

Prevalence and Persistence of *Chlamydia pneumoniae* Antibodies in Healthy Laboratory Personnel in Finland

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The rates of *Chlamydia pneumoniae* seroconversions suggesting acute primary infections or reinfections and the prevalences of antibodies were followed up among healthy laboratory workers. Annual serum samples were collected from 47 persons in Helsinki from 1958 to 1990 and from 40 persons in Oulu from 1994 to 1999. *C. pneumoniae* species-specific immunoglobulin G (IgG), IgA, and IgM antibodies were measured by microimmunofluorescence (MIF) in 407 sera from Helsinki. The 185 sera collected in Oulu were tested both by MIF and by commercial enzyme immunoassay (EIA). During the follow-up periods of 31 years in Helsinki and 6 years in Oulu, seroconversions were demonstrated by MIF in 45% and 15% of the study groups, respectively. In Helsinki 9% of the persons seroconverted twice during the follow-up period. By MIF, the total incidence rate per 100 person-years at risk was 6.9 in Helsinki and 4.9 in Oulu, and annual incidence rates varied from 0 to 15.4. By EIA, annual incidence rates in Oulu varied from 0 to 10.8. The seroconversions by MIF were usually not confirmed by EIA and vice versa. Prevalence and persistence rates, respectively, of IgA antibodies were higher in EIA (62% and 26%) than in MIF (26% and 17%), whereas the figures for IgG were quite similar. The prevalence of IgG and IgA antibodies was higher in older persons than in younger ones. The presence of antibodies did not offer protection from reinfection.

Chlamydia pneumoniae is a common respiratory pathogen, and almost all people are infected by the age of 20. Seroepidemiological studies have shown that the antibody prevalence rises with age in adult populations (39). Frequent reinfections or persistent infections might explain the higher antibody prevalence in older age groups (13, 28, 50). *C. pneumoniae* causes upper and lower respiratory tract infections in humans and causes about 10% of the pneumonia cases in adults worldwide (14, 38). Persistent *C. pneumoniae* infections have been associated with several chronic diseases, such as asthma (16, 17), chronic obstructive pulmonary disease (47), and coronary heart disease (34, 40, 41).

The epidemiological situation affects the prevalence of *C. pneumoniae* immunoglobulin G (IgG) and IgA antibodies (25, 38). Seroepidemiological surveys have shown that both epidemic and endemic *C. pneumoniae* respiratory tract infections occur in different parts of the world, and epidemics are more common in sparsely populated areas (38). It has also been suggested that asymptomatic infections or infections with mild respiratory symptoms are common (14). Most transmissions seem to take place at schools, military bases, and workplaces, although the spread of infections at home has also been reported (13, 26, 39). After an acute infection, IgM titers usually fall within 2 months and normalize within 4 to 6 months. Elevated IgG levels may persist for several years and occasionally be detectable over 3 years after the acute infection (29).

At the Department of Virology, University of Helsinki, and at the National Public Health Institute in Oulu, annual serum samples are collected from both laboratory and office person-

nel. We followed the prevalence and persistence of *C. pneumoniae* antibodies in the sera of healthy employees for 31 years in Helsinki and for 6 years in Oulu. Our aim was also to follow the kinetics of IgG and IgA antibodies in multiple sera obtained from the same individuals and compare antibody findings obtained by conventional microimmunofluorescence (MIF) and commercial enzyme immunoassay (EIA) in part of the sera.

MATERIALS AND METHODS

The study subjects belonged to the personnel of the Department of Virology, University of Helsinki, and the National Public Health Institute in Oulu. The annual serum samples were collected for occupational health surveillance and as preinfection sera. A total of 592 serum specimens from 87 persons, 67 women and 20 men, were tested. Informed consent was obtained from all study subjects. In Helsinki, 407 sera were collected from 47 persons. All subjects included in Helsinki had at least three serum samples taken from 1958 to 1990. The follow-up time varied from 3 to 31 years. From 77% of the persons, blood samples were obtained every year. The sera from 55 subjects in Oulu were collected during the period from 1994 to 1999. One serum sample was available from 11 subjects, two sera were from 4 subjects, and at least three sera were from 40 subjects (185 sera) for the measurement of *C. pneumoniae* antibodies. The follow-up time varied from 3 to 6 years. The mean age of 35 women and 5 men was 41 years (range, 22 to 57 years) at the beginning of 1994. The personnel were divided into two age groups: under 40 (16 cases) and over 40 (24 cases) years at the baseline in 1994.

