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Coinfections of *Mycoplasma pneumoniae* and *Legionella pneumophila* with Influenza A Virus

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Serum samples from patients with documented influenza A virus infections were examined for antibodies to *Legionella pneumophila* and *Mycoplasma pneumoniae* to determine whether simultaneous or sequential infections with *L. pneumophila* and *M. pneumoniae* were complicating factors in influenza. When the frequency of copositivity of sera to influenza A virus and *L. pneumophila* was compared with the expected frequency for each infection alone, the difference was not statistically significant. However, when the frequency of copositivity of sera to influenza A virus and *M. pneumoniae* was compared with the expected frequency for each infection alone, there was a statistically significant ($P < 0.005$) absence of coincident titers. Seasonal variations and differences in relative age frequencies for the two infections may partially explain the absence of coinfections. These data also suggest that in patients with either *M. pneumoniae* or influenza A virus infection, some type of protective mechanism which prevents coinfections with these organisms is present.

The most frequently recognized complications of influenza infections are concomitant or sequential bacterial infections of the respiratory tract (1, 4, 7, 12, 13, 17). To assess whether concurrent infections with *Legionella pneumophila* and *Mycoplasma pneumoniae* are complicating factors in influenza A virus infections, we reviewed the seroreactivity specimens from 1,060 patients with acute respiratory tract infections to these antigens.

MATERIALS AND METHODS

Sera. Acute and convalescent sera were selected from 1,060 patients with acute respiratory tract infections. Sera had been submitted to the hygienic laboratory at the University of Iowa, Iowa City, between 1971 and 1980 for diagnostic serological testing. Sera received between 1971 and 1977 were assayed upon receipt for antibodies to *M. pneumoniae* and influenza A virus and were then stored at −25°C in tightly sealed, screw-capped vials until they were tested in 1978 for antibodies to *L. pneumophila*. Sera submitted after 1978 were assayed upon receipt for antibodies to *M. pneumoniae*, *L. pneumophila*, and influenza A virus.

Antibody assays. Antibodies to influenza A virus and *M. pneumoniae* were assayed by standard complement fixation methods (8, 9). Antibodies to *L. pneumophila* were measured by the indirect influorescent antibody test (19). Reagents for this test were supplied by the Centers for Disease Control, Atlanta, Ga.

For the purpose of analysis, seroconversions were defined as sera with titers that increased at least fourfold between acute and convalescent phases of illness to levels of at least 1:32 for influenza A virus and *M. pneumoniae* and 1:128 for *L. pneumophila*. Sera with presumptive antibody titers were defined as those serum pairs in which the standing titers of at least one serum of the pair were 1:32 or more for *M. pneumoniae* and influenza A virus and 1:256 or more for *L. pneumophila*.

Statistical analysis. The G test for independence was used for statistical analysis.

RESULTS

The frequency of *L. pneumophila* titers in sera with and without seroreactivity to influenza A virus was as follows. Of the 1,060 serum pairs, 91 (8.5%) showed seroconversions to influenza A virus and 49 (4.6%) showed presumptive titers to influenza A virus. Of the 91 serum pairs with seroconversions to influenza A virus, 1 (1%) demonstrated a simultaneous seroconversion to *L. pneumophila*, and 10 (11%) showed presumptive titers to *L. pneumophila*. One seroconversion to *L. pneumophila* occurred in the 49 sera with presumptive titers to influenza A virus and 9 sera showed presumptive titers to both agents. Among the 920 paired sera without seroreactivity to influenza A virus, there were an additional 33 seroconversions and 116 presumptive titers to *L. pneumophila*.

When the percentages of seroconversions to *L. pneumophila* in sera with concomitant seroconversions (1%) or elevated titers (2%) to influenza A virus were compared independently with

the percentage of seroconversions (3.6%) in sera without titers to influenza A virus, the differences were not statistically significant ($P > 0.10$). Presumptive titers to *L. pneumophila* occurred in only 9 (18.4%) of the 49 sera with presumptive titers to influenza A virus and in only 116 (12.6%) of the 920 influenza A virus-negative sera. These differences were not statistically significant ($P > 0.10$).

