MYCOPLASMA PNEUMONIA[1,2]

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Introduction

In the late 1930's and early 1940's, a group of pneumonias that could be distinguished clinically from typical pneumococcal pneumonia was described (1, 2). This syndrome was designated primary atypical pneumonia (PAP) and it could not be associated with either bacterial or influenza virus infection by the laboratory methods available at that time. In 1943, it was first observed that many patients with the syndrome of primary atypical pneumonia developed cold agglutinins during the course of their illness (3). Epidemiological studies revealed that cold agglutinin pneumonia was a distinct entity. Although individuals with such illness did not exhibit a uniform clinical pattern, it was observed that cold agglutinin-positive pneumonia had a longer incubation period and a more severe and prolonged course than did cold agglutinin-negative pneumonia (4). Volunteer studies performed during World War II by the Commission on Acute Respiratory Disease demonstrated that the etiologic agent of cold agglutinin-positive primary atypical pneumonia was filterable (5).

In 1944, Eaton reported the isolation of an agent from patients with PAP (6). This agent was recovered in embryonated eggs that had been inoculated with filtered sputum from patients with PAP. The agent could be passed serially in embryonated eggs, but it did not produce a pathologic effect in this host; however, material from successive egg passages produced pneumonitis in cotton rats and hamsters. In addition, cotton rats and hamsters inoculated directly with filtered sputum from PAP patients developed pneumonitis. This effect could not be serially passaged in these animals. All of these experiments were hindered by the failure of all of the inoculated animals to develop pneumonia and by the presence of indigenous pneumonia-producing viruses in these animals. Using the techniques just described, the Eaton agent was shown to have a size of 180 to 250 mμ by gradacol membrane filtration and to be inhibited by chlortetracycline and streptomycin (6, 7, 8).

In 1957, it was shown that specific Eaton agent antigen could be localized in the bronchial epithelium of infected chick embryo lung by immunofluorescence (9). This technique made it possible to quantitate the organism and

[1] The survey of the literature pertaining to this review was concluded in September 1965.

[2] The following abbreviation will be used: PAP (primary atypical pneumonia).

also to detect and quantitate specific antibody. Further studies demonstrat that the Eaton agent could be propagated in primary chick embryo yolk s and monkey kidney tissue cultures (10, 11, 12).

For many years, the Eaton agent was thought to be a virus. The inhibiti of this organism by chlortetracycline and streptomycin made it less like that it was a true virus. Subsequently, it was observed that coccobacilla bodies could be visualized on the mucous layer of infected chick embr bronchial epithelium and that these bodies contained specific antigen th could be detected by immunofluorescence (13, 14). In addition, stain Eaton agent-infected tissue cultures contained extracellular "colony-lik structures and these structures corresponded to areas of specific immun fluorescence (12). These findings suggested that the organism might be mycoplasma. The identity of Eaton agent as a mycoplasma was establish when it was successfully propagated on a cell-free agar medium (15). T morphologic, histochemical, and nutritional properties of the agar-gro organism indicated that it was a member of the genus *Mycoplasma*. T identity of the agar-grown mycoplasma with the Eaton agent was establish by immunofluorescence, using specific rabbit sera and paired sera from p tients with Eaton agent pneumonia. Recently, the Eaton agent was giv the name *Mycoplasma pneumoniae* to connote its relationship to pneumon (16).

The findings from the earliest *M. pneumoniae* studies suggested that th organism was associated with PAP (6). The agent was recovered most oft during the early phase of illness and from patients who developed co agglutinins. Neutralization tests performed in hamsters as well as immun fluorescent studies provided serologic confirmation that patients with co agglutinin-positive PAP were infected with *M. pneumoniae* (17, 18). Despi these findings, some reservations were expressed concerning the relationsh of this organism to PAP.

Controlled epidemiologic investigations of naturally occurring respirato disease in two separate populations have provided convincing evidence th *M. pneumoniae* is a human respiratory tract pathogen. In studies utilizi the fluorescent antibody technique, serologic evidence of infection was d tected significantly more often among patients with moderate-to-seve respiratory tract disease than among comparable individuals free of respir tory disease (19, 20).

