CHLAMYDIAL SERUM IgG, IgA AND LOCAL IgA ANTIBODIES IN PATIENTS WITH GENITAL-TRACT INFECTIONS MEASURED BY SOLID-PHASE RADIOIMMUNOASSAY

P. TERHO AND O. MEURMAN

Department of Virology, University of Turku, Käinamyllynkatu 10, 20520 Turku 52, Finland

SUMMARY. A solid-phase radioimmunoassay (RIA) for IgG and IgA class antibodies to *Chlamydia trachomatis* was developed with *C. trachomatis* serotype L2 as antigen. The assay was sensitive, reproducible and correlated well with an immunofluorescence test ($r = 0.85$). Serum IgG antibodies were detected in 79% of *Chlamydia* isolation-positive versus 43% of isolation-negative male patients with urethritis, and serum IgA antibodies in 53% and 21%, respectively. Urethral IgA antibodies, measured from specimens taken for chlamydial isolation, could be detected in 94% and 38%, respectively. From 737 male urethral and 909 female cervical secretions screened for the presence of IgA antibodies, about half were isolation and IgA negative. Only 4% (6/151) of male and 5.4% (2/37) of female isolation-positive specimens were IgA negative. The determination of local IgA antibodies may be used as a screening test in chlamydial genital infections.

INTRODUCTION

During recent years, *Chlamydia trachomatis* has been proved to be the major aetiological agent in male nonspecific urethritis (NSU) and in postgonococcal urethritis (PGU) (Holmes *et al*., 1975; Oriel *et al*., 1975; Terho, 1978a and b) and in cervical disease of women (Oriel *et al*., 1978).

The most reliable way to diagnose chlamydial infection is to isolate the parasite in a cell culture, e.g., irradiated McCoy cells, after centrifuging the inoculum on to the cells to enhance infectivity (Schachter, 1978b).

However, drawbacks of the isolation method, such as loss of infectivity of the specimen due to transport delays or temperature or gross contamination by other micro-organisms, and the unavailability of the isolation techniques have emphasised the need for practical serological tests for chlamydial infections.

Several methods have been presented, the most useful until now being the microimmunofluorescence test (Wang and Grayston, 1970), which also allows the separate detection of antibodies belonging to different immunoglobulin classes (Treharne, Dines and Darougar, 1977; Wang *et al*., 1977). This
test has, however, its limitations; it requires highly skilled reading personnel and the reagents must be strictly controlled.

In our laboratory, sensitive and specific solid-phase radioimmunoassays (RIAs) have been developed and successfully used for measuring viral antibodies (Arstila et al., 1977; Meurman, 1978). The present report describes the adaptation of this RIA technique for the demonstration of chlamydial IgG and IgA antibodies in serum and in genital secretions from patients with genital-tract infections.

**Materials and methods**

*Patients and specimens.* The study material comprised the following groups.

I: 104 serum specimens from 52 patients with gonococcal urethritis (GU) including 31 patients who were chlamydia isolation positive and developed PGU. The remaining 21 patients, who served as controls, were isolation negative and did not develop PGU. These patients have been described earlier (Terho, 1978b).

II: 64 serum specimens and 58 urethral-secretion specimens from patients with chlamydia isolation-positive NSU (16 men) or chlamydia isolation-negative NSU (26 men). These patients were studied as reported earlier (Terho, 1978a).

III: 28 serum specimens and eight urethral-secretion specimens from eight male patients with a remote history of chlamydial NSU.

IV: Urethral secretion specimens from 151 chlamydia isolation-positive and from 586 chlamydia isolation-negative men, and cervical-secretion specimens from 37 chlamydia isolation-positive and 872 chlamydia isolation-negative women. These specimens had been submitted to our laboratory during March–November 1978 for routine isolation of *C. trachomatis*.

V: 14 serum specimens from seven patients with pulmonary psittacosis diagnosed by significant titre changes (four patients) or high titres (three patients) in the complement-fixation (CF) test. These sera were kindly supplied by Dr Jukka Suni, from the Aurora Hospital, Helsinki.

The urethral and cervical specimens were collected with a cotton-tipped endourethral or endocervical swab into 1.1 ml of 2SP transport medium (0.2 M sucrose, 0.02 M phosphate) supplemented with foetal calf serum (FCS) 3%, gentamicin 50 μg/ml and nystatin 25 units/ml. This method was found to result in an approximately 1 in 50 dilution of urethral secretion. The same 2SP specimen was used for isolation of *C. trachomatis* as described by Terho (1978c) and for antibody determination by RIA.