All the serum samples from one subject were tested simultaneously to minimize interassay variations in titers. The sera were tested for IgG, IgA, and IgM antibodies to *C. pneumoniae* by the MIF test (48, 49), using elementary bodies of the TW-183 strain in Helsinki and the Finnish strain Kajaani 6 (8) in Oulu. Twofold serum dilutions were used, starting from 1/8, and seroconversions suggesting acute infection during the preceding year were based on a fourfold titer rise in IgG and/or IgA between consecutive sera. IgG and IgA titers of ≥ 8 were defined as positive, and IgM-positive sera (a titer of ≥ 16) were retested after screening by Gullorsorb reagent (Gull Laboratories, Salt Lake City, Utah) to avoid false-positive reactions (22, 46). The presence of IgM antibodies in titers of ≥ 10 after Gullorsorb treatment was considered a marker of primary acute infection.

In addition to the MIF test, Oulu sera were also tested with commercial

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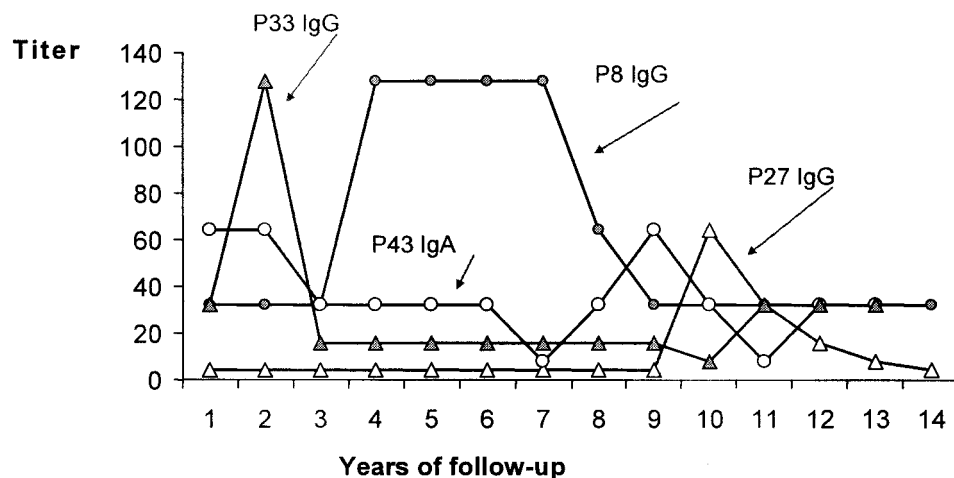


FIG. 1. *C. pneumoniae* antibody titers in four persons (P8, P27, P33, and P43) before and after seroconversion during a 14-year follow-up study.

species-specific *C. pneumoniae* IgG and IgA EIA kits (Labsystems, Helsinki, Finland). Levels of 30 enzyme immunounits (EIU) in IgG and 12 EIU in IgA were used as the cutoff values for seropositivity. When the EIUs were below 130 EIU in IgG and 50 EIU in IgA, a 1.5-fold change was considered significant, and with higher EIUs the diagnostic change was 1.3-fold.

Statistical methods. The differences in proportions were tested using the chi-square test or Fisher's exact test as appropriate. Spearman's correlation coefficients were used to compare the in-house MIF and the Labsystems EIA kits. The strength of agreement was analyzed by the kappa values as follows: poor, <0.2; fair, 0.21 to 0.4; moderate, 0.41 to 0.6; good, 0.61 to 0.8; and very good, 0.81 to 1.00.

