Serum specific IgA antibody to *Chlamydia trachomatis* in patients with chlamydial infections detected by ELISA and an immunofluorescence test

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SUMMARY Sera obtained from 34 men with *Chlamydia trachomatis* positive non-gonococcal urethritis, 34 men with *C trachomatis* negative non-gonococcal urethritis, 42 women with acute salpingitis, 38 healthy women, and 34 healthy men were studied for the presence of specific serum *C trachomatis* IgA and IgG antibodies. Serological results were correlated with *C trachomatis* isolation in cell culture.

An enzyme linked immunosorbent assay (ELISA) for *C trachomatis* specific serum IgA was employed using highly purified elementary bodies of *C trachomatis* serotype L2 grown in LLC-MK2 cells. Results obtained for *C trachomatis* IgA antibody by the ELISA test were compared with results obtained for the same sera by a single antigen immunofluorescence technique. A good correlation ($r = 0.91$) was found between two methods. Serum IgG antibody was also determined in the same sera by the immunofluorescence technique.

Patients with *C trachomatis* positive non-gonococcal urethritis had a significantly ($p < 0.0005$) higher prevalence (94.1%) of serum IgA antibody by ELISA compared with patients with *C trachomatis* negative non-gonococcal urethritis (20.5%) or healthy men (5.9%). Similarly, women with acute salpingitis had a significantly ($p < 0.005$) higher prevalence of serum IgA antibody (45.2%) compared with healthy controls (5.2%). Comparable results were obtained for *C trachomatis* serum IgA antibody using the immunofluorescence technique.

The prevalence of *C trachomatis* IgG antibody was significantly higher in patients with *C trachomatis* positive non-gonococcal urethritis (97.0%) compared with those with *C trachomatis* negative non-gonococcal urethritis (33.3%) and healthy controls (23.5%). A significantly ($p < 0.05$) higher prevalence of serum IgG antibody was also found in women with acute salpingitis (52.4%) compared with healthy controls (26.3%).

The importance of using specific *C trachomatis* serum IgA in the identification of chlamydial infection is discussed.

Chlamydia trachomatis is now generally considered the most common sexually transmitted agent. In addition to its recognised role in urethritis, cervicitis, salpingitis, proctitis, conjunctivitis, and pneumonia,¹⁻⁴ subclinical infections by *C trachomatis* appear to be a major cause of infertility in women.^{5,6}

The diagnosis of chlamydial infection is generally made after isolation in cell culture. Reliable cul-

tures, however, are generally available only in large medical centres with extensive experience in tissue culture techniques.

The microimmunofluorescence test of Wang and Grayston⁷ has been shown to be both sensitive and specific for the detection of chlamydial antibodies. Efforts to simplify the test have entailed the use of single broadly reacting antigen, usually L2. Serotype E or serotype L2 have also been used in a single antigen immunofluorescence assay.^{9,10}

More recently, the detection of chlamydial anti-

bodies by enzyme linked immunosorbent assay (ELISA),¹¹⁻¹³ radioimmunoassay,¹⁴ and immunoperoxidase assay¹⁵ has been described.

To date, little is known about the role of serum IgA antibodies in chlamydial and viral infections. With regard to viral infection, several studies¹⁶⁻²¹ have suggested that specific IgA antibody may be an indicator of an active infection.

In order to verify the feasibility of determining *C trachomatis* specific IgA serum antibody as an additional test for patients with chlamydial infections, a single antigen enzyme linked immunosorbent assay was adapted for specific *C trachomatis* serum IgA and compared with an immunofluorescence test. Serum IgG detection by an immunofluorescence technique was also performed on the same sera. Serological results were correlated with *C trachomatis* isolation in cell culture.

Material and methods

STUDY POPULATION

Serum samples and urethral or cervical cultures for *C trachomatis* were obtained from patients included in a multicentre study of sexually transmitted diseases. These included 34 men (mean age 25.4 years) with *C trachomatis* positive non-gonococcal urethritis, 34 men (mean age 24.4 years) with *C trachomatis* negative non-gonococcal urethritis, and 42 women (mean age 28.3 years) with acute salpingitis. The clinical diagnosis of acute salpingitis was based on common criteria, which included lower abdominal pain, abnormal vaginal discharge, adnexal tenderness, erythrocyte sedimentation rate ≥ 15 mm in the first hour, and usually fever ($\geq 38^\circ\text{C}$).

Thirty eight healthy women (mean age 27.2 years) and 34 healthy men (mean age 26 years) were also studied. Cultures for *C trachomatis* were not obtained from these two population groups.

Ten sera from children negative by the immunofluorescence test for serum IgA to *C trachomatis* were used as negative controls in the ELISA test.

