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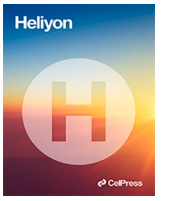

Review article

Enhanced insights into the neutrophil-driven immune mechanisms during *Mycoplasma pneumoniae* infection

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ABSTRACT

Mycoplasma pneumoniae (MP) infections represent a significant component of community-acquired pneumonia, especially in children, invoking a complex neutrophil-mediated immune response, crucial for host defense. This review consolidates current knowledge on the role of neutrophils in MP infection, focusing on their recruitment, migration and activation, as well as the molecular mechanisms underpinning these processes. Significant findings indicate that specific bacterial components, notably CARDS toxin and lipoproteins, intensify neutrophil recruitment via signaling pathways, including the IL-23/IL-17 axis and G-CSF. Furthermore, neutrophils engage in a series of responses, including phagocytosis, degranulation and NETosis, to combat infection effectively. However, dysregulated neutrophil activity can lead to exacerbated lung injury, highlighting the delicate balance required in neutrophil responses. Age and immunodeficiency also emerge as critical factors influencing the severity of MP infections. This review emphasizes the dual role of neutrophils in both defending against and exacerbating MP infections, suggesting that targeted therapeutic strategies could mitigate the adverse effects while enhancing beneficial neutrophil functions.

1. Introduction

MP, one of the smallest prokaryotic microorganisms capable of independent survival in inanimate media, is particularly remarkable for its unique absence of a cell wall. This distinctive characteristic not only sets it apart from bacteria, which possess cell walls, but also from viruses, which lack cellular structures altogether [1]. This unique characteristic not only influences its resistance to certain antibiotics but also plays a role in its pathogenicity [2,3]. In addition to causing respiratory disease, MP is associated with a variety of extrapulmonary infections. For example, it has been associated with the development of arthritis [4–6], diseases of the cardiovascular system [7,8], central nervous system infections and sometimes even with fatal consequences [9–11].

While tracheobronchitis remains the most frequently observed clinical manifestation of MP infection, *Mycoplasma pneumoniae* pneumonia (MPP) is the most significant clinical condition associated with MP infection [1,12,13], and it is a common form of community-acquired pneumonia in children, accounting for approximately 15–50 % of cases and up to 30–50 % of cases in epidemic

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years [1,14,15]. MP infections invade the respiratory tract and activate multiple immune cells to produce an inflammatory response [1]. Neutrophils are typically the first cells to extravasate into tissues in response to noxious stimuli and are highly responsive to a variety of stimuli, particularly pathogens and injury-associated molecular patterns [16]. These abundant leukocytes are the first line of defense against microbial threats, and once in the tissue, they drive inflammation and pathogen clearance by facilitating the recruitment of additional granulocytes and phagocytic monocytes, effectively controlling infection and maintaining mucosal integrity through processes such as degranulation reactions, oxidative bursts, and the release of neutrophil extracellular traps (NETs) [17,18]. During infection and homeostasis, neutrophils destroy pathogens and remove debris, and a rapid and effective response is necessary to promote wound healing [19,20]. However, excessive or dysregulated neutrophil responses have been implicated in the pathogenesis of multiple inflammatory conditions, autoimmune injury and obesity-related diseases [21–24]. Intriguingly, studies using animal models of MPP have shown that neutrophil depletion did not affect the number of MP in bronchoalveolar lavage fluid (BALF) but significantly reduce tissue damage, suggesting that neutrophils cause lung injury but play no role in MP clearance [25]. This paper aims to provide a comprehensive review of the latest advances in understanding the complex and multifaceted role of neutrophil-mediated immune responses during MP infection.

2. Neutrophil biology and immunological functions

Neutrophils represent a fundamental component of the innate immune system and are the predominant type of white blood cell found in peripheral blood, comprising approximately 50–70 % of all white blood cells. These cells are produced within the bone marrow and are noted for their short lifespan, typically enduring only a few hours to a couple of days in circulation [26]. Characterized by a segmented nucleus, neutrophils are also distinguished by their granules, which are rich in a variety of antimicrobial proteins and enzymes. These granules are crucial for the neutrophil's primary function: the engulfment and annihilation of pathogens through a process known as phagocytosis [20], a process where they engulf and digest pathogens within phagolysosomes using toxic enzymes

