Age-Related Prevalence of Complement-Fixing Antibody to 
*Mycoplasma pneumoniae* During an 8-Year Period

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We determined the age-related prevalence of complement-fixing antibody to *Mycoplasma pneumoniae* from the computerized laboratory results of our routine diagnostic department. The material consisted of about 58,000 sera from an 8-year period, 1971 to 1978. Among children less than 1 month old, the frequency of complement-fixing antibody of titers ≥8 was low (23%), and no decrease representing loss of maternal antibody was seen thereafter. An unexpectedly early increase in the antibody prevalence up to 62% was seen by 6 months of age. The frequency of antibody was high among young children from the age of 4 months, in whom symptomatic infection due to *M. pneumoniae* is rare. The frequency of higher titers (≥32) and the geometric mean titer increased more slowly, coinciding with the known age distribution of symptomatic *M. pneumoniae* disease: the frequency of high titers and the geometric mean titer peaked at the age of 7 to 10 years. Two explanations for the high frequency of complement-fixing antibody to *M. pneumoniae* in young children are discussed. It may be due to an asymptomatic infection caused by *M. pneumoniae* or to a nonspecific stimulus by lipids of other organisms, plants, or tissues leading to production of antibodies cross-reacting with *M. pneumoniae*, or it may be due to both of the above. During the study, two extensive epidemics due to *M. pneumoniae* occurred in Finland, but the prevalence of complement-fixing antibody bore no correlation with these peaks of occurrence.

The age distribution of symptomatic illness caused by *Mycoplasma pneumoniae*, one of the most common causative agents of pneumonia, is peculiar, with the incidence being highest among school-age children and young adults (14, 15, 21; G. Biberfeld, Ph.D. thesis, Karolinska Institutet, Stockholm, 1971). Thus, *M. pneumoniae* pneumonia is commonest in the age groups with the lowest total incidence of pneumonias (14). However, several studies have revealed that even infants and preschool children often have antibodies to *M. pneumoniae* (10, 15, 19, 21, 32, 33). The frequency of antibodies varies widely according to the test used. By sensitive mycoplasmacidal and radioimmunoprecipitating antibody tests, Brunner et al. (4) showed an unexpectedly high frequency of mycoplasmal antibodies among young children hospitalized in Washington, D.C., although when measured by the metabolism inhibition test, the frequency was low.

In this paper, we describe the age-related prevalence of complement-fixing (CF) antibody to *M. pneumoniae* obtained from the computerized results of some 58,000 screening tests performed at our routine diagnostic laboratory during an 8-year period, with emphasis on the frequency of the antibody among infants and young children and its age and titer distributions during the different years.

**MATERIALS AND METHODS**

**Patients and sera.** The patient material and the test results on antiviral antibodies have been described in detail elsewhere (P. Ukkonen, T. Hovi, C.-H. von Bonsdorff, P. Saikku, and K. Penttinen, submitted for publication). Briefly, during the 8-year period 1971 to 1978, about 58,000 sera were sent to the routine diagnostic laboratory at the Department of Virology, University of Helsinki, to be screened for antibodies to about 15 viruses and to *M. pneumoniae*, chlamydia, and toxoplasma. Since the proportion of acute infections caused by *M. pneumoniae* during the study was low (2.3%) compared with the total number of patients screened, and since the sera were sent from all kinds of treatment centers and hospitals in Finland, they can be taken to represent the normal Finnish population.

The computerized results were grouped into four 2-year periods consisting of 11,144 to 17,305 specimens and into 16 age groups, each comprising 559 to 9,514 specimens.

**Complement fixation test.** The complement fixation test was performed by a standard microtechnique. *M.
The frequency of CF antibody to *M. pneumoniae* by age in 1971 to 1978

