Coronary Angioplasty Induces Rise in \textit{Chlamydia pneumoniae}-Specific Antibodies

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\textit{Chlamydia pneumoniae} is frequently found in atherosclerotic lesions, and high titers of specific antibodies are associated with increased risk for acute myocardial infarction. However, a causative relation has not been established yet. We performed a prospective study of 93 patients undergoing percutaneous transluminal coronary angioplasty (PTCA) to investigate whether angioplasty influences \textit{Chlamydia}-specific antibody titers and whether there is an association with restenosis. Blood samples were obtained before and 1 and 6 months after angioplasty. Antibodies against chlamydial lipopolysaccharide and against purified \textit{C. pneumoniae} elementary bodies were measured by enzyme-linked immunosorbent assay (ELISA). After angioplasty, the prevalence of antibodies to lipopolysaccharide rose from 20 to 26% for immunoglobulin A (IgA), from 53 to 64% for IgG, and from 2 to 7% for IgM (P = 0.021, 0.004, and 0.046, respectively). There was a rapid increase of mean antibody titers of all antibody classes within 1 month of PTCA. During the following 5 months, antibody titers decreased slightly but were still higher than baseline values. Results of the \textit{C. pneumoniae}-specific ELISA were essentially the same. The rise of anti-\textit{Chlamydia} antibodies was not caused by unspecific reactivation of the immune system, as levels of antibodies against cytomegalovirus did not change. Neither seropositivity nor antibody titers were related to restenosis. However, increases in mean IgA and IgM titers were restricted to patients who had suffered from myocardial infarction earlier in their lives. In conclusion, we show that PTCA induces a stimulation of the humoral immune response against \textit{C. pneumoniae}. These data support the idea that plaque disruption during angioplasty might make hidden chlamydial antigens accessible to the immune system.

\textbf{MATERIALS AND METHODS}

\textbf{Patient population and blood sampling.} Between December 1994 and October 1995, 106 patients (68 men and 38 women; mean age, 62.9 years; range, 34 to 82) who were consecutive candidates for elective PTCA of a de novo lesion were enrolled in the study. One patient developed liver carcinoma during the follow-up period and was excluded from the study. From 12 other patients, we were not able to obtain blood samples at follow-up investigations for various reasons. Study analysis was done on the remaining 93 patients. All patients had given written informed consent to participate in this study prior to the PTCA procedure. Quantitative analysis of the lesions and of the PTCA result were performed using the Cardiovascular Measurement System (9). Blood was drawn immediately before PTCA and 1 and 6 months after PTCA. At the defined time points, patients also had clinical examinations, including bicycle exercise testing. In cases of suspected restenosis, repeat angiography and, if indicated, repeat PTCA was performed. After the 6-month observation period, each patient was classified by two experienced cardiologists who were unaware of the outcome of laboratory tests to define the clinical outcome of the study. Definitions of clinical end points were (i) recurrent ischemia, defined as either progression or recurrence of anginal complaints and/or a positive exercise test and (ii) restenosis that required repeat revascularization in the same segment as the primary stenosis.

\textbf{Measurement of chlamydial antibodies.} Serological analyses were carried out without prior knowledge of clinical data. All serum samples of a single patient were measured subsequently on the same microtiter plate.

(i) \textit{Chlamydial LPS ELISA.} Tests for antibodies (immunoglobulin G [IgG], IgA, and IgM) to chlamydial lipopolysaccharide (LPS) were done with a commercially available, recombinant enzyme-linked immunosorbent assay (ELISA) kit (MEDAC GmbH, Hamburg, Germany) on a fully automated ELISA proce-
sor (Libertas, Jason, Vienna, Austria). This ELISA includes a chemically pure structure of a recombinant LPS which contains a genus-specific epitope of the *Chlamydia* spp. pathogenic to humans (1). The IgG, IgA, and IgM cutoff values were calculated as prescribed by the manufacturer. Concentrations of chlamydial antibodies were expressed as an index calculated as the optical density of the sample/cutoff.

(ii) Chlamydial EB ELISA. Specific IgG and IgA antibodies to *C. pneumoniae* (strain TW-185) were determined by an in-house-developed enzyme immunosay as described for *C. trachomatis* (21). Briefly, *C. pneumoniae* was propagated in 6-well microtiter plates using optimal conditions (17). Chlamydial elementary bodies (EBs) were partially purified by differential centrifugation through a layer of 35% sodium diolate and used to coat microtiter plates. Serum specimens were diluted 1:1,000 for IgG determinations and 1:500 for IgA determinations. The absorbance of control antigen consisting of partially purified mock-infected cells was subtracted from the absorbance of the *C. pneumoniae* antigen. A titer was calculated by using a twofold dilution series of a positive control included on each plate. Pipetting was carried out using a robotic pipettor (Canberra Packard, Tilburg, The Netherlands) and the microtiter plates were processed with an immunoassay processor (Dynatech Immunoassay System, DPC Nederland BV, Apeldoorn, The Netherlands). Measured antibody titers as well as the sensitivity of the ELISA to detect seropositive individuals show a high correlation with results obtained by the microimmunofluorescence test (11, 20).