RESULTS

Helsinki sera. In Helsinki, the mean antibody prevalence was 69% (280/407) for IgG and 41% (168/405) for IgA, and IgM antibodies were not found. In men, the mean antibody prevalences (82% [116/141] for IgG; 59% [83/140] for IgA) were significantly higher ($P < 0.001$) than in women (61% [164/266] for IgG; 32% [85/265] for IgA). During the years 1958 to 1971, only 19 sera were available, and during 1989 to 1990, only 7 sera were available; we omitted these years from the analysis, because the prevalences were based on at least 11 sera (range 11 to 38). During 1972 to 1988, the annual antibody prevalences varied from 46% (5/11) to 87% (26/30) for IgG and from 27% (3/11) to 57% (17/30) for IgA antibodies. In 60% (244/407) of the sera, IgG titers ranged from 8 to 64, and 9% (36/407) had titers of ≥ 128 . In IgA antibody assays, 41.5% (168/405) of the sera were positive; high titers of ≥ 128 were seen in only 0.5% (2/405) of the sera. IgG seroconversions were seen at least once in 25% (8/32) of women and 33% (5/15) of men. Most of the 25 seroconversions in 21 persons were found in IgG or in the combination of IgG and IgA, and 12% (3/25) of the persons seroconverted only in the IgA test. A diagnostic IgA antibody response was found in 20% (3/15) of men and 13% (4/32) of women. Figure 1 shows the persistence of the IgG or IgA antibodies in four cases before and after seroconversion in 14 follow-up sera.

Only one person out of the 10 tested seroconverted between the years 1958 and 1969, at least partly due to the low number of serum specimens ($n = 10$) tested during these years. One or two seroconversions were generally noted annually, and the

incidence rate was 6.9 (25 seroconversions per 360 person-years of follow-up) during the whole follow-up period. The incidence rates per 100 person-years at risk during the years 1973 to 1988 varied from 0 to 15.4 (Fig. 2). In 1987, the incidence rate was highest, i.e., 15.4, suggesting a *C. pneumoniae* epidemic, which ended soon the next year, with the incidence rate declining to 3.3. Incidence rates over 10 were also recorded in 1980, 1981, and 1986. Among the persons who had high IgG titers (a titer of ≥ 64) and were followed up for several years after the seroconversion, the antibody levels decreased to a titer of 32 in 4 years. In fact, after the seroconversions, most of the high antibody titers (5/6) declined in 1 year to 32. Multiple seroconversions were seen in 6% (3/47) of the persons, and the cycle of reinfections ranged from 2 to 6 years. IgG titers of ≥ 32 persisted for over 10 years in 15% (7/47) of study subjects and IgA titers of ≥ 16 persisted in 4% (2/47) of the study subjects. IgA titers of ≥ 32 persisted for over 3 years in 11% (5/47) of the personnel, although after seroconversions, IgA antibodies decreased rapidly in 1 or 2 years. In one person, a stable IgG titer of ≥ 64 persisted for as long as 26 years.

Oulu sera. (i) MIF antibodies. In Oulu, the prevalence of IgG antibodies was significantly higher among persons ≥ 40 years old (72%, or 91/126) than among those under 40 (54%, or 32/59) ($P = 0.016$). IgA antibodies were not present in younger persons, but they were found in 26% (33/126) of the older ones (Table 1). All the sera were IgM negative. The annual prevalence of IgG antibodies varied between 33% (2/6) and 63% (5/8) in the younger group and from 54% (7/13) to 82% (18/22) in the older group. Annual prevalences of IgA antibodies varied from 15% (2/13) to 41% (9/22) in the older age group. Most (88%) study subjects were women ($n = 35$), and 14% (5/35) of them showed seroconversion. In 1998, two persons had seroconversions suggesting acute infections, whereas during the other years, only one person per year seroconverted. The incidence rates varied from 2.9 to 6.9 during 1995 to 1999 (Table 2). Fourfold or greater IgG decreases were seen in only 29% (7/24) of the age group ≥ 40 years (Table 1). IgA seroconversions occurred only in cases with high

