Original Article

Kinetic Study of Antibodies (IgG, IgA) to *Chlamydia trachomatis*: Importance of IgA Antibody in Screening Test for *C. trachomatis* Infection by Peptide-Based Enzyme Immunosorbent Assay

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SUMMARY: To assess the importance of only IgA antibody positivity in the peptide-based ELISA (P-ELISA) examination of kinetic behaviors of antibodies (IgA, IgG) to *Chlamydia trachomatis*, 426 sera from 52 follow-up antigen-positive patients were assayed. In part, a microimmunofluorescence (MIF) test and an immunoblot (IB) assay were also used for confirmation. The results showed that the positivity rates of IgA and IgG antibodies were 82.7 and 96.2%, respectively, at the first testing. One patient had both IgA- and IgG-negative antibodies at the first testing, but this became only IgA-positive and then IgG-positive. The patient was co-infected with *Candida albicans* and *C. trachomatis*, and saw a gynecologist for the symptom of itching. Although the major outer membrane protein was negative in IB assay, the results of the MIF test and absorption experiments were positive. MIF titters for IgA and IgG antibodies to *C. pneumoniae* were <1:8 and 1:32, respectively, at the peak level of P-ELISA. These findings seem to suggest that when only the IgA antibody is detected by P-ELISA, *C. trachomatis* infection may be present at an early stage, so confirmation via testing for *C. trachomatis* is needed.

INTRODUCTION

Recent advances in *Chlamydia* serology have enabled the use of many enzyme-linked immunosorbent assays (ELISAs) that were developed by matching the results of microimmunofluorescence (MIF) test (1) as a standard method throughout the world. Although the presence of an antibody does not always indicate a present infection, serologic tests are useful for pelvic inflammatory diseases (PID) in which pathogenic organisms could not be detected, and for the screening of *C. trachomatis* infections at health centers. Each assay kit has its own characteristics. However, this has not been thoroughly understood, so data have been evaluated against each other under the same consideration. Therefore, results among the different methods have been inconsistently positive or negative in borderline-level antibodies. For instance, peptide-based ELISA (P-ELISA) found that only the IgA antibody, not the IgG antibody, is positive. Few reports provide information regarding the quality of each kit for the sero-diagnosis of *C. trachomatis* infection (2-4). It seems to be considered a temporary nonspecific reaction having no confirmation. We have confirmed that P-ELISA, using a synthetic peptide antigen, has relatively high sensitivity (5-9) and specificity in terms of no reaction with *C. pneumoniae* antibody (10).

In this study, we tried to assess the significance of only IgA antibody positivity by analyzing the kinetic behaviors of serum IgA and IgG antibodies from long-term follow-up adult female patients with *C. trachomatis* infection using P-ELISA, MIF, and immunoblot (IB) assay.

MATERIALS AND METHODS

Sera: Serum samples were collected from 1992 to 1996 from 52 adult female patients with clinically diagnosed cervicitis and/or PID. The patients allowed me to take multiple blood samples. A total of 426 sera samples from 52 patients were used. The cervical swabs of all patients were antigen-positive by a DNA probe (Chugai Pharmaceutical, Co., Ltd., Tokyo, Japan) and/or the IDEIA-*Chlamydia* (Kyowa Medex, Co., Ltd., Tokyo, Japan) method. The rectum swab samples were also positive in some patients tested. The sample sera were taken from patients who agreed to be tested for *C. trachomatis* infection after being briefed on informed consent. After testing, the serum samples were kept at ~80°C until used. The Ethics Committee of Kyorin University approved the use of these sera (No17-6) for the experiments.

ELISA: Peptide-*Chlamydia*-IgA and -IgG [Meinyu] (P-ELISA; Meiji Dairies, Co., Ltd., Tokyo, Japan) and/or the IDEIA-*Chlamydia* (Kyowa Medex, Co., Ltd., Tokyo, Japan) method. The rectum swab samples were also positive in some patients tested. The sample sera were taken from patients who agreed to be tested for *C. trachomatis* infection after being briefed on informed consent. After testing, the serum samples were kept at ~80°C until used. The Ethics Committee of Kyorin University approved the use of these sera (No17-6) for the experiments.

Preparation of *C. trachomatis* and *C. pneumoniae*: *C. trachomatis* (L2/434/Bu (L2)) and *C. pneumoniae* TW183 were grown in HeLa229 cells and HEP-2 cells, respectively, and were purified by Caldwell’s method (purified *Chlamydia*). Then they were passed through Millipore filters (20, 10, 5, 1.6 μm) and harvested as filtrate solutions containing highly purified *Chlamydia* particles (11). The purified *Chlamydia* were suspended in 0.02% formalin-Dulbecco’s PBS (pH 7.3) were kept at ~80°C until used. Before that, a solution was tested to determine protein concentration by the

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BCA method (Pierce Biotechnology, Rockford, Ill., USA), using human serum albumin as the standard.