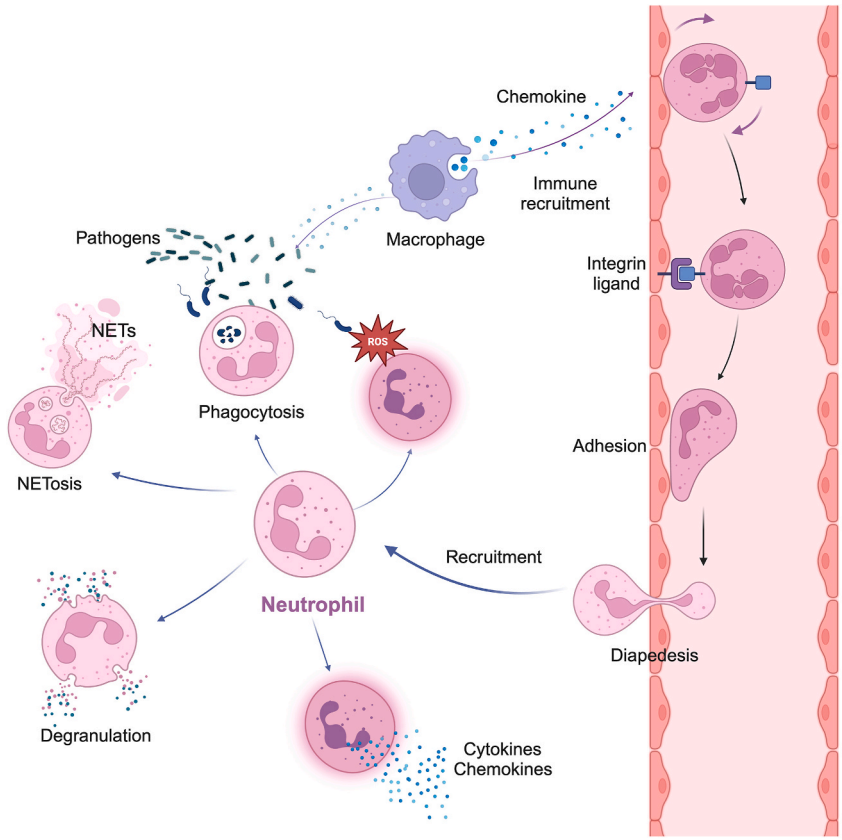

Fig. 1. Neutrophils biology and immunological functions. Neutrophils participate in phagocytosis, where they engulf and digest pathogens, as well as in degranulation, during which they release antimicrobial peptides and enzymes, such as myeloperoxidase (MPO), to combat infections. Neutrophils also produce reactive oxygen species (ROS), which play a key role in pathogen destruction but can cause tissue damage if not properly regulated. The recruitment of neutrophils to sites of infection or injury is a highly coordinated process involving chemotaxis, where macrophages release chemokines and cytokines that guide neutrophils towards the affected area. During this process, neutrophils interact with integrin ligands on the endothelial cells, which facilitate their adhesion and transmigration into tissues. Once at the site, neutrophils contribute to the immune response by releasing neutrophil extracellular traps (NETs) and further amplifying the inflammatory response.

and reactive oxygen species (ROS) [27]. Additionally, neutrophils engage in degranulation, releasing antimicrobial peptides, enzymes like myeloperoxidase (MPO), and other proteins that directly kill bacteria and fungi or modulate the immune response [28]. Another critical function is NETosis, where neutrophils expel their chromatin to form NETs that trap and kill pathogens extracellularly. However, excessive NETosis can contribute to tissue damage and immunopathology [29].

Immunologically, neutrophils are active participants in the modulation and resolution of inflammation (Fig. 1), swiftly migrating to sites of infection or tissue damage through chemotaxis. Research on the molecular mechanisms of neutrophil recruitment by pathogens, including MP, has shown that specific Pathogen components can trigger a robust neutrophil response. This response involves the adhesion of neutrophils to endothelial cells and their migration to the site of infection [30]. Upon arrival, they execute several key functions: (1) Phagocytosis: Engulfing and digesting pathogens. (2) Degranulation: Releasing granule contents to kill extracellular pathogens. (3) NETosis: A unique form of cell death in which neutrophils release NETs, composed of DNA and antimicrobial proteins, to ensnare and kill invading microbes [29]. As primary responders in inflammatory scenarios, neutrophils secrete cytokines and chemokines that recruit and activate other immune cells, thereby intensifying the inflammatory response. While this process is crucial for clearing infections, it may also lead to tissue damage if not meticulously regulated [31].

Beyond their established role in innate immunity, neutrophils also interact with components of the adaptive immune system. They can influence the function of T cells and B cells, contributing to the shaping of adaptive immune responses. For example, neutrophils can present antigens and produce cytokines that affect the differentiation and activation of adaptive immune cells [32]. This interaction underscores the versatility of neutrophils, highlighting their capacity not only to respond immediately to infections but also to modulate longer-term immune responses.

3. Neutrophil recruitment and migration in MP infection

Several clinical studies have shown that the proportion of peripheral blood neutrophils is increased in patients with MP infection and this increase is more pronounced in patients with severe MP infection [33–35]. Animal studies have also shown that MP infection leads to a strong early neutrophil response, as evidenced by significant neutrophil recruitment and infiltration in the lungs at 24 h after MP infection, a significant increase in the neutrophil ratio in BALF, and a large neutrophil infiltrate visible in lung histopathology. After MP infection, neutrophil migration and recruitment involve multiple signalling pathways [36–38].

In the healthy state, mature neutrophils are stored in the bone marrow through chemotaxis towards interleukin-4 (CXCL4), which is expressed on their surfaces, with stromal cell-derived factors in the bone marrow [39]. During infection or inflammation, locally produced granulocyte colony-stimulating factor (G-CSF) can inhibit the CXCL4 axis in neutrophils, thereby contributing to the migration of neutrophils from the bone marrow to the peripheral blood [5,40]. Increased levels of G-CSF are observed in both MP-infected patients and mice, suggesting that G-CSF may be associated with the migration of neutrophils from the bone marrow to the peripheral blood during MP infection [41,42]. However, the exact mechanism underlying the role of G-CSF in MP infection is poorly understood, and G-CSF knockout may help to determine the role of G-CSF in neutrophil recruitment after MP infection.