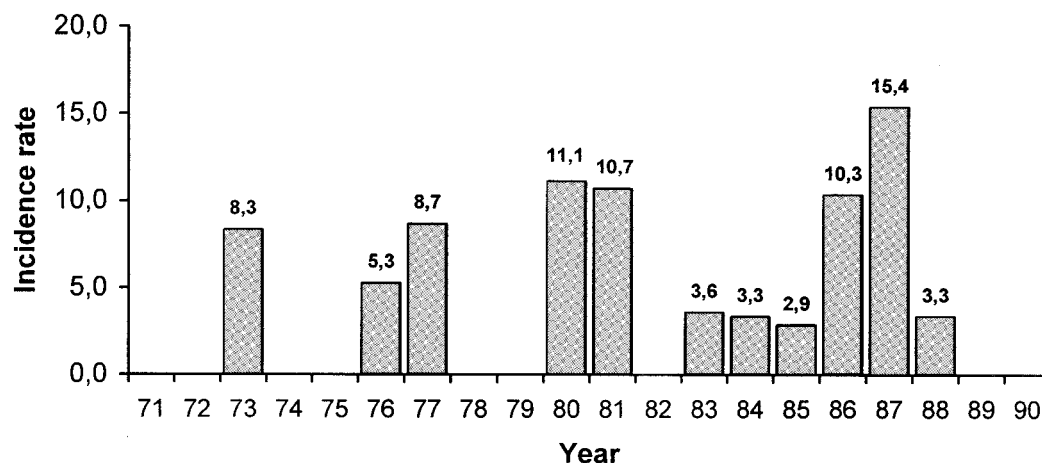


FIG. 2. The incidence rate per 100 person-years at risk of seroconversions, suggesting acute infections in the Helsinki personnel from 1971 to 1990.

IgG values (≥ 128), and only in one out of three cases did they decrease from 64 to 8 during the follow-up. Three cases had high IgG titers (≥ 128) for 4 years, and high IgA antibodies (≥ 64) persisted in only one woman throughout the follow-up. In half of the younger (8/16) and in 83% (20/24) of the older study subjects, IgG antibodies persisted at the level of ≥ 8 . Persistent IgA antibodies (≥ 8) were present in only 17% (4/24) of the older study subjects (Table 1).

(ii) **Antibodies and seroconversions in EIA.** IgG antibodies were found by EIA in 82% of the older (102/125) and in 63% of the younger age group (37/59) ($P = 0.005$). During the follow-up period, the annual prevalences of IgG antibodies varied from 50% (3/6) to 67% (8/12) in the younger group and from 69% (9/13) to 87% (20/23) in the older study group. Over half (60%) (75/125) of the older group had IgA antibodies, but the prevalence was only 7% (4/59) among younger persons ($P < 0.001$). Between 1994 and 1999, the annual prevalences of IgA antibodies varied from 50% (10/20) to 68% (15/22) in older subjects and from 0% (0/8) to 18% (2/11) in younger subjects. IgG antibodies persisted in 88% (21/24) and 63% (10/16), respectively, and IgA antibodies persisted in 63% (15/24) and in 13% (2/16), respectively (Table 1). Overall, five out of eight seroconversions suggesting acute infection were de-

tected in the older study subjects. The seroconversion rates were highest in 1997 and lowest in 1998, and seroconversions were seen in 9% (3/35) of women and in 60% (3/5) of men. It should be noted that the number of males was low ($n = 5$). In one younger person, three IgA seroconversions every other year were seen. After the seroconversions, all IgA antibodies decreased at least 1.5-fold, and in only one of three persons with IgG seroconversion did the antibody level decline significantly before the end of the follow-up. The persistence of IgA and the combination IgG and IgA antibodies were significantly higher among the older than the younger persons (Table 1). The IgG test revealed six cases and the IgA test revealed three cases with higher antibody levels than the positive control serum; these serum samples had not been rediluted, in accordance with the manufacturer's recommendations.

