

## Serological Response to *Chlamydia pneumoniae* in Adults with Coronary Arterial Fatty Streaks and Fibrolipid Plaques

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**The antigen-specific serological response to *Chlamydia pneumoniae* was studied in 45 adults with coronary artery atherosclerosis and compared with that in 40 adults with acute respiratory infection. *C. pneumoniae* antigen and DNA were detected in lesions more frequently in patients with low immunoglobulin G titers against *C. pneumoniae* than in those with high immunoglobulin G titers. Reactivities with the 42-kDa (46%) and 52-kDa (31%) proteins were observed more frequently in sera from seropositive individuals with atherosclerosis than in sera from patients with acute respiratory infection. Antibodies against the *C. pneumoniae*-specific 42- and/or 52-kDa protein may be a marker for chronic *C. pneumoniae* infection.**

*Chlamydia pneumoniae* is a recently characterized species of the genus *Chlamydia*. *C. pneumoniae* is a widespread respiratory pathogen. Seroepidemiological studies showed that >50% of the adult population worldwide have immunoglobulin G (IgG) antibody against the organism (2). While *C. pneumoniae* has been established as an etiological agent of acute respiratory infections, serological evidence has also implicated an association of *C. pneumoniae* with other diseases, e.g., coronary heart disease, myocardial infarction (8, 11, 12), and sarcoidosis (3). A prospective study from Finland has indicated that chronic *C. pneumoniae* infection is a significant risk factor for coronary heart disease (9). In that study, elevated microimmunofluorescence (micro-IF) antibody titers and the presence of circulating immune complexes containing the chlamydial lipopolysaccharide were used as indicators of chronic or past *C. pneumoniae* infection. Subsequently, *C. pneumoniae* has been demonstrated by electron microscopy, immunoperoxidase staining, and polymerase chain reaction in atherosclerotic lesions in the coronary arteries of autopsy patients from South Africa (4, 10). This study was undertaken to analyze the serological response against *C. pneumoniae* in these individuals by the micro-IF test and immunoblot and to determine any antigen-specific reactivities which might serve as indicators of *C. pneumoniae*-associated atherosclerosis.

Postmortem sera from 45 South African adults with coronary arterial fatty streaks ( $n = 15$ ) or fibrolipid plaques ( $n = 30$ ) were studied. The majority of deaths were due to accident, homicide, and trauma. Only one died of coronary heart disease. Eighty-eight percent were male, and 61% were Caucasian, with the remaining being black and Asian Indian. Forty percent were 35 years old or younger. *C. pneumoniae*-specific DNA and/or antigen was detected in atherosclerotic lesions of coronary arteries in 22 of these adults (4). There was no correlation between the detection of *C. pneumoniae* in the lesions and stage of disease. Of the 45 serum specimens, 34 had chronic *C. pneumoniae* antibody (IgG titers of  $\geq 8$  and  $\leq 256$ ) and 5 had acute antibody (IgG titers of  $\geq 512$  and/or IgM titers of  $\geq 16$ ). *C. pneumoniae* was detected in arterial lesions of two individuals with acute antibody. In

addition, sera from patients ( $n = 10$ ) with acute *C. pneumoniae* respiratory infection proven by isolation and/or serology (a seroconversion, a fourfold rise or drop in antibody titers, IgG titer of  $\geq 512$ , or IgM titer of  $\geq 16$ ) and sera from patients ( $n = 30$ ) with acute respiratory infection who were antibody negative or who had low IgG antibody titers (8 to 16) against *C. pneumoniae* were analyzed. The patients were University of Washington students. *C. pneumoniae* antibodies in these patients were determined by the micro-IF test (14). Statistical analysis was done by using Fisher's exact test.