The frequency of seroreactivity to *M. pneumoniae* in sera with antibodies to influenza A virus was as follows. One simultaneous seroconversion to *M. pneumoniae* occurred among the 91 serum pairs with seroconversions to influenza A virus, and no seroconversions occurred among the 49 sera with elevated titers to influenza A virus. None of the 91 serum pairs with seroconversions to influenza A virus showed a presumptive titer to *M. pneumoniae*, and 2 showed presumptive titers to both agents. Among the 920 serum pairs without elevated titers to influenza A virus, there were an additional 127 seroconversions and 58 presumptive titers to *M. pneumoniae*. When the frequency of copositivity of sera to influenza A virus and *M. pneumoniae* was compared with the expected frequency for each infection alone, there was a statistically significant ($P < 0.005$) absence of coincident titers.

DISCUSSION

The results of this study indicated that confections of *L. pneumophila* with influenza A virus occur. Of the 140 patients with serological evidence of influenza A virus infections (91 seroconversions and 49 presumptive titers), 21 (15%) also had confirmed or presumptive evidence of *L. pneumophila* infections. This frequency appeared to depend on the relative incidence of two infections since the incidence of serum copositivity was not significantly greater than that expected for each infection alone. Similar observations on the frequency of concurrent infections have been reported for beta-hemolytic streptococci, viruses, and *M. pneumoniae* (15), and recently for *L. pneumophila* and *M. pneumoniae* (16). It is not known if mixed infections have an effect on clinical manifestations or if they enhance illness.

Our results agree with those of Foy et al. (4), who reported that among 381 adult patients who had pneumonia during the same time period, 47 (12.3%) had evidence of *M. pneumoniae* infections, and 84 (22%) had evidence of influenza A virus infections. However, only 1 (2.1%) of the 47 patients with confirmed *M. pneumoniae* infections had an increase in influenza A virus titer. The absence of coinfections of adenovirus with *M. pneumoniae* was reported by Mogabgab (14), who concluded that coinfections, as evidenced by antibody response, were very unusual, and he suggested that mutual antagonism existed between the organisms. Conversely, in many studies of disease due to *M. pneumoniae*, cultural or serological evidence of concomitant or sequential infections with other infectious agents has been noted (2, 3, 5, 6, 10, 11, 18). In these studies, there was no evidence of synergistic or antagonistic roles of the infectious agents for one another.

Primary influenza pneumonia and pneumonia due to secondary bacterial infections are pulmonary complications which frequently follow infections due to influenza A virus. During an outbreak of influenza, many cases of pneumonia occur, but they respond to antibiotics and are clinically less distinct than either of the pulmonary complications (7, 13). The degree to which concomitant *M. pneumoniae* or *L. pneumophila* contribute to these pulmonary complications is not known. Our results indicated that coinfections with *L. pneumophila* occur and should be considered in patients with suspected bacterial complications, especially when a diagnosis cannot be made from results of Gram staining or culturing of sputum or transtracheal aspirate or both.

Our findings also showed that *M. pneumoniae* infections rarely complicate influenza. At present we have no explanation for the absence of coinfections. Seasonal variations and differences in relative age frequencies for influenza A virus and *M. pneumoniae* infections may partially explain this absence. Rates of infection with *M. pneumoniae* are greatest in school-aged children and young adults, with the majority of infections occurring between June and December (1a, 6a). Although the incidence of influenza A virus infection is also highest in children, pneumonia associated with this infection is most commonly seen in subjects at the extremes of the span of life and occurs almost exclusively between December and March (4, 6a). There is, however, a definite overlap in the seasonal occurrence of and age frequencies for infections (6a). If cross-immunity is an explanation, the nature of this system remains to be defined.

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