(iii) **Comparison of MIF and EIA.** Spearman's nonparametric correlation coefficients of in-house MIF and the Labsystems EIA were better in the IgG ($r = 0.76$; $P < 0.0001$) than the IgA ($r = 0.57$; $P < 0.0001$) tests. The agreement between the in-house MIF and the Labsystems EIA was moderate in IgG ($\kappa = 0.52$) and IgA ($\kappa = 0.43$). In both age groups, the prevalence of IgG EIA antibodies was about 10% higher than that obtained by the in-house MIF, and quite similar IgG persistence

TABLE 1. Prevalence and persistence of IgG and IgA antibodies, with increases and decreases in incidence, among Oulu personnel

Antibody and parameter ^a	<40 age group ($n = 16$) ^b		≥ 40 age group ($n = 24$) ^b		P^c	
	MIF %	EIA %	MIF %	EIA %	MIF	EIA
IgG prevalence	54 (32/59)	63 (37/59)	72 (91/126)	82 (102/125)	0.016	0.005
IgA prevalence	0 (0/59)	7 (4/59)	26 (33/126)	60 (75/125)	<0.001	<0.001
IgG persistence	50 (8/16)	63 (10/16)	83 (20/24)	88 (21/24)	0.037	0.120
IgA persistence	0 (0/16)	13 (2/16)	17 (4/24)	63 (15/24)	0.136	0.002
IgG + IgA	0 (0/16)	6 (1/16)	4 (1/23)	63 (15/24)	1.000	<0.001
IgG rise	0 (0/16)	0 (0/16)	17 (4/24)	13 (3/24)	0.136	0.262
IgA rise	0 (0/16)	6 (1/16)	13 (3/24)	8 (2/24)	0.262	1.000
IgG decrease	0 (0/16)	0 (0/16)	29 (7/24)	17 (4/24)	0.029	0.136
IgA decrease	0 (0/16)	13 (2/16)	4 (1/24)	29 (7/24)	1.000	0.272

^a The criteria for persistence were as follows: for MIA, a titer of ≥ 8 in IgG and IgA antibodies for ≥ 3 years; for EIA, EIU values ≥ 30 in IgG and EIU values ≥ 12 in IgA antibodies for ≥ 3 years.

^b n , number of subjects in group. The number of positive samples out of the total number of samples tested is given in parentheses.

^c P values were determined by Fisher's exact test or a chi-square test.

TABLE 2. Incidence of seroconversions suggesting acute *C. pneumoniae* infection and the annual prevalences of IgG and IgA antibodies among Oulu personnel

Assay and year	No. of subjects	No. of acute infections ^a	Person-years	Incidence ^b	% Prevalence by antibody	
					IgG	IgA
MIF						
94	21	NA		0	57	10
95	34	1	21	4.8	71	24
96	36	1	32	3.1	72	25
97	37	1	35	2.9	68	16
98	29	2	29	6.9	62	14
99	28	2	28	7.1	64	14
EIA						
94	21	NA		0	67	38
95	34	2	34	5.9	79	50
96	36	1	36	2.8	78	42
97	37	4	37	10.8	78	41
98	28	0	28	0	71	36
99	28	1	28	3.6	75	50
Total						
MIF		7	145	4.8		
EIA		8	144	5.6		

^a NA, not applicable.

^b Rate per 100 person-years at risk.

rates were obtained by MIF and EIA. The prevalences of IgA antibodies in the older group were 60% (75/125) in EIA and 26% (33/126) in MIF, and the persistence rates were 63% (15/24) versus 17% (4/24), respectively (Table 1). MIF seroconversions were not detected by EIA tests and vice versa.

DISCUSSION

The annual prevalence and persistence rates of *C. pneumoniae* antibodies were followed for over 3 decades in Helsinki and for 6 years in Oulu. Repeated reinfections, every fourth year in IgG and every second year in IgA, were needed for the persistence of high antibody titers (a titer of ≥ 64), but low IgG and IgA antibody levels could persist for over a decade. The highest prevalences of IgG and IgA antibodies were demonstrated after the peak incidence year 1987. The prevalences of *C. pneumoniae*-specific IgG and IgA antibodies were higher in older than in younger persons. In addition, the prevalence of IgG EIA antibodies was about 10% higher and of IgA antibodies was about 30% higher than by the in-house MIF.