IL-23 expression and downstream signalling pathways play equally important roles in the recruitment of neutrophils to sites of inflammation. After MP infection, alveolar macrophages begin to secrete IL-23, which causes a significant increase in IL-23 content during early infection (4 h) [38]. IL-23 further stimulates T cells to secrete IL-17, which in turn promotes the production of the neutrophil-related chemokines KC and G-CSF [22,38,43]. Using an anti-IL-23p19 antibody to block the IL-23 signalling pathway in MP-infected mice, it was found that the expression of IL-17 was significantly reduced and that the recruitment of neutrophils to the lungs was significantly inhibited. Therefore, in acute MP infection, the production of IL-17 is dependent on IL-23 expression and contributes to the recruitment of neutrophils [38].

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is another important growth factor that plays a key role in neutrophil production and recruitment during MP infection [22]. Both GM-CSF and TNF-α alter neutrophil function [44,45]. These changes include increased lifespan, increased expression of major histocompatibility complex class II (MHC-II) and T cell costimulatory molecules, increased ROS levels in response to formyl-methionyl-leucyl-phenylalanine (fMLP), decreased chemotaxis towards fMLP and chemotaxis towards interleukin-8 (CXCL8) [21,44].

MP produces a community-acquired respiratory distress syndrome (CARDS) toxin with ADP-ribosylation activity and vacuolisation activity, and CARDS toxin levels in BALF correlate positively with the severity of lung disease in mice [46]. The recombinant CARDS toxin has been demonstrated to induce the release of inflammatory cytokines and chemokines, such as interleukin IL-1β, IL-6 and TNF-α, as well as vacuolisation and cytotoxicity of epithelial cells in mouse lungs. Notably, it induces neutrophil infiltration into BALF and is not dependent on TLR2 or IL-1α expression, which leads to lung injury [46]. Thus, CARDS toxin plays an important role in neutrophil infiltration and lung injury in MPP patients.

Mice were intranasally infected with MP in the presence of antibodies against CARDS toxin to determine the amount of MP in BALF and neutrophil infiltration due to MP infection. These results suggest that CARDS toxin promotes the persistence of MP and the infiltration of neutrophils after MP infection. Up to 10 % of the CARDS toxins were located on the MP surfaces [46]; therefore, it can be speculated that CARDS toxins located on the MP surfaces may promote the attachment and persistence of MPs on epithelial cells, thus promoting MP infiltration of TLR2-dependent neutrophils [46]. This finding has implications for the development of anti-MP therapies and vaccines. For example, therapies that can inhibit IL-1α and IL-12p40 signalling could help prevent the exacerbation of MPP. Therefore, designing an antibody and administering a vaccination using CARDS toxin as a vaccine antigen is a suitable approach.

MP lipoproteins induce exuberant neutrophil recruitment through IL-17A-driven keratinocyte-derived chemokine (KC) production [25]. The potent neutrophil chemotactic agent KC and expression of the inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-17A were significantly elevated in the BALF of an animal disease model inoculated with MP lipid-associated membrane proteins (LAMPs, which

contain lipoproteins and other transmembrane proteins), and a strong positive correlation existed between the proportion and number of neutrophils and the concentrations of IL-17A and KC; the strongest correlation was found with the severity of lung lesions [25]. Together with IL-1β and IL-17A, TNF-α induces the production of KCs, leading to neutrophil recruitment loop positivity [25], which results in immunopathological impairments such as localised inflammation, airway remodelling, airflow obstruction, emphysema and decreased lung function.

Galectin-3 is involved in the inflammatory response to the refractory MPP (RMPP) airway, which includes the adhesion and activation of neutrophils [47]. Galectin-3 levels in the BALF of children with RMPP in the acute phase were strongly positively correlated with the percentage of neutrophils, indicating that galectin-3 plays a role in promoting neutrophil lung tissue recruitment and intra-alveolar luminal transfer in the acute phase of RMPP due to MP infection [47]. During pneumonia in galactose-3-deficient mice, neutrophils can aggregate in the interstitial tissue of the lung but cannot migrate to the alveolar cavity, thus reducing the immune and inflammatory responses of neutrophils [47]. Moreover, galectin-3 can cross-link cell surface glycoproteins and activate a variety of innate immune responses, such as respiratory bursts in neutrophils [47]. Taken together, these findings indicate that galectin-3 is involved in the airway inflammatory response in RMPP and is closely associated with neutrophil inflammatory site chemotaxis and luminal infiltration.