Measurement of antibodies to CMV. Quantitative determination of cytomegalovirus (CMV)-specific IgG antibody was performed as described previously (27). Briefly, microtiter plates were coated with protein extracts made from late-stage CMV-infected fibroblasts and with extracts from mock-infected fibroblasts as a control. Serum samples were added in serial twofold dilution beginning with 1:200. To standardize the test run, selected CMV-positive serum pools were prepared and included on each plate as standard serum. Undiluted pool serum was arbitrarily assigned to 100%. The amount of antibody present in the patient’s serum was expressed as a percentage of the antibody present in the reference serum. This procedure resulted in highly reproducible values of antibody concentrations, because dose response curves (optical density versus dilution) of unknown samples were related to the dose response curve of the standard pool. The previously determined cutoff value for CMV seropositivity is >1% for IgG.

Statistical methods. Statistical analysis was performed on a personal computer using the Systat software package version 5.01 (Systat Inc., Evanston, Ill.). The increase in seroprevalence was statistically tested using McNemar’s symmetry chi-square test. Analyses of frequency counts were performed with the use of Fisher’s exact test for small samples. Because of lack of normal distribution, we used the chi-square test. Analyses of frequency counts were performed with the use of Fisher’s exact test for small samples. Because of lack of normal distribution, we used the chi-square test.

RESULTS

Distributions of age, sex, and several known risk factors for atherosclerosis in the 93 patients included in the study are shown in Table 1. In a total of 103 lesions, the residual diameter increased from a mean (standard deviation [SD]) of 0.95 (0.37) mm to 2.04 (0.59) mm. The percentage of diameter stenosis decreased from a mean (SD) of 63.2% (14%) to 26.5% (11%). The reference diameter did not change [mean (SD), 2.66 (0.66) mm to 2.77 (0.71) mm]. The prevalence of several potential risk factors for restenosis, such as diabetes, a lesion in the left anterior descending artery, small vessel diameter (<2.5 mm), hypertension, hypercholesterolemia, and male sex did not differ according to the status of IgG and IgA antibodies to LPS of the patients (data not shown).

At the time of angioplasty, 19 (20%), 49 (53%), and 2 (2%) of the 93 patients had antibodies to LPS of classes IgA, IgG, and IgM, respectively. Within the follow-up period, seroprevalence rose significantly to 29, 64, and 7% for IgA, IgG, and IgM (P = 0.021, 0.004, and 0.046), respectively. There was a rapid increase of mean antibody concentrations of all classes within 1 month after PTCA (Fig. 1). During the following 5 months, antibodies decreased slightly, but were still higher than baseline values. Applying the criteria for acute *Chlamydia* infection proposed by Verkooyen et al. (29), four patients had acute infections during the study period. None of them experienced restenosis and only one had myocarial infarction prior to PTCA.

We also measured antibody titers against purified EBs of *C. pneumoniae*. In this EB ELISA, 72 of 93 patients (77%) tested positive for IgG. Mean titers of IgG antibodies before and 1 and 6 months after PTCA were 3,599, 4,294 (P < 0.00001), and

![FIG. 1. Concentrations of antibodies to chlamydial LPS (IgA, IgG, and IgM; solid line) and to human CMV (IgG and IgM; dotted line) at baseline and 1 and 6 months after PTCA. Concentrations of chlamydial antibodies are expressed as an index (calculated as the optical density of the sample/cutoff) and antibodies to CMV are expressed as a percentage of antibodies present in the reference serum. Error bars indicate standard errors of the means. *P < 0.05; **P < 0.005 versus baseline levels.](http://jcm.asm.org/)

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<th>TABLE 1. Patient characteristics (n = 93 patients)</th>
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* CABG, coronary artery bypass graft; LAD, left anterior descending coronary artery; CX, circumflex artery; RCA, right coronary artery; LM, left main coronary artery.
The conclusion of this paper that angioplasty induces an immunologic reaction to \textit{C. pneumoniae} by releasing or exposing chlamydial antigens requires that these antigens be present in the plaque. Indeed, \textit{C. pneumoniae} is constantly found in time points were 2.6, 3.0, and 3.1, respectively. The differences between the two groups were not statistically significant. Seropositivity and antibody titers of the other Ig classes were also not related to clinically diagnosed or angiographically verified restenosis (data not shown).