Incidence and epidemics. In Helsinki, the highest incidence rates of acute infections were seen in the years 1973, 1977, 1980 to 1981, and 1986 to 1987, suggesting that *C. pneumoniae* epidemics in Finland occur at intervals of 4 to 5 years. The annual incidence rate per 100 person-years at risk for acute infection varied from 0 to 15.4 by the MIF test. In Oulu between 1994 to 1999, the incidence rates varied from 0 to 7.1 by MIF and from 0 to 10.8 by EIA. The laboratory population may have elevated risk for *C. pneumoniae* infections compared to the general population, and even laboratory infections have been described (44). It has been suggested earlier that in Finland and the other Scandinavian countries, *C. pneumoniae* causes widespread epidemics every 5 to 7 years (25, 30), but

shorter interepidemic periods were suggested by Gnarpe and Gnarpe (11). Interestingly, in 1977, when an increased incidence of acute infections was observed in Helsinki, epidemic pneumonia outbreaks were also seen in Northern Finland. These outbreaks were first considered to be caused by a peculiar *Chlamydia psittaci* strain (42), but this observation later led to the discovery of *C. pneumoniae* (15). In 1985 to 1987, a countrywide *C. pneumoniae* epidemic was seen in military recruits (23) and in the civilian population in northern Finland (27), and the highest incidence figures were seen in Helsinki in 1986 to 1987.

Prevalences of *C. pneumoniae* antibodies. The IgG and IgA antibody prevalences in Helsinki varied from 46% (5/11) to 87% (26/30) and from 27% (3/11) to 57% (17/30), respectively, and the highest IgG and IgA prevalence rates were observed in 1988 after the countrywide epidemic. In Oulu, the highest IgG and IgA MIF prevalences were measured in 1995 and 1996. In EIA, the prevalences were about 10% higher for IgG and two- to fourfold higher for IgA antibodies compared to MIF. The mean prevalences of IgG and IgA antibodies (by MIF and EIA) were significantly higher in persons ≥ 40 than in those < 40 years old.

According to previous publications from Finland, as early as 1958, the mean antibody prevalence rates were 56% in adults and 26% in children based on samples representing the whole rural population (24). Unfortunately, in the present study, we did not have enough sera from the years 1958 to 1970 to allow comparison. Karvonen et al. (25) have shown that, between 1972 and 1977, the antibody prevalence increased from 57% to 66% in eastern Finland and then decreased over the next 5 years to 41% in 1982; in 1987 it increased again to 59%. In the years 1971 to 1982 and 1984 to 1987 in Finland, chlamydial antibodies were screened from over 160,000 sera by the complement fixation (CF) test. Puolakkainen et al. (37) analyzed these findings from over 17 years and showed that the prevalence of chlamydial antibodies was low in the early 1970s (less 2%), increased to 18% in 1976, and later varied annually between 12% and 22%, except in 1984, when over 31% of the sera contained chlamydial antibodies. Even though the CF test is less sensitive than MIF and measures chlamydial genus-specific antibodies instead of species-specific antibodies, the changes in the chlamydial CF positivity rate have most likely been caused by *C. pneumoniae* (TWAR) epidemics. In our study, the lowest prevalence was 46% (5/11) and the highest was 74% (17/23) between 1972 and 1977, whereas from 1982 to 1987, our prevalence rates were higher than in eastern Finland, varying from 53% (8/15) to 77% (30/39). In the 1990s, the prevalence rates by MIF and EIA were 57% (12/21) and 67% (14/21) at their lowest and 72% (26/36) and 79% (27/34) at their highest, respectively. In Sweden, evidence of two waves of *C. pneumoniae* infection was demonstrated between 1990 and 1996 based on the increased antibody prevalence in first-time blood donors throughout the 7-year period (12). Our results are also in line with the recently published methodological work by Hoymans et al., according to which IgG antibody prevalences in healthy study subjects ranged from 65 to 85%, depending on the serological test used (20). In a recent study by Tuuminen et al. (45), the prevalence rates of IgG EIA antibodies in a healthy population in 1996 to 1999 were at the same level as in our study, with the exception of IgA EIA

antibodies, which are much lower in our study in the age group of <40 years old (40% versus 7%).

