Review

Recent Progress in *Mycoplasma pneumoniae* Infection

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Abstract

Mycoplasma pneumoniae (Mp) is a definite respiratory pathogen affecting people of all ages. This organism is adsorbed on host cell surface through extreme adherent organelles, generating peroxide ions and possible exotoxins. Mp may directly invade host cells and cause latent infections. Induced immunoreactive injury is one of the main factors resulting in clinical symptoms of Mp infection. Polymerase chain reaction (PCR) and serological detection should be combined for diagnosis of Mp infection when nasopharyngeal and oropharyngeal specimens are simultaneously obtained and detected by PCR. The most reliable basis for diagnosis can be obtained with two serological tests in different courses of the disease. Culture methods also bear significance in diagnosing Mp infections and *in vitro* drug sensitivity tests.

Currently, more than 200 species of *Mycoplasma* are known. However, only several are pathogenic for humans. Among these species, *Mycoplasma pneumoniae* (Mp) is the most studied. However, further explorations are required to determine the manner of Mp interaction with host cells, its effects on the immune system, and extrapulmonary diseases that it causes. Currently, Mp acts as a definite respiratory pathogen affecting people in all age groups, and its compatibility with host cells is considered the main virulence factor. To date, controversies surround whether Mp can parasitize cells and cause latent infection. Autoimmune and extrapulmonary expression rates induced by Mp infection reaches up to 25% in infected populations [1]. Mp lacks standardized *in vitro* drug-sensitivity test. Currently, multiple investigation reports are available on mutations of macrolide drug-resistance gene. Compared with macrolide-sensitive Mp strain infections, people infected with drug-resistant Mp strain present significantly prolonged stage of effervescence. Mp infection outbreaks occur every four to seven years; inactivated Mp vaccine can reduce 40% of Mp-related respiratory tract infections [2].

Mp pathogenic cell and molecular basis

Interaction between Mp and host cell is achieved by an

adsorbed organelle, which comprises P1 protein containing 170 ku, high molecular weight (HMW) 1, 2, and 3, and proteins P90, P40, and P30. HMW is responsible for formation and maintenance of adhesive structure with extreme protrusions, enabling the entire Mp to form flask-like extensions. Proteins B, C, and P1 form adhesive complex and bind with host cell receptors, such as sialic acid glycosyl conjugates, glycoproteins, and sulfated glycolipids. Successful adherence to respiratory tract epithelium of host is a prerequisite for Mp-induced infection. Epidemiological studies showed that P1 of Mp features at least two major genotypes, and changes in P1 may be related with generation of genotype-specific antibodies, short duration of immunity, and clinical recurrence of infections. Successfully adhered Mp produces superoxide anions, making host cells susceptible to peroxide damage by inhibiting host catalase. HPr kinase plays a major regulatory role in Mp infection. Mp degrades phospholipids in host cell membrane to obtain glycerol. Glycerol activates HPr kinase to activate HPr protein, thereby producing peroxide and superoxide anions and subsequently leading to host cell damage, which is mainly manifested as disappearance of respiratory epithelial cell cilia, vacuolization, decreased sugar utilization, amino acid uptake, and synthetic ability manifested as persistent dry cough in clinical practice. Mp may become pathogenic by synthesizing and releasing exotoxins [3]. Mp genome-

wide scans showed a protein that is highly homologous to diphtheria bacillus toxin subunit S1, and it specifically binds to surface-active protein A. Synthetic protein function demonstrates that this molecule exhibits adenosine diphosphate ribosyltransferase activity with potential exotoxin effects. Thus, the protein was named community-acquired respiratory distress syndrome toxin. However, host cell receptor of this protein remains undiscovered, and in terms of toxin function, the role of its specific properties in binding with surface-active protein A remains unclear. Mp does not invade host cells, but genomic scans indicated that Mp lacks some genes that are essential for independent living; this condition is similar to that of strict intracellular parasites [4]. Although laboratory analysis showed Mp growth and reproduction in cells, and clinical findings also revealed Mp chronic or latent infections, requirement of long-term treatment, and other phenomena associated with intracellular parasite infections, further studies are required to clarify mechanism of Mp parasitism in natural circumstances.

Congenital and adaptive immune response

When Mp reaches the lower respiratory tract, it is phagocytosed by activated macrophages through antibody and complement conditioning. Neutrophils and lymphocytes also show chemotaxis toward infected sites. Duration of immune response induced by Mp infection is short, and secondary infection is very common. Some individuals become long-term carriers. Considering that congenital hypoglobulinemia patients are prone to chronic mycoplasma respiratory tract infections or extrapulmonary mycoplasma-disseminated infections, humoral immunity bears significance in controlling Mp. Through P1 adhesion unit, Mp binds to sialic acid receptor in mastocyte membrane and induces mastocytes to generate interleukin (IL)-4 [5]. Mp-derived lipoproteins can bind to Toll-like receptor on macrophage membrane and activate macrophages to generate tumor necrosis factor α , IL-8, and IL-1 β . Intensified cell-mediated immune response results in increased production of cytokines, more serious clinical symptoms of lung injury, and possible autoimmune response. Up to 25% of Mp-infected persons may develop extraperitoneal manifestations because of autoimmune reactions, which include the skin, nerves, cardiovascular system, kidney, gastrointestinal tract, skeletal muscle, and hematopoietic systems. Neurological

complications are the most common as Mp cell membrane contains several glycolipids and liposaccharide antigens, which can form cross antigens with neuronal components. Antiganglioside GM1 antibody and galactose brain glycoside antibody were detected in Mp-related encephalitis [6], along with increased cerebrospinal fluid cytokines IL-6 and IL-8. Mp nucleic acid was detected by nucleic acid hybridization in brain tissues. Mp antigen was immunohistochemically detected in brain tissues. Mp was detected in cerebrospinal fluid using polymerase chain reaction (PCR) and culture [7]; all results suggest that Mp not only can pass through immune-damaged cells but also spread infection to the central nervous system. Evidence of Mp infection was reported in PCR or culture in blood, synovial fluid, pericardial fluid, and skin lesions. Thus, immune damage and direct invasion of Mp may cause extrapulmonary manifestations of Mp infection.