The antigen-specific reactivity of sera was analyzed by immunoblotting as described earlier (1). Briefly, lysates of purified elementary bodies of *C. pneumoniae* (AR-39), *Chlamydia trachomatis* (L<sub>2</sub>/434/Bu), and *Chlamydia psittaci* (6BC) and lysates of HeLa 229 cells (in which these organisms were grown) were electrophoresed in sodium dodecyl sulfate-13% polyacrylamide gel electrophoresis with a discontinuous buffer system (5). After electrophoresis, separated proteins were transferred to nitrocellulose (13). Subsequently, the filters were incubated in 3% bovine serum albumin (BSA) in Tris-buffered saline (TBS) for 1 h and in human sera diluted 1:20 in 1% BSA-TBS overnight. After being washed six times for 5 min each time in TBS-0.05% Tween 20, the nitrocellulose strips were incubated in horseradish peroxidase-conjugated anti-human immunoglobulin diluted 1:500 (Sigma Chemical Co., St. Louis, Mo.) for 1 h at 20°C. The nitrocellulose strips were washed with TBS-0.05% Tween 20 six times for 5 min each time and in TBS two times for 5 min each time. Color was developed by the addition of  $\alpha$ -chloronaphthol (6 mg in 10 ml of TBS with 20% methanol) and 10  $\mu$ l of H<sub>2</sub>O<sub>2</sub>. The positions of low-molecular-weight markers (Pharmacia, Piscataway, N.J.) and the efficiency of the transfer were checked by staining with Ponceau S solution (0.5% Ponceau S in 1% glacial acetic acid).

A high prevalence (87%) of *C. pneumoniae* micro-IF IgG antibodies was found in postmortem sera from South African individuals with atherosclerotic changes in their coronary arteries. Although there was no difference in the detection rate of *C. pneumoniae* in the lesions between the cases with early changes, i.e., fatty streaks (44%), and those with more advanced pathology, i.e., fibrolipid plaques (45%), individu-

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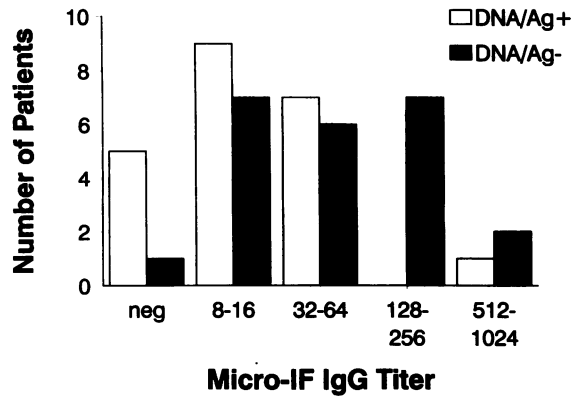


FIG. 1. Correlation of serum antibody titers against *C. pneumoniae* and the presence of *C. pneumoniae* antigen (Ag) and/or DNA in atherosclerotic lesions of coronary arteries. Serum antibody was assayed by the micro-IF test. The GMT of IgG antibody in the group in which *C. pneumoniae* antigen or nucleic acid was detected was  $13.6 \pm 4.2$ , and that in the group in which *C. pneumoniae* was not detected was  $53.4 \pm 4.3$  ( $P < 0.001$ ).

als with early stages of the disease tended to have lower IgG titers (geometric mean titer [GMT],  $12.1 \pm 3.4$ ;  $n = 15$ ) than individuals with more-advanced lesions (GMT,  $41.4 \pm 5.0$ ;  $n = 30$ ) ( $P < 0.001$ ). *C. pneumoniae* was also detected in arterial lesions in individuals who were seronegative (Fig. 1). Five of the six seronegative individuals were *C. pneumoniae* positive (4). Micro-IF IgG titers in cases with *C. pneumoniae* in the atherosclerotic lesion tended to be lower (GMT,  $13.6 \pm 4.2$ ;  $n = 22$ ) than in those with no *C. pneumoniae* in the atherosclerotic lesion (GMT,  $53.4 \pm 4.3$ ;  $n = 23$ ) ( $P < 0.001$ ). The significance of this observation is unknown. One possible explanation is that antibodies in these individuals were bound in immune complexes, as suggested by Saikku and colleagues (9), and thus were not detectable in the micro-IF test. Alternatively, IgG antibodies may be protective and high IgG titers could assist in eradicating or suppressing the infection.

In immunoblots, the 42- and 52-kDa proteins were more often reactive with sera from subjects with atherosclerosis than with sera from patients with respiratory infection ( $P < 0.05$  for the 52-kDa protein and  $P > 0.05$  for the 42-kDa protein). Other proteins often recognized by sera from patients with acute *C. pneumoniae* infection (98, 75, 68, 60, and 30 kDa) (1) were also recognized, but at a lower frequency ( $P < 0.05$  for the 60-kDa protein,  $P < 0.01$  for the 75-kDa protein,  $P < 0.001$  for the 68- and 30-kDa protein, and  $P > 0.05$  for the 98-kDa protein) (Table 1). The immunoblot profiles were similar between atherosclerosis groups who were positive and negative for *C. pneumoniae* except for reactivity against the 68-kDa protein. This protein was recognized only by sera from individuals in whom *C. pneumoniae* was not found in arterial lesions ( $P < 0.05$ ). The immunoblot profile of the five serum specimens from individuals with atherosclerosis who had acute antibody showed a pattern similar to that of the sera from the college students who had acute respiratory infection. The 75- and 60-kDa proteins were recognized frequently by these sera.