Persistence of *C. pneumoniae* antibodies. In the Helsinki study group, IgG antibodies (≥ 32) persisted for over decade in 15% (7/47) and IgA antibodies (≥ 16) persisted in 4% (2/47) of the study subjects. After suspected acute infections, high antibody titers decreased to a titer of 32 or lower in 4 years. In EIA, the persistence of IgA and the combination of IgG and IgA antibodies were more common in older workers than in younger ones, whereas MIF IgG decreases were only seen in older workers (Table 1). After acute infections, follow-up studies have shown that IgG antibodies may persist for over 3 years after primary infections, while IgM antibodies disappear in a few months (29). It has also been shown earlier that, after an acute infection, antibody titers peak at around 6 months before stabilizing or decreasing (35). In Sweden, the persistence of *C. pneumoniae* infection was followed up for 2.5 years by serology and PCR. All the members of the family showed serological evidence of *C. pneumoniae* infection by seroconversion and persistence by high and rising antibody titers (10). After unresolved infections, repeated infections or persistence of the organism may affect recurrent chlamydial disease (19). In the Seattle family study, Aldous et al. indicated that antibodies persisted longer after reinfections than after primary infection (1). We also showed here that reinfections are needed for the persistence of antibodies.

Comparison of MIF and EIA. To our surprise, the kinetics of EIA antibodies differed from that of MIF antibodies in the detection of seroconversions. Furthermore, both tests detected seroconversions in 15% of the persons tested, but always in different persons even though the same sera were tested by both methods. In our study, the strength of agreement between MIF and EIA was moderate in both IgG and IgA tests. We tested the seroconversions between sera taken about 1 year apart, and it is thus possible that, due to the different kinetics of MIF and EIA antibodies, we have missed seroconversion by one of the tests and that the annual incidence is actually higher than that obtained by one test only. At present, the MIF test is the recommended one for the measurement of *C. pneumoniae* antibodies. The commercial EIAs have not been fully validated; they seem to be less specific than MIF (7).

Especially in the older age group, the IgA EIA test overestimated the prevalence (62% versus 26%) and the persistence (63% versus 17%) of IgA antibodies compared to the in-house MIF (Table 1). The sensitivities of EIAs and enzyme-linked immunosorbent assays are higher than the sensitivity of MIF in acute-phase sera, and high *C. pneumoniae*-specific IgG titers are common in healthy individuals (18, 32, 36). Possible serological cross-reactions with *Chlamydia*-like microorganisms (23) might affect the results, although the Labsystems kits are free of the cross-reactive lipopolysaccharide component common to all chlamydia, and the lipopolysaccharide reaction is also excluded by the fluorescence pattern in MIF (20). The use of an IgG-removing reagent before the measurement of IgA antibodies in EIA would be worth testing. In both tests, IgG and IgA antibodies increase in older age but at different rates (13, 45).

Although MIF is the recommended method for the measurement of *C. pneumoniae* species-specific antibodies in acute infection, the method has not been validated for persistent or

chronic infection (7). In seroepidemiological studies, the criteria for the chronicity and adjustment for possible confounding factors are individually defined (4). In a critical meta-analysis, IgG and IgA titers had no predictive value for coronary heart disease (5, 6). The association between *C. pneumoniae* IgG titers and atherosclerosis has been significant in cross-sectional studies but not in prospective studies (3). The use of a single IgA antibody titer as a marker of chronic infection is discouraged in the recommendations of the Centers for Disease Control and Prevention (7). However, several studies have suggested that IgA seropositivity is associated with cardiovascular diseases and is a better marker of chronic infection than IgG seropositivity (21, 31, 43). The present study, by showing that IgA antibodies decrease more rapidly than IgG antibodies after the seroconversion, also suggests that the presence of IgA antibodies in a single serum sample may be a better marker for chronic infection. However, for the persistence of both elevated IgG and IgA titers, reinfections which might be associated with the development of chronic infections were needed. Interestingly, in mouse models, at least two reinfections are needed for the development of chronic infection (2, 9, 33).

In conclusion, elevated IgG and IgA titers to *C. pneumoniae* do not persist for half a decade without reinfection or reactivation. After seroconversion, IgA antibodies disappear rapidly, and their persistence may therefore represent ongoing chronic processes better than IgG antibodies. The choice of the serological test may have a remarkable effect in both diagnostic and epidemiological studies.

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