MIF test: MIF was used as the standard assay. The *Chlamydia* antigens were spotted in a 15-circle area on the glass slide (ICN Biomedicals, Aurora, Ohio, USA). They were dried and fixed with acetone, and reacted with a two-fold dilution of sera and FITC-conjugated anti-human IgA (IgG) antibody (Biosource, Camarillo, Calif., USA). Positive results were expressed as a final serum concentration of positive fluorescence. The positive result for IgA (IgG) was expressed as $\geq 1:16$.

IB assay: The assay method used in these experiments was slightly modified but essentially the same as described previously (11,12). The highly purified chlamydial elementary bodies were prepared successively as described above. *Chlamydia* was dissolved with 2% sodium dodecylsulfate (SDS) at 100°C for 5 min. Two micrograms of protein per lane was electrophoretically separated on 4 - 12% SDS-polyacrylamide gradient gel (SDS-PAGE; TEFCO, Co., Ltd., Tokyo, Japan). The protein fractions were transferred onto nitrocellulose membrane (NCM) by a transblotter (TEFCO) for 75 min. The NCM was washed twice with PBS (NaH$_2$PO$_4$ 0.44g, Na$_2$HPO$_4$ 12H$_2$O 2.58g, NaCl 8.76g/l pH 7.2) by gentle stirring and immersion in 5% skim milk (Difco Laboratories, Detroit, Mich., USA) in PBS at 4°C for 1 h to inhibit nonspecific adsorption of antibodies. The NCM was washed twice with PBS (–) containing 0.05% Tween 20 (PBST) for 5 min and then incubated with the patient’s serum (1:10 for IgA, 1:100 for IgG) diluted with PBS (–) containing 0.5% skim milk at room temperature for 2 h. After five washings for 5 min each, the NCM was incubated with peroxidase-conjugated goat anti-human IgG (Protos Immuno-Research, Burlingame, Calif., USA) at room temperature for 1 h, washed five times for 5 min each, and incubated with a color-developing substrate solution containing 3,3-diaminobenzidine (Dojindo Laboratories, Kumamoto, Japan).

Absorption experiment: Purified *C. trachomatis* (L2) organisms and *C. albicans* cultured on agar plates were suspended in PBS (–). After washing three times with PBS (–) and centrifuged at 12,000 rpm for 10 min, 90 µl sera were added to the bacterial pellets (corresponding to 1.1 × 10$^7$ IFU: 400 µg for L2 and 68 µg for *Candida*) by blending every morning. The mixtures were then left standing at 4°C for 2 days. As a control, separate serum was also kept under the same conditions. Statistical analysis was done using the *t* test.

**RESULTS**

Kinetic behaviors of serum antibody (IgA, IgG) to *C. trachomatis*: Of the 52 cases, 50 were positive for either IgA antibody, IgG antibody, or both (96.2%) at the first testing. Fifty and 43 of the 52 cases were IgG- (96.2%) and IgA- (82.7%) positive, respectively. Kinetic patterns of antibodies after antibiotic treatment were found to be distributed in the four groups at first testing, as summarized in Table 1. Antibiotic treatment was started at least 5 days after the first visit to the clinic. Fourteen of 44 cases (31.8%) of IgA antibody became negative within the observation period (20 to 400 days). Typical cases were further analyzed by the IB method. In one case, the MIF titers of IgA and IgG were $\geq 1:128$, 1:32, 1:32, and 1:64, 1:64, 1:16, respectively. The MOMP band was seen in the IB profile (Fig. 1).

Kinetic behavior of IgA-positive/IgG-negative antibodies: As shown in Fig. 2, this patient visited the clinic at an early stage of *C. trachomatis* infection, because of itching due to co-infection with *C. albicans*. Both *C. trachomatis*