*Antigen production for RIA.* The antigen was *C. trachomatis* serotype L2 (strain 434-B, originally obtained from the Institute of Ophthalmology, London, through the courtesy of Dr Sohrab Darougar and Dr John Treharne). It was passaged in our laboratory in Roux bottles in irradiated McCoy cells until 80–90% of cells contained visible inclusions by inverted light microscopy. After incubation for 72 h, the infected cells were collected in phosphate-buffered saline (PBS), pH 7.4, disrupted by ultrasonic treatment, and the cell debris was pelleted by centrifugation at 500 g for 10 min. From the supernate the antigen was pelleted at 24 000 g for 30 min (Sorvall RC2-B). The pellet was resuspended in PBS and was used as antigen for RIA. The protein concentration was determined by the method of Lowry et al. (1951) and was adjusted to 1000–2000 μg/ml. The infectivity of this semipurified antigen was $10^3$–$10^4$ inclusion-forming units/μg.

*Antisera.* The heavy-chain specific antihuman IgG, IgM and IgA immunoglobulins were isolated by immunoabsorption column chromatography from sera obtained from Orion Diagnostica (Helsinki), and the isolated homogeneous antibodies were labelled with $^{125}$I (Radiochemicals Centre, Amersham, Bucks) by the method of Hunter and Greenwood (1962). The specific activities of the labelled antihuman immunoglobulins ranged from 5 to 10 μCi/μg.

The labelled antihuman immunoglobulins were diluted in Eagle’s minimum essential medium containing heat-inactivated calf serum 10%, lactalbumin hydrolysate 0.5%, Tween 20
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1%, and Na3N 0.1%. They were standardised to give 5000 cpm bound when 0.2 ml of the labelled antibody preparations were incubated with balls coated with purified human IgG, IgM and IgA for antihuman IgG, IgM and IgA, respectively.

RIA procedure. Polystyrene balls of 6.4 mm diameter (Precision Plastic Ball Co., Chicago, Ill, USA) were coated with the antigen by incubating the balls submerged in an antigen solution (PBS) containing antigen 5 µg/ball at room temperature overnight.

Serum and secretion specimens were diluted in PBS containing bovine serum albumin (BSA) 0.5% and Tween 20 0.5%. Serial dilutions of specimens in 0.2 ml portions were incubated in polystyrene tubes with an antigen-coated ball for 2 h at 37°C. After washing, the balls were incubated with 0.2 ml of 125I-labelled antihuman IgG, IgM or IGA immunoglobulins for 2 h at 37°C. After a final wash, the balls were placed in clean tubes and assayed for bound radioactivity. Appropriate controls, including a positive and a negative reference serum and buffer blanks, were included in each assay.

The results were usually expressed as endpoint titres, estimated as the highest dilution of the test specimen where the cpm value was three times that of the negative control at the same dilution, with the proviso that the cpm of the test specimen should be 150 or more. The urethral and cervical secretion specimens of group IV were tested solely in dilution 1 in 100, i.e., a twofold dilution from the 2SP specimen, and the results were expressed directly as cpm values.

Immunofluorescence test. An indirect immunofluorescence (IF) test with infected (serotype L2) irradiated McCoy cell monolayers as antigen was used (Punnonen et al., 1979).

Statistics. The detection rates of antibodies in chlamydia isolation-positive and negative patients were compared by the Yates's chi-square test, and the mean antibody titres by the t-test. A comparison of antibody titres obtained by RIA and IF test was made by calculation of Spearman's correlation coefficient.

RESULTS

Development of the assay

Initial experiments were done with egg-grown C. trachomatis serotype L2 as antigen. However, extensive purification of the antigen by density-gradient ultracentrifugation proved necessary before it could be used in RIA. When antigen from infected McCoy cells was used in RIA, this purification step was unnecessary, but the semipurified lysate antigen reacted as well in the test. This method proved to be a very convenient and economical way to prepare the antigen; about 1 mg of semipurified antigen could be obtained from one Roux bottle of infected McCoy cells.

When the antigen was adsorbed to the polystyrene balls used as the solid phase, a concentration of 5 µg/ball was found to be optimal for saturation. The pH of the reaction diluent was not critical, hence PBS, pH 7.4, was used. Two hours was selected as the incubation time for specimens and labelled antisera. This resulted in a better assay sensitivity than 1-h incubations, and still allowed the assay to be completed during one working day. An overnight incubation would have increased the sensitivity, but it was considered to be inconvenient.

The sensitivity of the IgG RIA was compared with that of the IF by parallel testing of 104 paired serum specimens from the 52 patients in group I (fig. 1). The RIA titres were on average four times higher than the IF titres, but some individual variation was observed. None of the specimens negative in the IF had antibodies by the RIA test, but five specimens negative in the RIA had a
low level of antibodies when tested by the IF. There was a good agreement between the two tests ($r = 0.85$).

The reproducibility of the assay was good as shown by the inter-assay variance in RIA for IgG antibodies (fig. 2). For a positive serum, the mean coefficient of variation of cpm of dilutions 64–4096 was 15.5%. A twofold variation was observed in the endpoint titres obtained.

