by the host [32].

Nuclease

After infected with *M. pneumoniae*, neutrophils can rapidly accumulate at the infected area by chemokines chemotaxis, become highly phagocytic and eventually undergo morphological changes, which lead to Neutrophil extracellular traps (NET) and a variety of bactericidal substances release, ultimately effectively eliminate pathogenic microorganisms. *M. pneumoniae* produces some extracellular nucleases capable of degrading NETs. The magnesium-dependent nuclease encoded by *M. pneumoniae mpn491* is a major extracellular nuclease which improves the survival rate of *M. pneumoniae* and helps the pathogen to escape the host immune response by degrading NETs [33], resulting in further damage to the host (Fig. 1d).

Elongation Factor Thermo Unstable (EF-Tu)

Factor H is a negative regulator of the complement system in host, which helps avoiding unexpected complement activation [34]. C3 convertase cleaves complement component C3 into C3b, which is the main effector molecule of the complement system that can furtherly activate the complement system. Yanfei Yu et al. have revealed that *M. hyopneumoniae* could bind factor H via EF-Tu, which contributes to decreased C3 deposition on the *M. hyopneumoniae* surface, and ultimately blocks further complement activation. Meanwhile, many mycoplasmas, including *M. pneumoniae,* could hijack factor H via EF-Tu and then simulate host molecular to escape from complement attack [34] (Fig. 1d). In addition to helping mycoplasmas escape from complement killing, EF-Tu also strengthens adherence between mycoplasmas and tracheal epithelial cells (Fig. 1a).

Immunity Disorder

M. pneumoniae infection can cause innate immunity and adaptive immunity disorder in host. Stelmach et.al have shown that there were no increases of IgG, IgM, and IgA immunoglobulins in *M. pneumoniae* infected patients during a 1-year observation, which indicated immunity damage caused by *M. pneumoniae* infection. The levels of C3 and C4 increased significantly in the acute stage of infection, while immune suppression caused by *M. pneumoniae* infection could lead C3 and C4 to a normal or lower level in the later stage. In addition, *M. pneumoniae* infection induces pro-inflammatory cytokines and chemokines released in respiratory tract and activates variety of immune cells, causing T cells overactive exhausted and access to the verge of apoptotic progress [35]. Furthermore, adhesins and metabolites of *M. pneumoniae* can cause immune damage to respiratory epithelial cells lymphocytes, resulting in decreased activity and accelerated apoptosis of lymphocytes. Mainly, the decrease of $CD4^+$ function leads to the imbalance of immune function in patients infected by *M. pneumoniae*, ultimately causing antigen presentation disturbance, B cells maturation disorder, and the relative reduction of antibody production. The damage of respiratory system is further aggravated by disorders of humoral immunity, cellular immunity and innate immune system caused by *M. pneumoniae*.

Extrapulmonary Infection Mechanism

In addition to typical respiratory symptoms, *M. pneumoniae* can also cause some extrapulmonary complications. Importantly, extrapulmonary manifestations due to *M. pneumoniae* infection sometimes occur in the absence of pneumonia and even respiratory symptoms [36]. There is a myriad of extrapulmonary manifestations of *M. pneumoniae* infection that can potentially involve all systems and organs [2, 37]. The concomitant occurrence of mycoplasmaemia was obtained in the mycoplasmal central nervous system involvement, which proved *M. pneumoniae* could transfer to distant organs by blood transmission to cause disease. The extrapulmonary pathogenic mechanisms of *M. pneumoniae* can be divided into three parts: direct damage, indirect damage and vascular occlusion (Fig. 3). Early-onset extrapulmonary complications may be related to direct damage caused by the blood transmission of *M. pneumoniae*, while late-onset disease may be associated with indirect damage caused by autoimmunity, vascular damage and drug reaction.