**Table 1. Distribution of kinetic patterns of antibodies to *C. trachomatis* after antibiotic treatment**

| First serological test | Follow-up serum pattern | No. of cases (%)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1. IgA(–) IgG(–)</td>
<td></td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>2. IgA(+) IgG(–)</td>
<td>IgA(+) IgG(–) $\Rightarrow$ IgA(–) IgG(+)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>3. IgA(–) IgG(+)</td>
<td>IgA(–) IgG(+)</td>
<td>8 (15.4)</td>
</tr>
<tr>
<td>4. IgA(+) IgG(+)</td>
<td>IgA(+) IgG(+) $\Rightarrow$ IgA(–) IgG(+)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td></td>
<td>IgA(–) IgG(–)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td></td>
<td>IgA(–) IgG(+)</td>
<td>42 (80.8)</td>
</tr>
<tr>
<td></td>
<td>IgA(+) IgG(–)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td></td>
<td>IgA(–) IgG(+)</td>
<td>10 (19.2)</td>
</tr>
<tr>
<td><strong>Total 52 cases (426 samples)</strong></td>
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Fig. 1. Kinetic pattern of antibodies to *C. trachomatis* in an IgA cutoff index (COI) > IgG COI case in which COI of IgA decreased earlier than IgG and its immunoblotting profiles. The symbols are □, IgA; △, IgG; Ce, cervix; Re, rectum; +, antigen positive; −, antigen negative; m, marker; and a-c shows number of days (4, 37, 182) after first examination, respectively. MIF titers are expressed in Arabic numerals.
antibodies (IgA and IgG) were negative, but this became IgA-positive/IgG-negative and then became IgA-negative/IgG-positive. MIF was also positive, corresponding to the ELISA value. At the peak, MIF titers were 1:16 for IgA and 1:32 for IgG, respectively. Although 30-, 42-, 60-, and 90-KDa bands were detected for IgA antibody in the IB profile, MOMP was not seen clearly. The antibody to *C. pneumoniae* was also detected, but the titers were <1:8 for IgA and 1:32

![Graph](image)

**Fig. 2.** Kinetic pattern of antibodies to *C. trachomatis* in a case which was both IgA and IgG antibodies negative at first testing. The symbols are the same as described in Fig. 1, in which a-d show number of days (7, 17, 32, 69) after first examination.

![Graph](image)

**Fig. 3.** Kinetic pattern of antibodies to *C. trachomatis* in a suspected persistent infection case. The symbols are the same as described in Fig. 1, and a-d show number of days (0, 79, 135, 170) after first examination.

![Graph](image)

**Fig. 4.** Kinetic patterns of antibody to *C. trachomatis* in a re-infection case. The symbols are the same as described in Fig. 1, and a-h show number of days (19, 43, 131, 173, 194, 214, 261, 371) after first examination.
for IgG.

**Kinetic behaviors of antibodies in persistent and/or re-infections:** Persistent infection was conveniently designated in cases when the serum IgA antibody level tended not to decrease within about 6 months after antibiotic treatment, even though the antigen had been negative in cervical swab samples. As shown in Fig. 3, there was no change in the antibody level determined by P-ELISA. MIF titers were also maintained at 1:128-1:64 for IgA and 1:256-1:128 for IgG. MOMP was a major band and lasted throughout testing in the IB assay.

IgA and IgG antibodies were elevated in four patients within the process of decreasing from a high level to the baseline level. A typical case, shown in Fig. 4, seemed to be a re-infection, because the antigen was detected at least from the cervix on different days. The MIF titers also increased, from <1:8 to 1:64 for IgA and from 1:8 to 256 for IgG. In the IB assay, 30-, 60-, and 62-KDa bands were characteristically stained with anti-human IgA antibody. Especially, MOMP, 60-, and 62-KDa bands were stained strongly with anti-human IgG antibody.

**Absorption of antibodies by *C. trachomatis* and *C. albicans***: Though the absorption by *C. albicans* did not affect the intensity of absorbance of P-ELISA, *C. trachomatis* absorbed sera, resulting in one-third that of the control sera (Fig. 5).

**DISCUSSION**

Diagnostic and/or screening tests for detecting antibodies to *C. trachomatis* often have to be performed by using a single serum sample taken from the patient at their first visit to the clinic. To judge whether the data determined were truly positive or not in cases of borderline antibody levels, I investigated the kinetics of antibodies in sera collected from long-term follow-up patients using P-ELISA with high sensitivity and specificity. Our previous study demonstrated that even though *C. pneumoniae* IgG antibody was positive (MIF, 1:16 to 1:256), complete negative results were obtained in P-ELISA (9,10).

The positivity rates of IgA and IgG antibodies were 82.7 and 96.2%, respectively, at the first testing, suggesting that the patients had visited the out-patient clinic after a period of time that was at least long enough for the production of antibodies. IgA positivity (82.7%, day 0) tended to decrease within the term of about 200 days. The IgA antibody level decreased to the borderline level (Fig. 1), which led us to predict that IgA antibody may be detected earlier than IgG in the early stage of infections.