4. Neutrophil-mediated immune response in MP infection

The study found that the phagocytosis of MP by neutrophils can induce their apoptosis. Additionally, it suggests that neutrophil infiltration may contribute to the proliferation of MP following neutrophil degeneration [48]. Neutrophil-mediated immune inflammation requires a careful balance and although neutrophils survive for a long time in vivo, the phagocytosis of MPs can promote neutrophil degeneration [48]. When co-incubated with MP and A549 cells, neutrophil proliferation was accelerated in the high-to-medium granulocyte ratio group, neutrophil function was decreased, and extracellular IL-1β levels were increased in a time- and dose-dependent manner in neutrophils [48]. These results suggest that the release of intracellular nutrients from damaged cells and decreased neutrophil function can promote MP infection and play a role in activating the inflammatory response. Thus, lung injury and neutrophil infiltration are important factors influencing the development of MPP [48]. A summary of the experimental and clinical objective evidence for neutrophil-mediated immune responses to MP infection is presented in Table 1.

4.1. Secretion of granule proteins

Myeloperoxidase (MPO) is a key granule protein of neutrophils. In lung infections, activated neutrophils eliminate invasive pathogens by generating abundant ROS/nitrogen species while delivering MPO and other antimicrobial enzymes to the phagosome [49]. MPO has antimicrobial functions. Neutrophils with MPO deficiency exhibit normal maturation, migration and phagocytosis but exhibit antimicrobial defects. However, when MPO production exceeds the limits of local antioxidant defense protection, oxidative stress and local immune-inflammatory damage injury can occur [50]. A population study revealed that MPO expression was significantly greater in the BALF of children with acute MPP infection than in the BALF of control children, and MPO expression decreased during the recovery period. In addition, MPO was positively correlated with the duration of fever and hospitalisation [35], suggesting that the degree of MPO expression elevation may be correlated with the severity of the disease. The MPO activity in the lungs of MPP mouse model increased, whereas no significant difference in the copy numbers of MP in BALF was observed after immunisation inhibition of mice with cyclophosphamide; however, lung injury was reduced, as was MPO activity in lung tissue [36]. These findings suggest that abnormally elevated MPO may be related to MPP-induced lung injury [36].

4.2. Secretion of antimicrobial peptides

Upon MP infection, neutrophils secrete cathelin-related antimicrobial peptide to inhibit MP multiplication. LL-37 is the C-terminal peptide of human cathelin-related antimicrobial peptide and cathelin-related antimicrobial peptide (CRAMP) is the cathelin-related antimicrobial peptide isoform in mice. *In vitro* studies revealed that CRAMP has antimicrobial activity against MP, and coculture with MP and 10–20 μg/mL CRAMP resulted in a 100- to 1000-fold reduction in MP colonisation [51]. In the MPP population, LL-37 expression was elevated, and LL-37 levels were significantly greater in the severe pneumonia subgroup than in the mild disease subgroup. In an animal model of MP infection, the expression of the CRAMP protein in neutrophils was significantly increased in BALF, while the CRAMP concentration in the supernatant was 10-fold greater in the BALF of MPP patients than in the control group after 5 h of direct stimulation of neutrophils with MP [52]. The above study suggests that cathelin-related antimicrobial peptides play an antimicrobial role in MP infection and that neutrophils are the main source of cathelin-related antimicrobial peptides in MP-infected BALF.

4.3. S100A8/A9 release

In a population-based study, the protein profile of BALF from children with MPP was examined, and S100A8/A9 was found to be highly expressed; moreover, both BALF and serum samples showed elevated levels of neutrophil-releasing S100A8/A9, and serum levels of S100A8/A9 were significantly greater in children with MPP than in children without MPP and in children with viral and bacterial pneumonia [53]. Similarly, animal experiments showed that S100A8/A9 induced apoptosis in human alveolar basal epithelial cells in a concentration-dependent manner, and this apoptosis may be related to the onset and development of MPP. Further

Table 1
Neutrophil-mediated immune response in MP infection.