Volunteer studies provided the final proof that *M. pneumoniae* can cau respiratory disease in man (21, 22, 23). Twenty-six per cent of 69 voluntee without detectable fluorescent-stainable antibody developed a febrile respir tory disease, with or without pneumonia, following experimental infecti with this organism. A significant proportion of the infected individuals d veloped cold agglutinins. Volunteers with naturally acquired fluorescen stainable antibody for the agent did not develop febrile respiratory illne when challenged with this organism.

Biologic Properties

M. pneumoniae shares a number of properties with the other mycoplasma species that infect man (*M. hominis* type 1, *M. salivarium*, *M. orale* type 1, *M. orale* type 2, and *M. fermentans*). *M. pneumoniae* can reproduce in a cell-free medium (15). The organism is fastidious in its nutritional requirements. It requires cholesterol and native protein for growth (24, 25). Colonial growth on agar is characteristic of the genus *Mycoplasma* in that colonies grow predominantly beneath the surface of the agar (15). The smallest reproductive units are 125 to 150 mμ in diameter (6, 26, 27, 28). Thus, *M. pneumoniae* and the other mycoplasmas are the smallest free-living microorganisms. *M. pneumoniae* is highly pleomorphic since it lacks the rigid cell wall that surrounds bacteria (28, 29). Instead, it is bounded by an external triple-layered "unit" membrane (28, 29). The organism is absolutely resistant to penicillin, but is readily inhibited by the tetracyclines (30). In contrast to most bacterial species and consistent with the properties of other mycoplasmas, growth of *M. pneumoniae* can be inhibited by specific antibody (31). *M. pneumoniae*, like other mycoplasmas, appears to be an independent entity. There is no convincing evidence that this organism, or any other mycoplasma, is derived from or reverts to a parental bacterial form.

M. pneumoniae is unique among the human mycoplasma species in producing complete lysis of certain mammalian red cells within 24 to 48 hours (32, 33, 34). Circular areas of complete (beta) hemolysis develop when agar plates containing colonies are overlaid with a 3 per cent suspension of guinea pig red cells in agar. In addition, when erythrocytes are added to an agar plate containing colonies of *M. pneumoniae*, the cells are adsorbed directly onto the mycoplasma colonies (35). Subsequently the adsorbed erythrocytes lyse. *M. pneumoniae* is the only human mycoplasma species which adsorbs erythrocytes. *M. pneumoniae* is also unique among the human mycoplasma species in reducing tetrazolium under aerobic conditions (36).

M. pneumoniae has more fastidious nutritional requirements than *M. hominis* type 1, *M. salivarium*, and *M. fermentans*. In addition to cholesterol and native protein, *M. pneumoniae* requires a heat-stable dialyzable factor present in fresh yeast extract for optimum growth (15, 37). *M. orale* type 1 and *M. orale* type 2 also require this factor for growth (38–40).

The importance of *M. pneumoniae* as a human pathogen has stimulated study of the antigenic structure of the organism. Chemical and chromatographic fractionation have yielded protein, lipid, and polysaccharide antigens (42). Most of the serologically reactive antigens are present in the lipid fractions (43, 44). There are at least three lipid fractions, separable by thin-layer chromatography, that possess complement-fixing activity (43). In addition to their complement-fixing activity, these lipid components appear to be involved in indirect hemagglutination and the growth inhibition phenomenon. Proteins also appear to be necessary components of the antigens

that attach to tanned erythrocytes in the indirect hemagglutination test. The most immunogenic antigens appear to be lipoprotein complexes which stimulate complement-fixing, indirect-hemagglutinating, and growth-inhibiting antibodies (43). Agar gel diffusion studies, using antigens derived from sonically disrupted suspensions of *M. pneumoniae*, have failed to demonstrate any antigenic relationship between *M. pneumoniae* and other human mycoplasma species (45). Of all the human mycoplasmas studied by this method, *M. pneumoniae* appears to be the most antigenically distinct.