CHLAMYDIAL ANTIGEN AND ELISA PROCEDURE

The LLC-MK2 cell line derived from monkey kidney cells was used for growing *C trachomatis*. The cells (which are highly susceptible to *C trachomatis*) were grown in 75 cm³ tissue culture flasks with Eagle's minimum essential medium containing 10% fetal calf serum and 10 $\mu\text{g/ml}$ gentamicin. When confluent monolayers had formed, the medium was replaced with the same medium containing 5% fetal calf serum, 10 $\mu\text{g/ml}$ gentamicin, 1 $\mu\text{g/ml}$ cycloheximide, and 30 μM glucose. LLC-MK2 cell mono-

layers were infected with *C trachomatis* serotype L2 (434-BU strain, provided by J Schachter, San Francisco, California). The infected cultures were incubated at 37°C for 48 h.

Elementary bodies of *C trachomatis* were purified by the method of Caldwell *et al.*²² by Renografin gradients, with minor modifications. Briefly, infected cells were ruptured by sonication and centrifuged. The supernatant was layered over 8 ml of a 35% (vol/vol) Renografin (ER Squibb and Sons Inc, Princeton, New Jersey, USA) solution in 0.01 M HEPES and centrifuged at 43 000 g for 1 h at 4°C in a SW 27 rotor (Beckman Instruments Inc, Fullerton, California.) The pellet was resuspended in 0.01 M sodium phosphate (pH 7.2) and layered over discontinuous Renografin gradients (13 ml of 40%, 8 ml of 44%, and 5 ml of 52% Renografin, vol/vol), centrifuged at 43 000 g for 1 h at 4°C in a SW 27 rotor. The elementary body band was collected by Pasteur pipette, diluted in 0.01 M HEPES, and then centrifuged at 30 000 g for 30 min. The protein content of preparations was determined by the method of Lowry *et al.*²³

Control antigen was prepared in the same way from uninfected LLC-MK2 cells.

Micro ELISA plates (Dynatech M129A) were coated with 100 μl of elementary bodies or control antigen in carbonate-bicarbonate buffer pH 9.6 for 1 h at room temperature. The final protein concentration of elementary body or control antigen preparations was 20 $\mu\text{g/ml}$. Coated plates were washed five times with phosphate buffered saline (PBS)-Tween (Sigma Chemical, St Louis, Missouri) and used immediately. Serial twofold dilutions of human sera in PBS-Tween were made in the test plate. Each serum dilution was tested in elementary body and control antigen coated wells. A known positive and one negative serum sample were assayed on each plate. The plate was then incubated at 37°C for 1 h and washed 10 times with PBS-Tween. Antihuman IgA (α -chain) peroxidase labelled (Dako, Copenhagen, Denmark) diluted 1/400 in PBS-Tween was added in a volume of 100 μl per well. The plate was incubated for 1 h at 37°C , washed 10 times with PBS-Tween. Thereafter, 100 μl of the citric acid phosphate buffer (pH 5.6) containing 40 mg 1.2 phenyldiaminedihydrochloride (Merck, Darmstadt, West Germany) and 20 μl of 30% H_2O_2 per 100 ml buffer was added per well. The enzymatic reaction was stopped after 15 min by adding 100 μl of 2 N H_2SO_4 per well. The result of the test was read at 492 nm by a Dynatech Micro-elisa autoreader MR, 580. All readings were made against a blank well, which received all of the above treatments except that human serum was replaced by PBS-Tween.

The reproducibility of the results was in the range of twofold dilutions.

IMMUNOFLUORESCENCE TEST

All sera were tested for chlamydial antibodies of the IgG and IgA classes by a single antigen test using the L2 strain of *C trachomatis* inclusions in LLC-MK2 cells, as previously described.⁹ Fluorescein labelled antihuman IgG or IgA antibodies (Dako) and dilutions of 1/40 or 1/10 respectively were used.

CHLAMYDIAL CULTURES

Isolation of *C trachomatis* from clinical specimens was done in McCoy cells, which were pretreated with 5'-iodo-2'-deoxyuridine as previously described.⁹

STATISTICAL ANALYSIS

Statistical significance was evaluated by the χ^2 test with Yates's correction, or by Student's *t* test.

Results

STANDARDISATION OF ELISA FOR

DETERMINATION OF *C TRACHOMATIS* IgA

An optimal concentration of 20 $\mu\text{g/ml}$ for elementary bodies was used for coating the microtitre plate. At this antigen concentration serial twofold dilutions (from 1/80 to 1/512) of high, medium, and low

Fig. 1 Titration curves for serum chlamydial IgA antibody by ELISA. Results from high positive (●), intermediate positive (▲), low positive (■), and two negative sera (◇) are shown.

positive sera showed a linear relation to absorbance (Fig. 1).