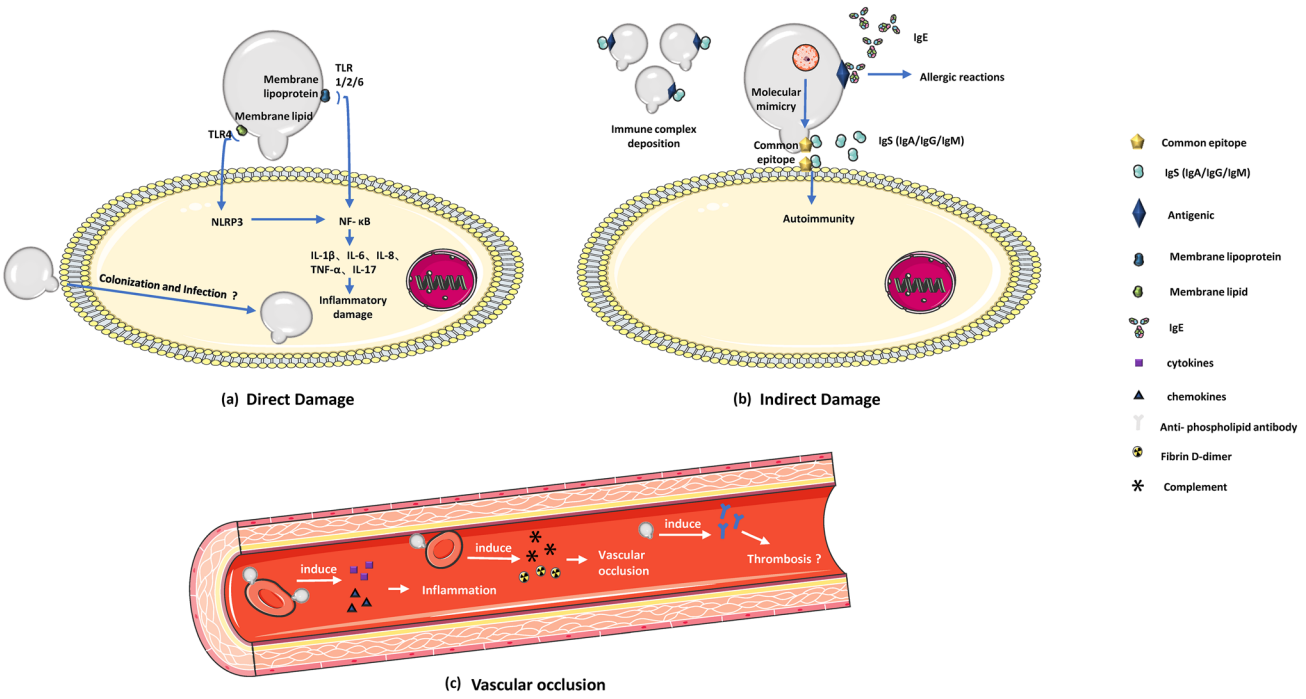

Fig. 3 Pathogenic mechanisms of *M. pneumoniae* extrapulmonary infection. **a** Direct invasion and inflammatory damage induced by cytokines lead to direct damage to host cells. **b** *M. pneumoniae* antigens mimic host cell components or cause changes in the structure of host cell membrane antigens to stimulate host autoimmunity. Immune complex deposition is considered responsible for *M. pneumoniae* extrapulmonary infection. Self-reactive IgEs promote the occurrence of allergic reactions and cause certain damage to tissue cells. **c** *M. pneumoniae* locally induces cytokines and chemokines to affect the vascular wall, causing vasculitis and thrombotic vascular occlusion through medical mediators (such as complement and fibrin D-dimer)

Direct Damage

M. pneumoniae existed in blood, pericardial fluid, synovial fluid and skin lesions by PCR and culture testing. Therefore, there is the possibility of direct invasion and damage in the extrapulmonary pathogenesis caused by *M. pneumoniae*. Some patients with an immature or damaged immune barrier on the respiratory tract surface are not sufficient to develop into pneumonia after *M. pneumoniae* infection, and the pathogen can be passively transferred into the circulation through weak gaps between injured lungs epithelial cells [38]. Since erythrocytes carry sialoglycoproteins, *M. pneumoniae* is of hemadsorption and has the ability to absorb to erythrocytes. Therefore, *M. pneumoniae* is considered to cause systemic infection after invading into the blood system (Fig. 4). The occurrence of *Mycoplasma* bacteremia constitutes a direct extrapulmonary manifestation.