Function of Mp in asthma and chronic lung disease

Mp infection is sometimes difficult to treat and causes chronic clinical processes that play certain roles in adult rheumatoid arthritis, adolescent idiopathic arthritis, Crohn's disease, and asthma, because of the presence of host cell infections, immunoregulatory effects, and surface antigen variations [8]. Compared with healthy controls, asthmatic people present significantly higher detection rate of Mp, whereas macrolide antibiotics significantly improve lung function in Mp-infected asthma patients; enduring Mp infection was observed during long-term monitoring of children with impaired respiratory function; experiments also showed that Mp-induced inflammatory mediators, such as IgE, substance P, neurokinin 1, and IL-5, can aggravate asthma; all these observations indicate that Mp is pathogenic in asthma [9].

Mp laboratory testing

Mp culture identification requires up to six weeks. Serological experiments aid in crowd epidemiological investigation. However, such methods feature weak reliability for individual diagnosis. Thus, effective clinical diagnosis of Mp remains inadequate. Specific antibodies are detected within one week after Mp infection, peak within three to six weeks, and then reduce afterward. Increase in serum antibodies in acute and recovery stages is reliable for diagnosis of Mp

infection. However, this variable cannot be used for early diagnosis. IgM or IgA detection aids early diagnosis, but a positive proportion exists among healthy people. She *et al.* ^[10] performed investigations on culture of 24,677 cases of respiratory tract Mp specimens, among which 10 cases were positive (0.04%). PCR results of 8,509 respiratory tract Mp specimens showed 167 positive cases (1.9%). IgM antibody detection of 92,507 serum Mp specimens detected 6,049 positive cases (6.6%). Considering low Mp culture rate of clinical specimens, PCR combined with serological detection may serve as feasible diagnostic method. Nasopharyngeal and oropharyngeal specimens simultaneously obtained for PCR may provide reliable basis for diagnosis. For sputum patients, positive rate of PCR in sputum samples is higher than that in nasopharyngeal specimens and can well reflect infections in the lower respiratory tract ^[11]. Although culture method features poor sensitivity, it manifests excellent specificity. Especially for Mp infections manifested outside the lungs, culture positivity is the best evidence for direct invasion of Mp. Clinical isolates can also be used for further genotyping and drug resistance analysis. Gnarp *et al.* ^[12] observed that pharyngeal carriage rate of Mp reaches 4.6%–13.5% in healthy people. Thus, for patients who test positive in PCR and negative in serology, asymptomatic carrier, antibodies not produced by immune deficiency, or early successful inhibition of Mp by antibiotics should be considered. For patients who test negative in PCR and positive in serology, existence of substances contained in specimen and inhibits PCR material or immune memory should be considered.

Mp macrolide antibiotic resistance and drug-sensitivity test

Under normal circumstances, Mp is sensitive to macrolides, tetracyclines, and quinolones antibiotics. In cases of mutations in Mp 23S rRNA peptide transferase-binding region, macrolide exhibits decreased binding ability to Mp ribosome. Reported Mp 23S rRNA point mutations include A2063G, A2064G, and C785T. Liu *et al.* ^[13] reported that among 55 cases of clinical isolates in Shanghai, 44 cases (83%) were resistant to macrolide, and all resistant strains showed A2063G 23SrRNA mutation. Xin *et al.* ^[14] reported that among 50 cases of clinical isolates, 46 cases (92%) were resistant to macrolide; among these cases, 40 contained 23S rRNA A2063G; one case featured A2063C, and five cases presented A2064G. Suzuki *et al.* ^[15] reported that compared

with macrolide-sensitive Mp strains, stage of effervescence was significantly prolonged in people infected with drug-resistant Mp strains. However, no serious outcomes, such as respiratory failure, occurred, possibly because pathogenicity of Mp is based on immune injury, and macrolide drugs posed immunomodulating effects. Miyashita *et al.* ^[16] reported two cases of adult macrolide-resistant Mp-induced pneumonia, and Lung *et al.* ^[17] reported one case of severe macrocytosis caused by macrolide-resistant Mp in children. As tetracycline is an antimicrobial agent, and wide use of quinolones may probably result in resistant strains with single-point mutations, and as Mp infection occurs mostly in children, macrolide drugs are still the first choice of treatment. Currently, clinical practice requires development of reliable and reproducible Mp drug-susceptibility test. However, no standardized Mp *in vitro* susceptibility test is available because of long incubation period and harsh cultivation conditions for Mp and lack of definite standard test strains for minimal inhibitory concentrations.

Summary

In the future, studies on Mp infection may focus on the following aspects: development of standardized Mp *in vitro* culture and drug-sensitivity test, discovery of new Mp virulence factors through genome scanning and protein identification, and exploration of congenital and adaptive immunity of hosts to Mp infection.

Declarations

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No.

Competing interests

The author declare that he has no competing interest.

Authors' contributions

AQ Zhong made the literature analysis and wrote, discussed and revised the manuscript of this review.

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