In experiments like this, it would have been ideal if we had been able to compare certain controls: for example, normal women and/or patients without antibiotic treatment. Antibody levels of two normal women (7 months and 12 years) had follow-ups, and the results found baseline level antibodies for those periods (unpublished data). However, there was no chance to make comparisons with ill patients. Kasamatsu et al. (13) have reported that both antigen and antibody had persisted from 11 to 34 weeks after pregnancy without antibiotic treatment. Puolakkainen et al. (14) found that *C. trachomatis* culture-negative patients were stable in negative IgA antibody to *C. trachomatis*. These findings may suggest that the IgA antibody level may not decrease in the presence of *C. trachomatis* antigen.

Although almost all antigen-positive female patients have antibody (IgA and/or IgG) to *C. trachomatis* at their first visit to the clinic, I found a case that seemed to be at the early stages of both *C. albicans* and *C. trachomatis* infection. The patient visited the gynecologic clinic mainly because of the symptom of itching. This was negative for IgA antibody according to P-ELISA and MIF test at the first testing, but IgA antibody became positive after 17 days, and then IgG antibody became positive after 32 days. The absorption experiments led to decreased antibody levels, specifically to *C. trachomatis* serovar L2, but antibodies to MOMP were negative in the IB profile. These results may show that the case was at an early stage of infection, and they strongly suggest that P-ELISA detected the *C. trachomatis*-specific antibody by virtue of its high sensitivity.

In Japan, IgA-positive/IgG-negative cases, such as that discussed above, have tended to be recognized as nonspecific reactions because there are quite a few reports like ours. It has been considered that *C. trachomatis* IgM antibody was detected first, followed by the IgG and then the IgA antibodies (15), although antibody production will be affected by the activity of related cytokines. (i) Animal experiments using monkeys showed that the IgA antibody was detected after the IgG antibody (16). (ii) Almost all of the patients’ sera were MOMP-positive in IB profiles (17), and as the sera of patients who had had an infection much earlier have been tested, the IgA and/or IgG antibodies were usually positive. These reports may suggest that IgG antibody was detected earlier than IgA antibody. Although the IB assay is a useful method for detecting antibodies, its sensitivity may be lower than that of ELISA. As far as we know, there are no reports comparing the sensitivity between ELISA and IB assay in *Chlamydia* research. However, our previous data show that antibodies detected by P-ELISA were positive when the IB assay gave negative results (10,11).

Clad et al. (3) have shown cases that were only IgA-positive in peptide-based ELISA (Labsystems, Helsinki, Finland), but that was negative in MIF, showing that the ELISA is more sensitive than others assays. Hejnar and Koukalova (18) also reported only IgA antibody-positive cases determined by a lipopolysaccharide-coated ELISA assay, in which cases were regarded as IgG production failures. These reports may sug-
gest that the sera of C. trachomatis-infected patients contain various antibodies to different epitopes at different stages of infection, so that P-ELISA seems to detect antibody to the C. trachomatis-specific epitope (variable domain IV) without steric hindrance.

Fortunately, the antigen of P-ELISA is coated with peptides derived from serovars E, G, L2, and C, which are representative of three groups: C (C, A, H, I, Ia), B (B, Ba, D, Da, E, L1, L2, L2a), and intermediate G (G, F, K, L3), so that it can detect antibodies to all serovars of C. trachomatis infections, although both serovars D and E are the most common organisms isolated from patients with sexually transmitted infections in Japan.

We conclude that the synthetic peptide-based ELISA test used in this experiment can detect low-level C. trachomatis-specific IgA antibody at the early stage of infection. Thus an only IgA antibody-positive case should not be neglected when P-ELISA was used in the screening assay. Highly sensitive assay methods, such as PCR and ligase chain reaction (LCR), should be conducted to confirm whether C. trachomatis organism is positive or not.

Typical re-infection cases and cases that seem to be persistent infections were found in this study. Anti-C. trachomatis antibodies (IgA, IgG) increased from the baseline level to the plateau level, and showed strong reactions with MOMP, 60-, and 62-KDa proteins (Fig. 4). Similarly, a case that seemed to be a persisting infection, having undergone no change and a high antibody level for about 6 months, showed a strong reaction to MOMP but a very weak reaction to others for IgA (Fig. 3). A few reports have appeared about the comparative evaluation of serum antibody levels (COIs) and/or IB profiles in long-term follow-up patients (3,12,19-21) Especially, IgA antibody has reacted only with MOMP in our persistent cases. These findings may provide basic information for the evaluation of laboratory data obtained in a single serum sample.

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Parts of these results have been presented at the 13th Conference of the Japanese Association of Sexually Transmitted Diseases (December 2000), the 78th to 80th Conferences of the Japanese Association of Infectious Diseases, and the 101st and 106th Conferences of the American Society for Microbiology (May 20-24, 2001, Orlando, Flor. and June 7-9, 2006, Atlanta, Ga.).

REFERENCES