There are two possible forms of direct injury, firstly, the direct *M. pneumoniae* invasion outside the respiratory tract (Fig. 3a). Since early-onset hepatitis sometimes develops in the absence of pneumoniae, it may be associated with a direct-type extrapulmonary manifestation [39]. *Mycoplasma pneumoniae* is speculated to directly colonize and infect liver epithelial cells, but this situation has not been proven. Secondly, inflammatory damage induced by *M. pneumoniae* (Fig. 3a). In tissues riched in cytokine-producing cells, the membrane lipoprotein of *M. pneumoniae* can induce local cytokines production which leads to inflammatory damage in tissues and organs [25]. According to reports, IL-17 is an important immune mediator in the systemic immune response, which may be related to the disease severity and extrapulmonary pathogenesis [40].

Indirect Damage

It has been hypothesized that recognition of *M. pneumoniae* by innate immune cells and consequent activation of the cells may be considered as main candidates to induce some serious *M. pneumoniae* complications [41]. Fink et al. using indirect immunofluorescence and PCR detection for serum IgM, IgA, IgG and cerebrospinal fluid (CSF) samples of patients with sudden neurological diseases. They found that the damage to the nervous system did not seem to be caused by direct invasion of *M. pneumoniae*, and most likely an immune response to infection. Immune-mediated mechanisms have been mainly implicated in *Mycoplasma pneumoniae*-related extrapulmonary diseases (MpEPDs) [6].