Ecology, Epidemiology, and Clinical Characteristics of *Mycoplasma pneumoniae* Infections

In man, the effects of *M. pneumoniae* infection range from inapparent infection to upper respiratory illness to bronchopneumonia. The majority of infections apparently do not result in clinical pneumonia and it has been estimated that only 3 to 10 per cent of infected individuals develop pneumonia (20, 21, 22). In addition to respiratory tract involvement, volunteers have been noted to develop myringitis, often bullous, following experimental infection with *M. pneumoniae* (21, 22). Although *M. pneumoniae* infection has been demonstrated in patients with naturally occurring myringitis, controlled epidemiologic studies are needed before a definite etiologic association between *M. pneumoniae* and myringitis can be considered established (46).

In both the civilian and military populations studied, it has been observed that *M. pneumoniae* infections occur throughout the year, generally with increased rates during the fall and early winter (17, 19, 32, 47). Epidemics of *M. pneumoniae* illness have been described only in military populations. These epidemics are generally of long duration and do not have a sharp epidemic profile. *M. pneumoniae* appears to spread slowly throughout the civilian population. Fluorescent-stainable, growth-inhibiting, and indirect hemagglutination antibodies are usually not detected during infancy (32, 48). The proportion of persons with such antibodies increases slowly with increasing age (48, 49). These antibodies are found most frequently in individuals in the third and fourth decades (48, 49).

Generally, spread is most efficient within small epidemiologic units, such as the family or the military training platoon. Intensive and prolonged exposure of susceptible persons to infected individuals appears to be necessary for transmission of infection. Such conditions are present during military recruit training (20, 50). In addition, military training provides a large population of susceptibles. Similar environmental conditions apparently are also present in prison populations since 90 per cent of federal prison inmates were found to possess *M. pneumoniae* antibody (23). College populations also offer the special conditions which favor the spread of this organism (51).

M. pneumoniae appears to be an important cause of pneumonia in the civilian population. Pneumonia caused by *M. pneumoniae* occurs most frequently during late childhood and during the second and third decades; how-

ever, the organism can cause pneumonia in persons of almost any age (47, 51–55). In a recent study in the Seattle, Washington area, 20 per cent of 215 patients of all ages with a diagnosis of nonbacterial pneumonia were infected with *M. pneumoniae* (47). Studies in Great Britain, Finland, and Sweden have implicated this organism as the cause of between 10 per cent to 33 per cent of all pneumonic illnesses (53, 54, 55).

M. pneumoniae was associated with 8 to 39 per cent of the pneumonias that occurred in three military recruit populations (20, 32, 50). Serologic evidence of *M. pneumoniae* infection was also demonstrated in 26 per cent of 69 cold agglutinin-negative pneumonia illnesses which occurred among military personnel and their dependents at various army camps over a 12-year period (49). In an eight-year study, 22 per cent of University of Wisconsin college students with pneumonia developed antibody to *M. pneumoniae* (51).

All estimates of the importance of *M. pneumoniae* infection as a cause of pneumonia should take into account the wide fluctuations in prevalence of this organism which can occur in any given area or population. During the past 20 years, many observers have commented upon the cyclic occurrence of cold agglutinin-positive, primary atypical pneumonia. Recently, specific serologic methods have been used to document this fluctuation in the prevalence of *M. pneumoniae* infection in two populations. During the years 1957–1959, 10 per cent of pediatric lower respiratory illness at the Children's Hospital of Washington, D.C., was associated with *M. pneumoniae* infection, while during 1962–1964, only 1 per cent of similar patients had serologic evidence of such infection (19, 56). In 1959, 67 per cent of the Marine recruits studied at Parris Island, S.C., had serologic evidence of *M. pneumoniae* infection. Although there have been no detectable changes in environment or in the methods of recruit training, the incidence of serologically positive pneumonia decreased to 7 per cent in 1963 (20, 57). The reasons for these wide fluctuations remain obscure at the present time.