Author	Type of Study	Objective	Method	Conclusion	Reference
Mara AB et al.	Experimental	To explore the immune response triggered by MP membrane lipoprotein vaccination and whether it leads to vaccine-enhanced disease through IL-17A-driven neutrophil infiltration	Animal experiments, using MP membrane lipoprotein vaccination to analyse immune response and IL-17A expression changes post-vaccination, assessing neutrophil recruitment and its relationship with vaccine-enhanced disease	The vaccination caused IL-17A-driven neutrophilia, leading to enhanced inflammatory response and triggering vaccine-enhanced disease	[25]
Chen Z et al.	Experimental	To explore the role of the IL-8/neutrophil axis in the pathogenesis of pneumonia during MP infection	Laboratory study to evaluate the role of IL-8 production and neutrophil recruitment induced by MP infection in the development of pneumonia	MP infection plays a key role in pneumonia pathogenesis through IL-8-mediated neutrophil recruitment, suggesting that this pathway may be a potential therapeutic target in the future	[35]
Shi S et al.	Experimental	To explore the role of immunosuppression in lung injury induced by MP infection and to evaluate MPO as an indicator of neutrophil activity in the process of lung injury	Mouse model simulating MP infection, using immunosuppressants and measuring MPO activity to assess neutrophil activity and its effect on lung injury	Immunosuppression reduced neutrophil-mediated inflammatory responses and lung injury by lowering MPO activity, suggesting that MPO could be a therapeutic target for regulating neutrophil activity in MP infections	[36]
Wu Q et al.	Experimental	To investigate the role of IL-23-dependent IL-17 in neutrophil recruitment and activity, and its importance in immune defense against MP infection	Animal experiment, using gene knockout technology to block IL-23 and IL-17, and infecting with MP through airway perfusion to study lung immune response	IL-23 promotes the production of IL-17, regulating neutrophil recruitment and activity, and enhances lung defense against MP infection in mice	[38]
Tamiya S et al.	Experimental	To explore the role of neutrophils in lung injury after MP infection through both TLR2-dependent and TLR2-independent pathways	Using a mouse model, evaluating TLR2-dependent IL-1α and IL-12 p40 production, and the effects of TLR2-independent CARDS toxin on lung injury	Neutrophil-mediated lung injury occurs via two mechanisms: TLR2-dependent IL-1α and IL-12 p40 production and TLR2-independent CARDS toxin action. Both pathways play important roles in MP-induced lung injury	[46]
Zhao Q et al.	Clinical	To explore the role of neutrophils in the prognosis of MP infection across different age groups	Analysis of neutrophil functional changes in patients with MP infection across different age groups and their relationship with disease prognosis	Age significantly affects neutrophil function, particularly its ability to respond to infections, leading to poor prognosis in older patients	[49]
Wang M et al.	Clinical	To explore the characteristics of refractory MPP in children, especially the role of neutrophils in inflammation and their relationship with disease severity	Analysis of laboratory data and clinical presentations of hospitalized children, focusing on the correlation between neutrophil count and disease severity	Neutrophils play a key role in the immune pathology of refractory MPP in children. High neutrophil counts are associated with severe inflammation and disease	[50]
Banov L et al.	Clinical	To explore the clinical manifestations and outcomes of MP-induced pneumonia in children with cancer and neutropenia	Study of the impact of MP infection on children with cancer and neutropenia, and its pathological manifestations in immunodeficient patients	MP infection can lead to severe pneumonia in neutropenic children, with immunocompromised patients showing a poor response to infection	[87]
Bai S et al.	Experimental	To explore the role of IL-17 in stimulating neutrophils to release S100A8/A9 proteins in MP-induced pneumonia in children and its effect on lung epithelial cell apoptosis	In vitro and animal model experiments analysing how IL-17 stimulates neutrophil-mediated S100A8/A9 release, leading to lung epithelial cell apoptosis in MP infection	IL-17 promotes lung epithelial cell apoptosis through neutrophil-released S100A8/A9 proteins, playing an important role in MP-induced pneumonia and potentially serving as a future therapeutic target	[53]
Yamamoto T et al.	Experimental	To explore the role of Mpn491 in NETs and its effect on MP evasion of immune killing	Using gene knockout technology to analyse the role of Mpn491 in degrading NETs, combined with studies on neutrophil interaction with MP and NETs formation and degradation	Mpn491 degrades NETs, helping MP evade neutrophil-mediated killing, indicating its key role in bacterial survival and persistent infection	[59]
Mu D et al.	Clinical	To explore early predictors of airway mucus, plug formation during MP infection, especially the role of neutrophils	Analysis of inflammation indicators such as changes in neutrophil/lymphocyte ratio, combined with clinical characteristics for early prediction	Early changes in neutrophils can serve as a predictor of bronchial mucus plug formation in MP infection, providing a basis for early intervention	[63]

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Table 1 (*continued*)

Author	Type of Study	Objective	Method	Conclusion	Reference
Schaunaman N et al.	Experimental	To explore the role of ST2 in neutrophil recruitment and its impact on airway inflammation and immune response during MP infection	Using mouse models and in vitro cell experiments to evaluate the function of ST2 during MP infection, particularly its effect on neutrophil recruitment and inflammatory response	ST2 plays a key role in neutrophil-mediated inflammatory responses during MP infection, suggesting this pathway as a potential target for treating airway inflammatory diseases	[67]
Li D et al.	Clinical	To explore NLR as a predictor of poor prognosis in patients with MP infection	Analysis of the relationship between NLR levels and disease severity and prognosis in patients with MP infection	Elevated NLR levels are significantly associated with increased severity and poor prognosis of MP infection, indicating its potential as a prognostic biomarker	[88]
Xu X et al.	Experimental	To explore the role of histamine released by neutrophils in the inflammatory response during MP infection and its impact on pathological inflammation	Using a mouse model to induce MP infection, assessing the effect of neutrophil-released histamine on inflammatory response, lung injury, and immune response	Neutrophil-released histamine plays a key role in promoting the inflammatory response after MP infection. Histamine regulation may become a potential therapeutic target for reducing inflammatory lung injury	[89]
Kuwahara M et al.	Clinical	Investigate the role of neutrophils in MpEPDs, particularly in neurological disorders	Retrospective clinical analysis of patients with neurological complications post-MP infection and laboratory testing for anti-glycolipid antibodies	Neutrophils contribute to systemic inflammation, leading to extra-pulmonary effects like neurological disorders, and anti-glycolipid antibodies were linked to neurological symptoms	[83]
Medina JL et al.	Experimental	Investigate how CARDS toxin elicits an immune response during MP infection, focusing on neutrophil involvement and IgE production	Laboratory study using mice exposed to CARDS toxin. Immune responses, including neutrophil activity and IgE production, were analysed	CARDS toxin induces a noticeable immune response, attracting neutrophils and producing IgE, which triggers a neutrophil-driven inflammatory response. Neutrophils are involved in the inflammatory reactions caused by CARDS toxin, including those in MpEPDs	[86]