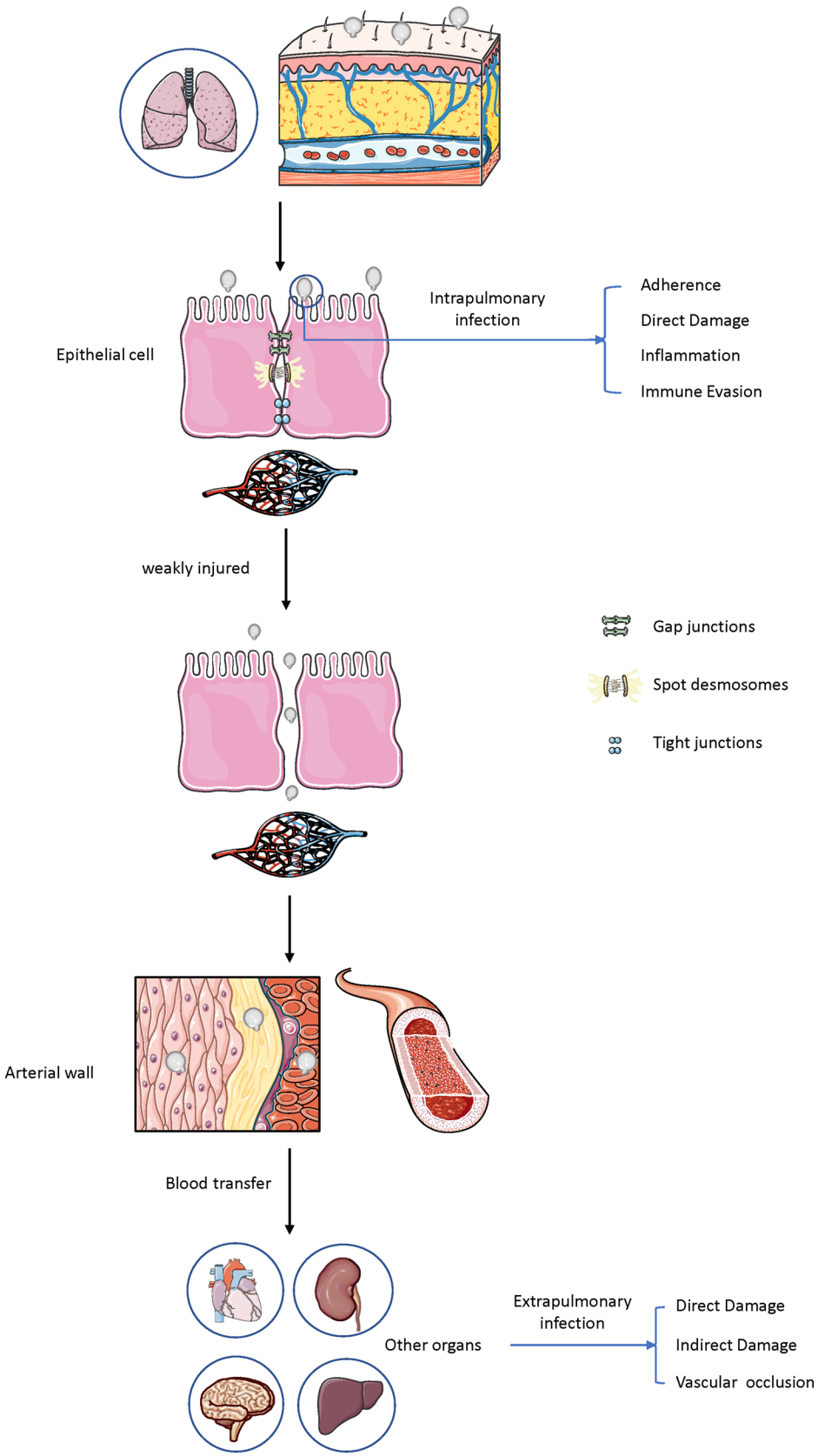

Fig. 4 *M. pneumoniae* can be passively transferred into the circulation through weak gaps between injured epithelial cells in some patients with an immature or damaged immune barrier on the respiratory tract surface and transfer to other organs by adhering to erythrocytes to cause extrapulmonary infection

Autoimmunity Caused by Molecular Mimicry

Mycoplasma pneumoniae antigen could mimic host cell components and cause shifts in the structure of host cell membrane antigens to activate auto-immune responses which forms immune complexes with corresponding organs to activate complements, producing neutrophil chemotaxis factors and C3a, C5a, C3b. A large number of white blood cells infiltrate the diseased site, release the hydrolase in the lysosome, cause destructive injuries and disease in multiple organs (Fig. 3b). For example, the P1 and P30 proteins on the attachment organelles of *M. pneumoniae* show high levels of homology to troponin, cytoskeletal proteins, keratin and fibrinogen of the host. Antibodies in response to *M. pneumoniae* infections target various host tissues and form immune complexes to cause damage in various tissues and organs such as liver, kidney, brain, smooth muscle and lungs [5]. Conclusively, Autoimmunity plays an important role in extrapulmonary complications caused by *M. pneumoniae.*

Immune Complex Deposition

In cases of *M. pneumoniae* infection complicated by acute nephritis and renal failure, there are reports that the genome of *M. pneumoniae* and immune complexes containing *M. pneumoniae* antigens have been detected in the glomerulus, it may be associated with excessive immune complexes deposition and complement activation in the tissues (Fig. 3b). Circulating immune complexes are responsible for the pathological mechanism of glomerulonephritis and IgA nephropathy related to *M. pneumoniae* infection.

Non-Specific Antibody

M. pneumoniae can activate B lymphocytes and produce non-specific polyclonal antibodies that are not directly against *M. pneumoniae*. The experiment of Sauteur et al. has shown that the level of serum antibodies against *M. pneumoniae* proteins and glycolipids arise in *M. pneumoniae*-infected children and mice. The equal recovery of serum antibodies level from *M. pneumoniae* infection in Btk-deficient (a mice species developed *M. pneumoniae*-specific IgG responses to *M. pneumoniae* proteins but not to *M. pneumoniae* glycolipids) and wild-type mice suggests that pulmonary *M. pneumoniae* clearance is mainly mediated by IgG reactive with *M. pneumoniae* proteins, and *M. pneumoniae* glycolipid-specific IgG or IgM is not essential [42]. Cold agglutinins, a kind of IgM antibodies, are produced in 50% patients infected by *M. pneumoniae* and may persist for several weeks. Cold agglutinins can be used to confirm clinical suspicions of primary atypical pneumonia caused by *M. pneumoniae*. One theory is that cold agglutinins are the result of cross-reactive autoantibodies developed against the *M. pneumoniae* glycolipid antigen and I antigen of human erythrocytes during acute *M. pneumoniae* infection. This non-specific antibody can cause auto-immune hemolytic anemia (cold agglutinin disease), which is the most famous indirect extrapulmonary manifestation caused by *M. pneumoniae* infection.