There is no pathognomonic clinical syndrome that permits the diagnosis of *M. pneumoniae* infection. Individual cases of pneumonia caused by *M. pneumoniae* cannot be differentiated from pneumonia of other etiology. Generally, *M. pneumoniae* pneumonia has a longer incubation period and duration and is more severe than viral pneumonia. The diagnosis of *M. pneumoniae* infection can be made with certainty only by employing specific serologic techniques or by recovering the organism. In two studies of laboratory-proven *M. pneumoniae* pneumonia, fever, cough, headache, chills, and general malaise were present in over 70 per cent of the cases (47, 58). Nasal symptoms consisting of rhinorrhea and nasal obstruction developed early in the course of disease in 40 to 50 per cent of the patients. Rales were present upon physical examination in 50 to 80 per cent of the patients. Pharyngeal erythema and cervical adenopathy were present in a smaller percentage of patients. Most patients did not have any abnormalities of the peripheral leukocyte or differential count on urinalysis. Chest X rays usually revealed

minimal-to-moderate unilateral patchy pneumonitis in the lower half of the lung. Upper lobe involvement or bilateral lower lobe involvement occurred in approximately one quarter of the patients. The course of pneumonia in untreated individuals was variable. The clinical course in most individuals was characterized by a prolonged illness with persistence of abnormal chest X-ray findings, cough, and rales. Fever, headache, and malaise disappeared in most instances between the third and tenth day after onset of symptoms. The resolution of chest X-ray findings, cough, and rales proceeded at a slower rate and usually occurred between 7 and 21 days after onset. In about 50 per cent of *M. pneumoniae* pneumonias, progression of pulmonary infiltration occurred between the fourth and sixteenth day of hospitalization and 22 per cent of the patients had abnormal chest X rays four weeks after onset (58). These pulmonary infiltrates eventually resolved over the next four to six weeks. In young adults, *M. pneumoniae* pneumonia is rarely fatal. In only three instances has the organism been recovered from the lung at postmortem (9, 59). Prolonged illness with residual pleural abnormality due to *M. pneumoniae* infection has been reported (60). Other reported complications of *M. pneumoniae* infection include erythema nodosum, pericarditis, otitis externa, tympanitis, meningoencephalitis, pityriasis rosea, and interstitial emphysema (47, 58). These complications have been reported infrequently and their association with *M. pneumoniae* infection has not been firmly established.

Diagnosis

Isolation of M. pneumoniae *from clinical specimens.*—The agar medium consisting of seven parts Difco PPLO agar, two parts unheated horse serum, and one part 25 per cent fresh yeast extract, which was used in the first successful cultivation of *M. pneumoniae*, has been found to be an efficient system for the recovery of *M. pneumoniae* from naturally and experimentally infected individuals (61). Agar cultures are incubated at 34 to 37° C either aerobically or in an atmosphere of 95 per cent nitrogen and 5 per cent carbon dioxide. Most *M. pneumoniae* isolates are characterized by their slow growth in agar, and the characteristic homogeneous granular "mulberry" appearance of the colonies. Most *M. pneumoniae* colonies lack the light peripheral zone which characterizes colonies produced by the other mycoplasmas found in the oropharynx. The rapid beta hemolysis of guinea pig red blood cells by *M. pneumoniae* colonies, as well as hemadsorption by these colonies, are useful properties for presumptive identification. The identity of isolates can be confirmed in a number of serological tests, using specific antisera to *M. pneumoniae*. The tests most commonly used for this purpose are growth inhibition, complement fixation, and specific fluorescent staining of colonies.

Because of the time required for the microscopic observation of agar plates for mycoplasma colonies, other isolation methods have been developed. Recently a diphasic medium consisting of both agar and broth was found to be an efficient system for the isolation of *M. pneumoniae* (47). Since

M. pneumoniae ferments glucose, glucose and phenol red were added to the diphasic medium. The growth of *M. pneumoniae* was visualized by the change in the color of the medium during incubation. This color change represented a change in pH due to fermentation of glucose. The addition of methylene blue to this diphasic medium has been found to inhibit the growth of oropharyngeal mycoplasmas other than *M. pneumoniae*, and thus has aided in the isolation of this organism (36). Confirmation of the identity of isolates from diphasic media require the specific serologic methods described above.