Several studies have shown that high titers of antibodies to \textit{C. pneumoniae} are frequently found in patients suffering from acute myocardial infarction. Investigating this issue, we found a close correlation between the rise of chlamydial antibodies after PTCA and previous myocardial infarction (Fig. 3). Significant increases in mean IgA and IgM antibodies occurred only in patients who had suffered from myocardial infarction earlier in their lives. To elucidate if the rise in \textit{Chlamydia}-specific antibody titers was due to an unspecific stimulation of the humoral immune system by the angioplasty procedure, we also measured antibodies to human CMV in these samples. However, mean antibodies to CMV did not change during the observation period (Fig. 1).

**DISCUSSION**

In this study, we showed that PTCA induces a significant rise in mean anti-\textit{Chlamydia} antibodies of IgA, IgG, and IgM classes. This increase was specific for \textit{C. pneumoniae} and occurred primarily in patients with prior myocardial infarction. An association with restenosis was not observed. A rise in antibody titer is generally considered evidence of an acute chlamydial infection and/or reinfection. A twofold or more rise in titer has been proposed as a diagnostic criterion (29). In the present study, most changes in antibody titer were smaller. Only four patients showed serological evidence of acute infection during the surveillance period and thus the prevalence of acute chlamydial infection was not higher than in the general population (10). We believe rather that the observed rise in antibody level reflects an immunologic reaction induced by angioplasty. Balloon angioplasty causes serious damage to the target artery, including plaque disruption, fissuring, and endothelial denudation (18). As a consequence of this injury and of the upcoming healing process, antigens from \textit{C. pneumoniae} immunologic reaction requires that these antigens be present in the plaque. Indeed, \textit{C. pneumoniae} is constantly found in...
Atherosclerotic lesions. However, the reported rates of positive tissue findings vary considerably, mainly depending on the particular detection technique used. By comparing several of these techniques, we have recently shown that some of the known C. pneumoniae antigens are always present in advanced atherosclerotic lesions, but nearly never DNA or RNA (16). Therefore, we can assume the presence of C. pneumoniae antigens in the lesions of most if not all patients.

Acute chlamydial infections within the vessel walls may constitute a risk factor for a vascular event. The previously reported detection of high antibody titers and of Chlamydia-specific immune complexes in the months before and after an acute myocardial infarction and our findings are in agreement with this hypothesis (12, 24). It is interesting that the rise in mean antibody levels of IgA and IgM classes was restricted almost completely to patients who had had a myocardial infarction earlier in their lives. IgA and IgM classes are known to react quickly to acute reinfections (28). The data suggest that those patients who already suffered from an acute cardiac event may have a higher density of chlamydial antigen and/or a higher immunological activity within the plaques. Consequently, patients with unstable angina should have higher antibody titers than those with stable angina. A recent publication reported such an association between high chlamydial antibody levels and instability (26), and a study further exploring this issue is in progress in our laboratory. For detection of antichlamydial antibodies, we used initially a commercially available recombinant LPS ELISA (1). This LPS ELISA is not able to differentiate between antibodies against various Chlamydia species, as the LPS used as antigen is also part of C. trachomatis and C. psittaci. However, it is not very probable that these species contributed substantially to seropositivity in our study. Infections with C. trachomatis are rare in the age group of our patients, who furthermore do not belong to a high-risk population for C. trachomatis. In recent reports on the serological data of patients with coronary artery disease, IgG seroprevalence for C. trachomatis was less than 1%, and no cases of C. psittaci infection were identified (14, 29). Because of the relatively short half-life of anti-LPS antibodies and the quick increase in IgM and IgA reactivity after infection, this LPS ELISA may currently be the most sensitive serological method for the detection of acute chlamydial infection (28). In contrast, to determine the prevalence of C. pneumoniae infection, i.e., establishing the proportion of subjects with C. pneumoniae infection in the past, the microimmunofluorescence or the EB ELISA seems to be more appropriate, as the antibodies to protein antigens last much longer.

![Graph showing concentrations of antibodies to chlamydial LPS at baseline and 1 and 6 months after PTCA](http://jcm.asm.org/)

**FIG. 3.** Concentrations of antibodies (IgA, IgG, and IgM) to chlamydial LPS at baseline and 1 and 6 months after PTCA in patients who had suffered from myocardial infarction prior to PTCA (n = 35; solid line) and in patients who had not (n = 58; dotted line); concentrations of chlamydial antibodies are expressed as an index (optical density of the sample/cutoff). Error bars indicate standard errors of the means. *P < 0.05; **P < 0.005 versus baseline levels.
the idea that C. pneumoniae infection plays a role in the immunopathogenesis of atherosclerosis.

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REFERENCES