IL: Interleukin; MPO: Myeloperoxidase; TLR: Toll-like Receptor; CARDS: Community Acquired Respiratory Distress Syndrome; G-CSF: Granulocyte-Colony Stimulating Factor; NETs: Neutrophil Extracellular Traps; ST2: Suppressor of Tumorigenicity 2; NLR: Neutrophil/Lymphocyte Ratio; MpEPDs: Extrapulmonary manifestations of *Mycoplasma pneumoniae* infections.

studies showed that upregulated expression of S100A8/A9 proteins was involved in the IL-17 signalling pathway and that IL-17A induced the release of S100A8/A9 proteins from neutrophils [53]. Therefore, S100A8/A9 could be used as a new biomarker for clinical differential diagnosis as well as a therapeutic target in paediatric MPP [53].

4.4. Oxidative respiratory damage

The process by which neutrophils are stimulated to consume oxygen and produce superoxide anions and hydrogen peroxide through NADPH oxidase is known as an oxidative respiratory burst [54]. Superoxide anions and hydrogen peroxide play important roles in antimicrobial resistance, and excessive oxidative respiratory bursts can lead to immune damage associated with oxidative stress. An oxidative respiratory response can be induced in neutrophils during MP infection [55]. Oxidative respiratory damage and lung antioxidant capacity was assessed by detecting malondialdehyde (MDA) levels and superoxide dismutase (SOD) respectively, the MPP mouse model exhibited significantly greater MDA levels and lower SOD levels, after inhibition of neutrophil function with cyclophosphamide, lung tissue MDA levels were lower than those in mice with normal neutrophil function, while SOD levels were increased [36]. These findings suggest that neutrophils are involved in the pathogenesis of MPP through oxidative respiratory damage.

4.5. Secretion of the neutrophil extracellular trap network

NETs are reticular tissues released by neutrophils with a DNA backbone and a variety of active proteins attached. NETs are mainly derived from neutrophil granules, such as elastase, myeloperoxidase, calreticulin, antimicrobial peptides and defensins[3,4]. The release of NETs can be activated either by endogenous stimuli such as damage-associated molecular patterns (DAMPs) or by microbial pathogen-associated molecular patterns (PAMPs) [56].

NETs can capture and kill a variety of pathogens, including bacteria, viruses, fungi and parasites, but an abnormally increased abundance of NETs can also cause immune damage [57,58]. The level of cfDNA, a marker associated with NETs, is significantly greater in the peripheral blood of children with MPP than in that of healthy children, suggesting a role for NETs in treating MPP [52]. In *in vitro* experiments, MP directly stimulated neutrophils to produce NETs, but MP could secrete the extracellular nuclease Mpn491 to degrade

NETs and thus evade neutrophil immune killing. The powerful immune escape mechanism of MP against NETs could protect MP from the capture and killing of the NET reticulum [59], suggesting that NETs play a more immune-damaging role in MPP. In addition, NETs can upregulate the secretion of the inflammatory factor IL-1 through the enhancement of IL-8 secretion, thereby eliciting an inflammatory response, among other processes. The serum levels of the proinflammatory factor IL-8 in children with MP were significantly positively correlated with the percentage of neutrophils due to the selective upregulation by NETs of the IL-1 family cytokine response in bronchial epithelial cells produced by neutrophils, which enhances the production of the proinflammatory cytokine IL-8 and exacerbates immune damage [60].

5. Neutrophil and airway hypersecretion states

Under normal conditions, mucus not only serves as an airway barrier but also helps mucosal cilia to remove harmful substances or metabolites; persistent mucus hypersecretion leads to airway obstruction [61], and airway hypersecretion is also present in MP [62–65]. Several clinical studies have shown that an elevated peripheral blood neutrophil ratio is an early indicator of the formation of respiratory mucus plugs in children with MPP [62–65]. The mechanism by which elevated neutrophil ratios cause mucus plugs is poorly understood and may be related to the release of elastase from neutrophils. Elastase can increase mucin expression by activating protein kinase C and reactive oxidation products, which activate TGF-α-converting enzymes, promoting TNF-α release and epidermal growth factor receptor phosphorylation [66].

6. Pathways that promote the neutrophil inflammatory response

The IL-23/IL-17 axis is associated with MP-induced neutrophil-associated lung inflammation. In a mouse model of acute respiratory MP infection, lung IL-23p19 mRNA was significantly upregulated during early infection (4 h), and it was found that IL-23 induced IL-17 production in activated $CD4^+$ T cells and was involved in host defense against MP [38]. MP significantly upregulated the expression of the IL-17, IL-17C and IL-17F genes in lung tissues; however, IL-23 neutralisation had no effect on MP-induced lung IL-17C mRNA expression. These results suggest that IL-17/IL-17F production in acute MP infection is IL-23-dependent and the IL-23/IL-17 axis may act as an interface between innate and adaptive immunity in lung defense against MP (e.g., neutrophil recruitment and activation) [38].