Overall, the recognition of the *C. pneumoniae* major outer membrane protein in atherosclerosis sera was rare (18% of all seropositive atherosclerosis cases), and the reaction was genus specific because the sera also reacted with *C. trachomatis* and *C. psittaci* major outer membrane proteins.

TABLE 1. Immunoblot recognition of *C. pneumoniae* proteins by sera from patients with coronary artery atherosclerosis and isolation-proven acute *C. pneumoniae* respiratory infection

Protein recognized (kDa)	% Positive sera from patients with:		
	Atherosclerosis and <i>C. pneumoniae</i> in lesions <sup>a</sup>		Acute respiratory infection ( $n = 10$ )
	Negative ( $n = 23$ )	Positive ( $n = 22$ )	
98	22	5	30
75	13	5	60
68	22	0	80
60	43	23	80
52	26	27	0
42	48	36	20
MOMP <sup>b</sup>	13	18	40
30	4	5	60

<sup>a</sup> Detection by immunocytochemical stain and/or polymerase chain reaction.

<sup>b</sup> MOMP, major outer membrane protein.

Likewise, recognition of 60- and 75-kDa proteins was cross-reactive, as several of the sera reacted with proteins with similar molecular masses from the three species tested. Reactivity with the 42-, 52-, and 98-kDa proteins, and occasionally a 60-kDa protein, was *C. pneumoniae* specific. An example of an immunoblot with sera from patients with atherosclerosis and acute respiratory infection is shown in Fig. 2. Of the control sera tested, micro-IF-negative sera showed no reactivity in immunoblotting, and sera with low IgG titers occasionally reacted weakly with 98-, 68-, or 60-kDa protein. None of the sera reacted with HeLa cell proteins.

Sera from individuals with advanced atherosclerotic changes reacted most frequently with the 42-, 60-, and 52-kDa proteins of *C. pneumoniae*. Interestingly, these three proteins were also recognized most frequently by sera from patients with sarcoidosis (70, 65, and 50%, respectively) (7). Association of sarcoidosis with chronic *C. pneumoniae* infection has been reported elsewhere (3). *C. pneumoniae*

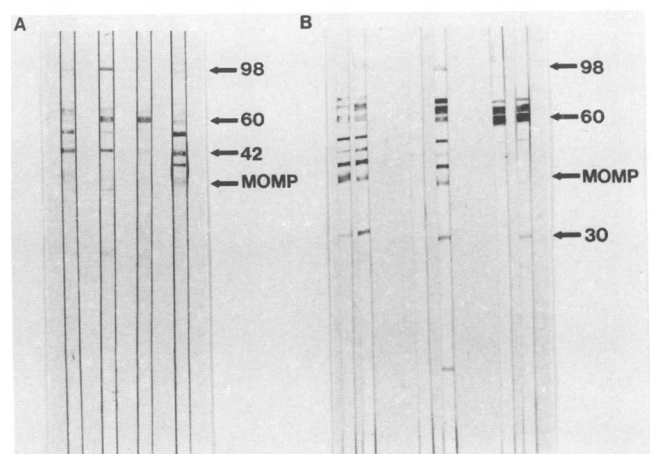


FIG. 2. A representative immunoblot pattern of sera from patients with coronary atherosclerosis (A) and acute respiratory infection (B) reacting against *C. pneumoniae*. Proteins recognized frequently are indicated with arrows (MOMP, major outer membrane protein; 98, 60, 42, and 30, sizes in kilodaltons).

antibodies dissociated from circulating immune complexes from patients with coronary heart disease have been identified to react specifically with the 42- and 98-kDa proteins of *C. pneumoniae* (6). In acute *C. pneumoniae* respiratory infection, reactivity with the 60-kDa protein was often seen, while the 42- and 52-kDa proteins were seldom recognized (1, 7). These results suggest that reactivity with a *C. pneumoniae* 42-kDa and/or 52-kDa protein may be associated with chronic *C. pneumoniae* infection and might serve as a marker of chronicity.

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