<table>
<thead>
<tr>
<th>Age</th>
<th>No. tested</th>
<th>Percent with antibody of titers:</th>
<th>Geometric mean titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥8</td>
<td>≥32</td>
</tr>
<tr>
<td>&lt;1 mo</td>
<td>1,578</td>
<td>22.9</td>
<td>1.6</td>
</tr>
<tr>
<td>1–3 mo</td>
<td>1,048</td>
<td>23.3</td>
<td>1.8</td>
</tr>
<tr>
<td>4–6 mo</td>
<td>559</td>
<td>61.7</td>
<td>8.8</td>
</tr>
<tr>
<td>7–11 mo</td>
<td>1,049</td>
<td>73.6</td>
<td>19.5</td>
</tr>
<tr>
<td>12–23 mo</td>
<td>1,916</td>
<td>73.1</td>
<td>20.2</td>
</tr>
<tr>
<td>2–3 yr</td>
<td>1,959</td>
<td>74.2</td>
<td>25.6</td>
</tr>
<tr>
<td>4–6 yr</td>
<td>2,324</td>
<td>73.3</td>
<td>32.4</td>
</tr>
<tr>
<td>7–10 yr</td>
<td>2,970</td>
<td>77.1</td>
<td>38.9</td>
</tr>
<tr>
<td>11–20 yr</td>
<td>5,798</td>
<td>74.4</td>
<td>32.7</td>
</tr>
<tr>
<td>21–30 yr</td>
<td>9,514</td>
<td>67.3</td>
<td>19.4</td>
</tr>
<tr>
<td>31–40 yr</td>
<td>7,426</td>
<td>58.4</td>
<td>13.8</td>
</tr>
<tr>
<td>41–50 yr</td>
<td>6,592</td>
<td>50.8</td>
<td>10.6</td>
</tr>
<tr>
<td>51–60 yr</td>
<td>6,302</td>
<td>45.3</td>
<td>8.8</td>
</tr>
<tr>
<td>61–70 yr</td>
<td>5,504</td>
<td>42.7</td>
<td>8.2</td>
</tr>
<tr>
<td>71–80 yr</td>
<td>2,789</td>
<td>39.1</td>
<td>7.2</td>
</tr>
<tr>
<td>&gt;80 yr</td>
<td>648</td>
<td>35.3</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* A titer of <1:8 was considered to be 1:4 for the calculation of the geometric mean.

*pneumoniae* lipid antigen was prepared by Orion Diagnostica, Espoo, Finland, according to the method described by Jansson (20). Lipid extraction of the antigen was performed as described previously by Kenny and Grayston (22).

**RESULTS**

*Age-related prevalence of antibody.* The prevalence of CF antibody to *M. pneumoniae* (titer ≥ 8) among infants under 1 month old was 23%, i.e., significantly less than among adults 20 to 40 years old (58 to 67%) (groups which include females of childbearing age) (Table 1). The proportion of positive reactors in the next age group, 1 to 3 month olds, was the same, and there was no decrease reflecting the disappearance of transplacentally acquired maternal antibodies. In fact, even among 4 to 6 month olds the prevalence increased sharply, being 62% in this age group.

In the age groups between 7 months and 20 years, the prevalence of antibody remained constant, varying between 73 and 77%. The frequency was highest among 7 to 10 year olds. After the age of 20, the prevalence declined steadily, being about 50% among 50 year olds and 35% among over 80 year olds.

The frequency of higher antibody titers (≥32) and the geometric mean titers increased more slowly than the total prevalence of measurable titers, and they peaked at the age of 7 to 10 years (Table 1). Thus, the prevalence of high titers obviously corresponds to the age distribution of symptomatic *M. pneumoniae* infection.

**Yearly distribution of antibody pattern.** The age-related prevalence patterns of *M. pneumoniae* antibody were unchanged in the four 2-year periods (Fig. 1), although there were extensive epidemics of mycoplasmal infection in Finland in 1971 to 1972 and 1977 (29). Mycoplasmal infections were also abundant during the inter-epidemic years. The epidemics did not affect the prevalence of antibody to *M. pneumoniae* in the study population, except for a slight increase in the group of high titers (≥128) (Table 2). The yearly geometric mean titer did not correlate with the epidemics.

**DISCUSSION**

Two peculiar features of these results were the low frequency of CF antibody to *M. pneumoniae* among 0 to 3 month olds as compared with that of the age group of the mothers, and its sharp increase at a very young age, among 4 to 6 month olds. In these respects, the prevalence differs from that of respiratory viruses, i.e., adeno-, parainfluenza, influenza, and respiratory syncytial viruses. Among infants less than 1 month old the prevalence of viral antibodies is equal to that of the age group of mothers, owing to transplacentally acquired maternal antibodies. Thereafter, their prevalence decreases as the maternal antibodies disappear and is lowest among 4 to 6 month olds. Then the prevalence increases steadily during childhood (Uukonen et al., submitted for publication).

Similar changes in *M. pneumoniae* antibodies have also been shown by some serological tests. Using sensitive mycoplasmacidal and radioimmunoprecipitating antibody tests, Brunner et al. (4) observed antibody to *M. pneumoniae* in 50 to 100% of 0 to 3 month olds. Thereafter, the frequency decreased temporarily, probably indicating loss of maternal antibody. A similar decrease in antibody frequency was observed by Taylor-Robinson et al. (33), using the tetrazolium reduction inhibition test, but not with the CF
test or with the indirect hemagglutination test for titers of 20 or higher. In contrast, Hornsleth (19), using the CF test, observed that the frequency of mycoplasmal antibody was lowest (10%) among 0 to 5 month old's. In our study, the frequency of CF antibody among children less than 1 month old was equal to that of 1 to 3 month old's, being only one-third of that among young adults of childbearing age; it thereafter increased rapidly without showing any decrease as observed by some other methods.