Antibody titres were expressed as the reciprocal of the highest serum dilution that yielded an optical density at 492 nm > 5 standard deviations over the mean of values obtained with sera from 10 children negative by immunofluorescence for serum IgA to *C trachomatis*, and tested by ELISA in a separate experiment. The IgA antibody titres ranged from 8 to 512.

The titration results for all sera tested with ELISA and immunofluorescence tests are shown in Fig. 2. Of the 182 sera tested 120 were negative and 59 were positive by both techniques. Three samples were positive by ELISA and negative by the immunofluorescence test (see below).

The correlation coefficient for IgA with the two methods was 0.91, showing good agreement.

C TRACHOMATIS IgA ANTIBODY

Antichlamydial serum IgA antibody was found by ELISA in 32 (94.1%) of 34 men with *C trachomatis* positive non-gonococcal urethritis, with a mean titre of 24.8, whereas only seven (20.5%) of 34 men with *C trachomatis* negative non-gonococcal urethritis ($p < 0.0005$) had IgA serum antibody with a mean titre of 28.7. Only two (5.9%) of 34 healthy controls had serum IgA to *C trachomatis*.

Women with acute salpingitis had a significantly ($p < 0.005$) higher prevalence of *C trachomatis* IgA

Fig. 2 Correlation between antibody titres obtained by ELISA and the immunofluorescence test ($n = 182$; $y = 1.58 \times + 0.085$; $r = 0.91$).

antibodies compared with control women: 19 (45.2%) of 42 women with acute salpingitis and two (5.2%) of 38 healthy women were positive for serum IgA to *C trachomatis*. The mean titre of IgA seropositive patients with acute salpingitis was 54.4. Of the 42 women with acute salpingitis, three (7.1%) had positive cervical cultures for *C trachomatis*. All three patients had serum IgA to *C trachomatis* with high titres ranging from 64 to 128. Comparable results were obtained by the immunofluorescence technique.

C TRACHOMATIS IgG ANTIBODY

Patients with *C trachomatis* positive non-gonococcal urethritis had a significantly ($p < 0.0005$) higher prevalence of *C trachomatis* IgG seropositivity than healthy controls (97.0% and 23.5% respectively). A higher prevalence of *C trachomatis* IgG antibody was also found in patients with *C trachomatis* negative non-gonococcal urethritis compared with healthy controls (33.3% v 23.5%), but this difference was not significant.

A significantly ($p < 0.05$) higher prevalence of *C trachomatis* IgG antibodies was also found in patients with acute salpingitis compared with controls (52.4% v 26.3%) (Table).

Discussion

The feasibility of the ELISA technique for chlamydial serology has been shown previously.¹¹⁻¹³ We have developed an ELISA test in which the elementary bodies of *C trachomatis* L2 serotype were used as antigen for the detection of specific serum IgA antibodies to *C trachomatis* in human serum. The results obtained by ELISA were compared with those obtained by the immunofluorescence technique: a positive correlation ($r = 0.91$) was found. Higher titres were obtained by ELISA than by immunofluorescence. With only three exceptions,

however, (three samples positive by ELISA and negative by the immunofluorescence technique) there was agreement as far as IgA seropositivity and IgA seronegativity was concerned.

Two infections which can be caused by *C trachomatis* have been considered in this study: acute salpingitis in women and non-gonococcal urethritis in men. Recent investigations^{3,24-26} have shown that *C trachomatis* can be associated with acute salpingitis. The relative importance of *C trachomatis* as a cause of acute salpingitis varies greatly depending on the population, ranging between 5 and 36% and 20 and 62% when cultural or serological^{25,27} data respectively are considered.

In the current study *C trachomatis* IgG antibodies were determined by the indirect immunofluorescence technique and IgA antibodies were determined by both ELISA and the immunofluorescence technique.

Women with acute salpingitis had a significantly higher prevalence ($p < 0.05$) of *C trachomatis* IgG antibodies compared with matched controls (52.4% v 26.3%). The mean IgG titre (82.0) of the patients with acute salpingitis was significantly ($p < 0.05$) higher than that of controls (13.1). Relatively high *C trachomatis* IgG titres (≥ 64) were noted in 30.9% of the acute salpingitis group, and none of the healthy controls had IgG titres ≥ 64 . These results are in agreement with previous studies using microimmunofluorescence techniques^{28,29} in which a higher prevalence of raised IgG titres was found in patients with acute salpingitis compared with controls. *C trachomatis* IgG titres ≥ 64 by the microimmunofluorescence technique have been suggested to be associated with current chlamydial infection.²⁸ *C trachomatis* IgG antibody, however, may indicate only previous chlamydial infection in sites other than the fallopian tube.