Serologic methods.—A rise in cold agglutination antibody titer was the first serologic method employed in the diagnosis of PAP (3). Following the isolation and identification of *M. pneumoniae*, it was observed that all individuals infected with the organism did not develop cold agglutinins during convalescence (20, 47, 62, 63). In addition, not all cold agglutinin responses were associated with *M. pneumoniae* infection. In two recent studies approximately one half of individuals with laboratory proven *M. pneumoniae* pneumonia developed a fourfold or greater rise in cold agglutinins during convalescence (47, 62). However, in the same studies, approximately 15 per cent of patients with pneumonia not associated with *M. pneumoniae* infection showed a similar increase in cold agglutinins. Therefore, a cold agglutinin response constitutes only presumptive evidence of *M. pneumoniae* infection.

Patients with *M. pneumoniae* infection also develop agglutinins for streptococcus MG, a nonhaemolytic streptococcus which is a normal inhabitant of the human oropharynx (64). Generally, a rise in streptococcus MG agglutinins occurs less often than does the development of cold agglutinins. It has been suggested that *M. pneumoniae* represents an L form of streptococcus MG (65). However, such a relationship could not be confirmed when the two organisms were compared serologically (66). Furthermore, recent studies utilizing the DNA homology technique have failed to reveal any relationship between these two microorganisms (67).

Prior to the identification of *M. pneumoniae* as a mycoplasma, immunofluorescence was the standard method for diagnosing infection caused by this organism. The technical difficulties involved in this test precluded its wide spread application to sero-diagnostic and sero-epidemiologic studies. Following the successful cultivation of *M. pneumoniae* in broth cultures several other serological tests became feasible (68).

Infected broth cultures contain complement-fixing antigens, which are in part separable from the intact organism by centrifugation. The antigens involved in complement fixation are lipid and are soluble in ether or chloroform (43, 44). Purified lipid antigens can be used in the complement fixation test.

Sonically disrupted organisms can be used to sensitize tanned sheep erythrocytes. These erythrocytes can then be used in the indirect hemagglutination test (IHA) to measure *M. pneumoniae* antibody (39, 69). Antibody to *M. pneumoniae* can also be measured by several growth inhibition techniques. In the tetrazolium reduction inhibition (TRI) test, inhibition of

growth of *M. pneumoniae* is indicated by the failure of tetrazolium to be reduced (70). In the fermentation inhibition (FI) test, inhibition of growth by antibody is indicated by the failure of the organism to metabolize glucose and thus change the pH and color of the phenol red indicator (71).

Immunofluorescence with chick embryo lung antigen remains the most efficient technique for the serodiagnosis of *M. pneumoniae* infection (68, 72). Complement fixation or IHA are approximately 80 per cent as efficient as immunofluorescence (72). Although the IHA technique measures higher levels of antibody than immunofluorescence, it is less efficient for the serodiagnosis of *M. pneumoniae* infections since many naturally infected individuals possess IHA antibody at the onset of symptoms. The TRI and FI tests have not yet been extensively evaluated in the serodiagnosis of naturally occurring *M. pneumoniae* infections, but preliminary information suggests that they may be useful for this purpose (70, 71, 72). Antibodies measured by growth inhibition or immunofluorescence (using chick embryo lung sections) correlate well with resistance to experimentally induced *M. pneumoniae* illness (72).

Therapy

The use of tetracycline drugs in the therapy of primary atypical pneumonia has been the subject of much controversy since 1954. Several studies demonstrated a therapeutic effect of these drugs on cold agglutinin-positive PAP patients (73, 74). Other workers failed to demonstrate a beneficial effect of these drugs in PAP. In retrospect, it is probable the variable results of tetracycline treatment can be related to differences in the proportion of patients infected with *M. pneumoniae* in the various studies (62, 73). In a study of serologically positive *M. pneumoniae* illnesses 0.9 g of demethylchlortetracycline daily for six days significantly reduced the duration of fever, cough, malaise, and rales when the demethylchlortetracycline-treated patients were compared with a similar group of patients who received a placebo (62). In addition, antibiotic treatment markedly accelerated the clearing of pulmonary infiltration. These clinical findings are in agreement with the known *in vitro* sensitivity of *M. pneumoniae* to tetracycline (7, 30).