In vitro, S100A8/A9 treatment significantly increased apoptosis in human alveolar basal epithelial cells. Bioinformatics analysis indicated that the upregulated S100A8/A9 protein was also involved in the IL-17 signalling pathway [53]. The elevated expression levels of S100A8/A9 in the BALF and serum of children with MPP may be due to IL-17-induced neutrophil release [53].

By using an ST2-deficient mouse model of MP infection, it was demonstrated that ST2 is essential for MP-induced lung neutrophil inflammation and that ST2-deficient mice have significantly lower neutrophil counts 24 h post infection (early stage of infection) and elevated levels of the host defense protein lactoferrin in BALF [67]. It was shown that ST2 plays a deleterious role in acute MP infection, including promoting inflammation [67].

The data suggest that ST2 increases the production of proinflammatory cytokines involved in neutrophil recruitment in MP-infected lungs and that the overexpression of ST2 in human epithelial cells leads to an increased load of MPs in cell supernatants and BALF, exacerbating the proinflammatory response in airways (e.g., neutrophils), and that blocking ST2 signalling broadly attenuates airway infection and inflammation [67]. It is unclear whether ST2 is associated with chronic or recurrent exposure to MP infection.

A study showed a significant increase in neutrophil TNF-α production after neutrophil stimulation with MP [68]. To verify whether MP-stimulated neutrophils produce TNF-α through increased TLR2 expression, the cell surface expression of TLR2 and TLR4 in MP-stimulated neutrophils was examined using flow cytometry. The results showed that TLR2 expression was significantly increased in neutrophils stimulated by MP compared to that in the uninfected group, while TLR4 expression was not significantly changed. RT-qPCR was used to determine TLR1, TLR2, TLR4 and TLR6 expression in MP-stimulated peripheral blood neutrophils. The mRNA expression of TLR2 was significantly greater in the MPP group, and the TLR1 and TLR2 mRNA expression levels were significantly greater in the RMPP group than in the MPP group [68].

Neutrophil TLR2 expression is upregulated after MP infection, and neutrophil TNF-α secretion may occur primarily through increased TLR2 production [53,68]. Although adhesion to epithelial cells is the first step in the pathogenesis of MP and epithelial cells secrete TNF-α, the development of pulmonary inflammation and systemic hyperinflammation may be mediated mainly by the inflammatory cascade response of neutrophils under co-stimulation with MP and TNF-α [68].

7. Effects of age on neutrophil function

An appropriate neutrophil response is essential for host defense, and age-related changes in neutrophil function may be one reason why young children and older adults are more susceptible to community-acquired pneumonia (CAP) [69]. It has been found that older patients exhibit an increase in the number of neutrophils through an increase in G-CSF during acute inflammation following MP infection. In addition, neutrophil activation can be influenced by promoting IL-8 secretion and is associated with adverse outcomes in patients with MPP [49]. A population-based study revealed that age and neutrophil counts were significantly greater in the RMPP subgroup than in the non-RMPP subgroup and that there was a linear correlation between age and the percentage of neutrophils in RMPP patients [49]. Further analyses revealed that neutrophil function was more active in older children with MP, and neutrophil apoptosis, nicotinamide adenine dinucleotide phosphate, mitochondrial function and oxidative stress levels varied among the different

age groups of MP-infected children [49].

Based on the conversion of human age to mouse lifespan, 3-week-old weanling and 8-week-old adolescent mice were selected for the establishment of an MPP model, and increased numbers of lung infiltrative neutrophils were observed in histopathological sections from 8-week-old MPP group mice compared with those from the 3-week-old MPP group of mice. Moreover, the levels of MDA, MPO and NETs were significantly increased in the lung homogenates of 8-week-old mice infected with MP. In addition, the levels of indicators related to lung injury, such as the ratio of the BALF protein concentration to the serum concentration and the dry-to-wet weight ratio of lung tissue, were significantly greater in the infected group than in the 3-week-old MP-infected group. Neutrophils isolated from aged humans also showed enhanced respiratory bursts and NET production [49]. Consequently, age-related aberrant infiltration and activation of neutrophils in the lung tissues of mice following MP infection resulted in varying degrees of lung injury. Age also correlates with the prognosis of MPP, and the increased counts and enhanced activation of neutrophils in children under 9 years of age could explain the difference in susceptibility to MP infection according to age [49].

8. Impact of MP infections in immunodeficient patients with neutrophil defects

There is no specific evidence that children with primary or secondary neutropenia or neutrophil defects are more susceptible to MP infection [70]. However, Research indicates that children with immune deficiencies, especially those impacting neutrophil function like Leukocyte Adhesion Deficiency (LAD), may face a higher risk of severe outcomes from MP infections. These patients are prone to recurrent severe infections which can be difficult to manage due to the inability of neutrophils to effectively phagocytose and kill MP, leading to prolonged infection and inflammation [53,71]. Severe manifestations can include refractory pneumonia that does not respond well to standard treatments [72].