Thus, the various tests obviously measure different antibodies to *M. pneumoniae*. CF antibody seems to penetrate through the placenta less easily than mycoplasmacidal and radioligand precipitating antibodies. An explanation for this might be that the CF antibodies in serum are mainly of the immunoglobulin M (IgM) class, which do not cross the placenta. Often after *M. pneumoniae* infection, especially among young persons, the IgM antibody response predominates (1, 8, 31). With time, the CF antibody to *M. pneumoniae* usually shows a gradual transition from IgM to IgG (1, 12). However, CF IgM antibodies can still be found as long as 4 years after infection (1).

The age distribution of symptomatic disease due to *M. pneumoniae* differs from that of antibody prevalence. Clinical mycoplasmal infections are most common among 5 to 19 year old's, and quite rare among children under the age of 5 years (2, 10, 14, 15, 18, 19, 21), although in our figures antibody to *M. pneumoniae* was frequent in the latter age group, too. Many earlier surveys have also revealed antibodies to *M. pneumoniae* among young children in various frequencies, depending, at least in part, on the test used (4, 7, 9, 10, 15, 19, 21, 32, 33). There are two possible explanations for the occurrence of mycoplasmal antibodies in high frequency in young children, although clinical infection is rare. First, infection in this age group may be asymptomatic (4, 13). This is supported by the finding of Fernald et al. (13) that 74% of *M. pneumoniae* infections among children in a daycare center were asymptomatic. Also, the isolation rate of *M. pneumoniae* from infants and preschool children with respiratory disease is almost indentical with that from the same age group free of respiratory disease: 3.1 and 3.0%, respectively. According to Brunner et al. (4), this similarity suggests that in this age group the organism does not cause respiratory illness. These findings have led to a suggestion that disease caused by *M. pneumoniae* may be in part immunologically determined in a host sensitized by one or more silent infections (4, 13).

Another alternative or concomitant possibility is that the elevations in *M. pneumoniae* antibody titers in young children are often nonspecific and caused by other organisms or factors. Various degrees of relatedness have been observed in the antigenic composition of different mycoplasmas, principally among arginine-utilizing species (27). However, *M. pneumoniae* is thought to be serologically distinct from all other human mycoplasmas even when tested with less specific techniques such as the CF and indirect hemagglutination tests (26). *M. pneumoniae* has been shown to have antigenic components similar to glycolipids from MG strain streptococci and to lipids from selected strains of group A streptococci and *Staphylococcus aureus* (4, 25), as well as to glycolipids from parsnips, carrots, and spinach (17, 25). Additionally, some animal and human tissues such as liver, brain, lung, and

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heart share antigenic components with *M. pneumoniae*, as does human erythrocyte I antigen (G. Biberfeld, Ph.D. thesis). Thus, it is possible that young children develop antibodies against these organisms, plants, or tissues, and the antibodies then cross-react in mycoplasmal antibody tests. Among adults, obviously nonspecific elevations in CF titers have been observed during pancreatitis and some central nervous system infections (23, 28, 30); these are possibly due to an antigenic stimulus by tissue components.

Whatever the origin of these antibodies in young children, Brunner et al. (4) showed them to be capable of killing *M. pneumoniae* in the presence of complement. Circulating antibodies do not, however, significantly prevent natural or experimental infection with *M. pneumoniae*, although they may prevent the spread of these organisms from the respiratory epithelium to the other tissues (3, 6, 11, 16). The resistance to infection is obviously more dependent on the local immunological defense of the respiratory tract. In the present study, no correlation was observed between the prevalence of antibodies in low titers and the epidemic occurrence of *M. pneumoniae*.

CF antibodies often persist in the serum for several years after *M. pneumoniae* infection (1, 24), but gradually disappear with time. Because over 60% of the children tested had developed antibody by the age of 4 to 6 months and the prevalence of antibody was still over 50% until the age of 50 years, it is obvious that boosting of the mycoplasmal antibody occurs with time, owing to specific reinfections or to the above-mentioned nonspecific mechanisms, or both.

Recently, new sensitive enzyme immunological techniques which can use the protein antigen of *M. pneumoniae* have been presented (5, 30). Seroepidemiological studies with these tests may possibly show whether the elevations in *M. pneumoniae* antibody titers commonly observed among infants and preschool children by older methods are nonspecific and caused by various naturally occurring glycolipids.

**LITERATURE CITED**