Atopy and Elevated IgE Levels

The body with atopy refers to an inherited tendency can produce IgE antibodies in response to small amounts of common environmental proteins [40]. Patel et al. observed 162 hospitalized children and found a significant increase in the total serum IgE level of children infected by MpEPDs, and it was significantly higher than that of children with only classic *Mycoplasma pneumoniae*-related respiratory illnesses, which indicates the existence of atopy in MpEPDs children [43]. The incidence of atopy in patients with extrapulmonary manifestations is higher than that of patients without extrapulmonary manifestations, so atopy may be associated with MpEPDs [40, 44]. Increased IgE levels are considered to be a sign of immune disorders. Self-reactive IgEs aggravate immune-mediated diseases and manifestations such as allergic reactions (Fig. 3b). For example, after infected by *M. pneumoniae*, the P1 protein could induce the production of P1-specific IgE in patients allergic to *M. pneumoniae*, and ultimately resulting in allergic symptoms and tissue damage [45]. Some patients who are prone to produce IgE, may be predisposed to develop extra-respiratory diseases associated with *M. pneumoniae* acute infections [46].

Vascular Occlusion

Extrapulmonary manifestations are not only directly related to the infection process and auto-immune, but also vascular complications. Thrombosis can appear in a vessel of any part of the body, pulmonary vessels are the most commonly involved sites, and accordingly chest pain was the most common symptom, followed by neurological symptoms and abdominal pain [47]. Jacobs et al. has reported a new case of pediatric priapism and proved that this symptom may be an extremely rare but reasonable type of vascular occlusion produced by *M. pneumoniae* infection.

The occurrence of extrapulmonary manifestations caused by vascular occlusion involves direct and indirect mechanisms. The direct type is that *M. pneumoniae* can be hematogenously transferred to distant organs, locally induced cytokines and chemokines (including TNF-α and IL-8) to affect the vascular wall, and eventually lead to local vasculitis or thrombosis without systemic hypercoagulability (Fig. 3c). The indirect type is systemic hypercoagulability through the activation of chemical mediators including

complement and fibrin D-dimer, which may result in thrombotic vessel occlusion (Fig. 3c).

Liu Jingwei has reported that some of the factors causing thrombosis are transient, and some are due to hereditary thrombophilia in patients with thrombosis caused by *M. pneumoniae* infection [48]. On the other side, some substances and phenomena have been suggested to increase the risk of thrombosis but transient, including cold agglutinin, vascular malformations, sickle cell trait, positive for anticardiolipin antibodies, β2-glycoprotein antibodies, lupus anticoagulant antibodies and anti-prothrombin antibodies. (Fig. 3c) [48, 49].

Others

The *M. pneumoniae* superantigen may be one of the factors of extrapulmonary manifestations. Superantigen produced by several bacteria could stimulate the production of a large number of T lymphocytes and lipid-related membrane proteins, leading to an uncontrolled immune response like the damage of Kawasaki disease [50].

Conclusion

Over the past few years, the understanding of the mechanisms by which *M. pneumoniae* causes intrapulmonary and extrapulmonary manifestations has gradually increased. The occurrence of related disease is the result of multiple pathogenic factors. Whether respiratory symptoms or extrapulmonary manifestations, although both are independent of each other, there are still similarities such as direct invasion, inflammatory damage and immune-mediated damage. The development of drugs targeting for the common pathogenic mechanism of intrapulmonary and extrapulmonary infection may support the *M. pneumoniae* infection prevention. For extrapulmonary complications caused by *M. pneumoniae*, more researches are required to construct a comprehensive treatment strategy including microbiology (antibiotics), hematology (anticoagulants) and immunology (immunomodulators). In addition, exploring alternative treatment options for macrolide antibiotics may improve the clinical symptoms of macrolide treatment failure. Vaccine development is the first choice for any infected disease control strategy. A safe vaccine that can provide protective immunity is essential to reduce *M. pneumoniae* infection. Coinfection with *M. pneumoniae* occurred in patients infected with other common respiratory pathogens. Clinicians managing patients with COVID-19 infection should be mindful of coinfections with *M. pneumoniae*, which may exacerbate clinical symptoms during this COVID-19 outbreak. To explore the synergistic mechanism between *M. pneumoniae* and other pulmonary pathogens may provide some strategies for prevention and treatment of *M. pneumoniae* co-infection.

Author Contributions JH drafted the original manuscript. YY modified the manuscript. XC and LX prepared a part of graphic and text materials. WX supervised the writing. PL conceived the idea. All authors have read and approved the manuscript.

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Declarations

Conflict of interest The authors have no conflict of interest to declare.

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