Prophylaxis

A preliminary investigation involving experimental infection of 50 human volunteers suggested that propagation of *M. pneumoniae* in a cell-free medium, either agar or broth, resulted in attenuation of this organism for man (23). These experiments suggest that it may be possible to develop a live attenuated vaccine for prevention of *M. pneumoniae* respiratory disease.

Progress has also been made in the development of an efficacious and safe inactivated *M. pneumoniae* vaccine. Recently, an experimental *M. pneumoniae* vaccine was developed (75). The preparation of this vaccine was dependent on the development of a new culture medium for the organism. In this new medium, a chloroform extract of egg yolk and a chemically defined

salt-amino acid-vitamin solution was substituted for the highly allergenic horse serum and beef infusion broth constituents of the standard medium. The experimental inactivated vaccine elicited the development of growth-inhibiting antibodies in rabbits and monkeys. In addition, vaccinated hamsters developed resistance to multiplication of *M. pneumoniae* in their lungs. Preliminary tests for antigenicity of this vaccine in man have yielded encouraging results. The majority of injected individuals developed growth-inhibiting antibodies following two injections of the vaccine. In addition, in a study in which vaccinated volunteers were experimentally challenged with *M. pneumoniae*, evidence was obtained that vaccine-induced antibody provided protection against respiratory disease caused by *M. pneumoniae* infection (76).

Current Problems

Following the delineation of primary atypical pneumonia as a distinct clinical entity, it was noted that patients convalescing from this syndrome developed a variety of antibodies. These included cold agglutinins, antibodies to streptococcus MG, and antibodies to extracts of mouse and human lung (2, 64, 77). These antibodies develop in addition to, and are not directly related to, specific *M. pneumoniae* antibodies. It is possible that these "nonspecific" antibodies represent an autoimmune response and that infection with *M. pneumoniae* modifies normal host constituents, thus rendering them antigenic. Recent studies on the properties of the hemolysin of *M. pneumoniae* suggest a mechanism which may explain the genesis of these "nonspecific" antibodies. The hemolysin of *M. pneumoniae* has been demonstrated to be a peroxide (78). This peroxide could act on the surface of erythrocytes, altering their antigenic structure. The altered erythrocytes could then serve as antigens and thus stimulate autoantibodies. Such a mechanism could also explain the hemolytic anemia that occurs in a small proportion of cold agglutinin-positive pneumonias (79). Similar phenomena are thought to be involved in the etiology of several autoimmune hematologic disorders (80). In addition to its action on erythrocytes, the peroxide hemolysin of *M. pneumoniae* might alter the antigenic nature of other host cells and thus cause them to stimulate the formation of autoantibodies. If these "nonspecific" antibody responses to *M. pneumoniae* infection do represent autoimmune responses, mycoplasma infection should be sought as the initiating event in other autoimmune diseases.

The identification of the Eaton agent as a mycoplasma has stimulated the investigation of the role of other human mycoplasmas as possible etiologic agents of nonbacterial pneumonia in man. In a recent study, serologic evidence of infection with the DC 63 strain of *M. hominis* type 1 was detected in 3 per cent of 346 pneumonia patients studied (52, 81). This represented significantly more infection than that detected in a comparable group of control patients without respiratory disease. In addition, experimental infection of human volunteers with the DC 63 strain of *M. hominis* type 1 produced

afebrile exudative pharyngitis in a significant proportion of individuals who lacked antibodies for the organism (81). This study suggests that *Mycoplasma hominis* type 1 may also cause respiratory disease in man.

Conclusion

M. pneumoniae is the first mycoplasma that has been shown conclusively to produce disease in man. The identification of this organism as a mycoplasma has made possible the application of new serologic and microbiologic techniques to the diagnosis of primary atypical pneumonia. In addition, effective prophylaxis through immunization is now feasible. The role of other mycoplasmas in human diseases, long a subject of controversy, is now receiving increased and intensive investigation.

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