MP infections in immunodeficient patients can lead to various complications, including extrapulmonary manifestations such as arthritis, skin eruptions and neurological symptoms. These complications are more frequent and severe in patients with compromised neutrophil function [73].

It's also crucial to consider the co-infection rates and the increased susceptibility to bacterial infections among these patients, which complicate the clinical picture and may require more intensive treatments and interventions [74]. These patients often present with atypical symptoms, and the standard diagnostic tests may not be as effective. Treatment is complicated by the need to balance managing the infection with the risk of exacerbating the underlying immunodeficiency [36]. The increased disease burden of MP in children with neutrophil defects necessitates heightened vigilance and tailored management strategies.

9. The neutrophil-mediated immune mechanisms in MpEPDs

The extrapulmonary manifestations of *Mycoplasma pneumoniae* infections (MpEPDs) are largely mediated by immune mechanisms rather than direct bacterial invasion. These can be classified into three categories: (1) direct local inflammation caused by cytokine production, Cytokines like IL-1, IL-6 and TNF-α play significant roles in triggering systemic inflammation, leading to damage in organs like the central nervous system, skin, heart, and kidneys (2) immune modulation such as autoimmunity or immune complex formation, These immune responses trigger damage across various organ systems. For example, neurological complications like encephalitis and Guillain-Barré syndrome (GBS) are often associated with antibodies against glycolipids like galactocerebroside, while conditions such as Stevens-Johnson syndrome (SJS) and vasculitis are linked to immune complex deposition and cytokine-mediated endothelial dysfunction. These mechanisms often manifest even in the absence of respiratory symptoms [73,75].

Neutrophils play a critical role in driving the extrapulmonary manifestations of MP infection. Not only do they directly engage in immune defense by phagocytosing and killing pathogens, but they also influence disease progression indirectly by modulating inflammatory responses and affecting the function of other immune cells. However, the overactivation of neutrophils can lead to immune-mediated tissue damage and systemic inflammation, impacting various extrapulmonary organs such as the heart, liver, and nervous system. This complex immune response mechanism may result in multiple organ dysfunction syndrome, making the extrapulmonary manifestations of MP more severe and difficult to control.

The specific mechanisms by which neutrophils mediate the MpEPDs involve several key aspects. First, M. pneumoniae can trigger a strong inflammatory response, including the activation and migration of neutrophils, facilitated by the production of inflammatory cytokines such as IL-1, IL-4, IL-6, and TNF-α, which further promote neutrophil recruitment and activation [76–79]. Moreover, the formation and deposition of immune complexes can lead to neutrophil aggregation and activation, intensifying inflammation and causing tissue damage [80–82]. Neutrophils also contribute to direct cellular damage, as M. pneumoniae invades host cells, causing their damage and death. This process may be driven by neutrophil-released enzymes and reactive oxygen species [78].

In some cases, hypersensitivity reactions such as drug allergy responses can occur, with neutrophils being activated and contributing to tissue damage, which may be linked to immune responses triggered by M. pneumoniae infection [81,82]. Additionally, neutrophils play a role in vasculitis and thrombosis by releasing inflammatory mediators and damaging vascular endothelial cells, leading to these vascular complications [73,83]. In neurological extrapulmonary manifestations, neutrophils participate in the inflammatory response, affecting the nervous system by releasing cytokines and chemokines [83–85]. Finally, M. pneumoniae infection may promote a Th2-driven immune response, involving neutrophils in the activation and regulation of immune cells, particularly in allergic and inflammatory diseases [86].

Thus, neutrophils have a dual role in MP infection: while they are essential in pathogen defense, their overactivation can result in significant organ damage. This multifaceted involvement of neutrophils in systemic inflammation, immune-mediated tissue damage, autoimmune reactions, and vascular lesions underscores their importance in the severity of extrapulmonary manifestations of MP

infection. Monitoring neutrophils and related indicators is, therefore, crucial for assessing disease severity and guiding clinical treatment.

10. Conclusion

In conclusion, the recruitment and migration of neutrophils in MP infection involve intricate and multifaceted molecular mechanisms that are vital for the host's defense against pathogens. The research highlights those specific bacterial components, such as the CARDS toxin and lipoproteins, significantly enhance neutrophil recruitment through various signaling pathways, including the IL-23/IL-17 axis and G-CSF. Neutrophils, as the first line of defense, undergo adhesion, migration and activation processes, producing reactive oxygen species, antimicrobial peptides and NETs to combat the infection. However, the balance of neutrophil activity is crucial, as excessive or dysregulated responses can lead to tissue damage, exacerbating lung and systemic injury, including vasculitis, thrombosis, and neurological complications. Additionally, factors such as age and immunodeficiency further influence the severity of MP infections, highlighting the need for tailored therapeutic strategies. Understanding these molecular pathways and their implications can inform the development of targeted treatments and vaccines, offering improved management and prognosis for patients with MP infections.

Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Lu Fan: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Nuo Xu:** Visualization, Validation. **Yun Guo:** Writing – review & editing, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Ling Li:** Writing – review & editing, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no competing interests.

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