We have shown by both ELISA and the immunofluorescence technique a higher prevalence

Prevalence of serum IgA antibody to *C trachomatis* detected both by ELISA and by an immunofluorescence technique compared with chlamydial IgG antibody detected by an immunofluorescence test in different study populations

Study group	No tested	IgA ELISA				IgA IFA test				IgG IFA test			
		No Positive	(%)	Mean titre	Titre ≥ 32 (%)	No Positive	(%)	Mean titre	Titre ≥ 16 (%)	No Positive	(%)	Mean titre	Titre ≥ 64 (%)
Men with NGU Ch+	34	32	(94.1)	24.8	41.6	31	(91.2)	5.5	14.2	33	(97.0)	28.0	30.4
Men with NGU Ch-	34	7	(20.5)	28.7	14.7	5	(14.7)	4.8	14.2	11	(33.3)	16.9	9.1
Healthy men	34	2	(5.9)	12.5	—	2	(5.9)	2.8	—	8	(23.5)	12.1	—
Women with AS	42	19	(45.2)	54.4	35.7	19	(45.2)	12.3	21.4	22	(52.4)	82.0	30.9
Healthy women	38	2	(5.2)	15.8	—	2	(5.2)	4	—	10	(26.3)	13.1	—

NGU Ch+ = *C trachomatis* positive non-gonococcal urethritis.

NGU Ch- = *C trachomatis* negative non-gonococcal urethritis.

AS = acute salpingitis.

IFA = immunofluorescence.

and raised *C trachomatis* IgA antibody titres in patients with acute salpingitis compared with matched controls. By the ELISA technique, patients with acute salpingitis showed a significantly ($p < 0.005$) higher prevalence of IgA antibodies to *C trachomatis* than the control group ($45.2 \text{ v } 5.2\%$). The mean IgA titre of women with acute salpingitis by the same technique was significantly ($p < 0.01$) higher than that in controls ($54.4 \text{ v } 15.8$). Relatively high *C trachomatis* IgA titres (≥ 32) were found in 35.7% of patients with acute salpingitis, while none of the control group had IgA titres ≥ 32 . Similar results were also obtained by the immunofluorescence technique.

Since the half life of *C trachomatis* IgA was five to six days in normal subjects²⁷ the presence of these antibodies may be a better marker for active *C trachomatis* infection than *C trachomatis* IgG antibody. Confirmation of this will require relating the presence of *C trachomatis* serum IgA antibody with the isolation of the agent from the fallopian tube.

The availability of a serological marker of *C trachomatis* infection in patients with acute salpingitis would be of considerable value and might obviate the need for invasive procedures in the diagnosis of acute salpingitis.

A clear cut association between *C trachomatis* specific serum IgA and active *C trachomatis* infection was found in men with *C trachomatis* positive non-gonococcal urethritis. Of these, 94.1% had *C trachomatis* serum IgA compared with only 20.5% of *C trachomatis* negative non-gonococcal urethritis patients ($p < 0.0005$) and 5.9% of healthy subjects ($p < 0.0005$). Ninety seven per cent of *C trachomatis* positive non-gonococcal urethritis patients also had serum IgG compared with 33.3% of *C trachomatis* negative non-gonococcal urethritis patients ($p < 0.0005$) and 23.5% of healthy men.

In a previous study, Thero and Meurman,¹⁴ using a radioimmunoassay technique, found serum IgA in only 53% of isolation positive as against 21% isolation negative men with urethritis. The different results obtained in the current study can be tentatively explained both by the different population examined and by the methods used. In this study specific *C trachomatis* IgA antibody was always found in association with serum IgG antibody: the higher the IgG titre the higher the IgA titre, the highest IgA titres being found in patients with acute salpingitis. In *C trachomatis* positive non-gonococcal urethritis patients serum IgA was also found in the presence of low (16–32) IgG titres.

In conclusion, the presence of serum IgA antibody to *C trachomatis* seems to be correlated with active *C trachomatis* infection, at least in patients with chlamydial non-gonococcal urethritis. Hence,

IgA detection may be a useful further test in chlamydial serology. However, the possibility that *C trachomatis* serum IgA can be used as a marker of a current *C trachomatis* infection needs further investigation. The appearance and persistence of *C trachomatis* IgA have to be examined in prospective studies and correlated with the development of symptomatic or asymptomatic *C trachomatis* infections.

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