**Chlamydial antibodies in male patients with urethritis**

The chlamydial RIA antibody titres of the initial specimens from patients in groups I and II are presented in table I. When patients with *C. trachomatis*
Fig. 2.—Inter-assay variance in the solid-phase radioimmunoassay for IgG antibodies to *Chlamydia trachomatis* (serotype L2). Positive and negative sera were tested on 10 different days during a 6-month period. The solid lines indicate mean values; the broken lines indicate standard deviation.
### Table I

**Distribution of chlamydial RIA antibody in sera and urethral secretions of male patients with chlamydia isolation-positive or isolation-negative urethritis**

| Result of chlamydia isolation test | Antibody | Number of patients | <64 (%) | 64 | 128 | 256 | 512 | 1024 | 2048 | 4096 | GMT |
|-----------------------------------|----------|--------------------|---------|----|-----|-----|-----|-----|-----|-----|-----|-----|
| Positive                          | Serum    | 47                 | 10 (21) | 1  | 0   | 2   | 11  | 10  | 12  | 1   | 932 |
|                                   | IgG      |                    |         |    |     |     |     |     |     |     |     |     |
| Positive                          | Serum    | 47                 | 22 (47) | 2  | 2   | 5   | 3   | 11  | 1   | 1   | 562 |
|                                   | IgA      |                    |         |    |     |     |     |     |     |     |     |     |
| Positive                          | Urethral | 16                 | 1 (6)   | 0  | 0   | 2   | 2   | 5   | 4   | 2   | 1123|
|                                   | IgA      |                    |         |    |     |     |     |     |     |     |     |     |
| Negative                          | Serum    | 47                 | 27 (57) | 0  | 3   | 8   | 7   | 0   | 1   | 1   | 375 |
|                                   | IgG      |                    |         |    |     |     |     |     |     |     |     |     |
| Negative                          | Serum    | 47                 | 37 (79) | 1  | 0   | 4   | 0   | 4   | 1   | 0   | 478 |
|                                   | IgA      |                    |         |    |     |     |     |     |     |     |     |     |
| Negative                          | Urethral | 26                 | 16 (62) | 1  | 3   | 1   | 4   | 0   | 1   | 0   | 294 |

GMT = geometric mean titre from patients with a titre ≥64.

Isolation-positive and negative urethritis are compared, 79% versus 43%, respectively had serum IgG antibodies ($\chi^2 = 11.4, p < 0.001$), 53% versus 21% had serum IgA antibodies ($\chi^2 = 8.9, p < 0.001$), and 94% versus 38% had urethral IgA antibodies ($\chi^2 = 10.4, p < 0.001$). The geometric mean titres (GMT) of serum IgG antibodies and urethral IgA antibodies of antibody-positive specimens were in isolation-positive patients significantly ($t = 3.95, p < 0.001$, $t = 3.53, p < 0.01$, respectively) higher than in isolation-negative patients. The GMTs of serum IgA antibodies in these two groups did not differ significantly ($t = 0.25$).

The antibody titres of subsequent specimens, which were taken 1–5 weeks after the initial specimens, were usually equal to the initial titres. In only four out of 40 isolation-positive patients with paired serum specimens was a fourfold or greater increase in antibody titres observed. The increase was detected in one case in serum IgG and in IgA titres, in two cases in serum IgG titres only, and in one case in serum IgA titres only. Paired urethral specimens were available from 10 patients with chlamydia isolation-positive urethritis. Significant increases in urethral IgA antibodies were not noticed.

From 42 patients of group II, serum and urethral secretion were available. In 13 patients (including 10 chlamydia isolation-positive) serum and urethral IgA antibodies were detected, in 17 patients (including one chlamydia isolation-positive) neither was detected, and in 12 patients (including four chlamydia isolation-positive) urethral IgA antibodies without serum IgA antibodies were detected. In no case was serum IgA antibody found without a simultaneous detection of urethral IgA antibody.

Forty-four male urethral specimens were tested by RIA for IgG antibodies. Urethral IgG antibodies were found in 12 out of 27 IgA-positive specimens. In
each case the IgG level was lower than the IgA level. In no case was IgG antibody detected in the absence of IgA antibodies.

To obtain information about the persistence of chlamydial antibodies, serum and urethral-secretion specimens from patients who had had remote chlamydia isolation-positive urethritis were tested. Chlamydial serum IgG antibodies were detected in seven out of eight patients in the initial serum specimens. During the follow-up of 2–4 years, when the patients remained clinically free from urethritis, four patients lost their IgG antibody and in one other case there was a fourfold decrease in titre. In two patients the serum IgG titres remained constant. Serum IgA antibodies were initially detected in three out of eight patients. During the follow-up, one patient lost his serum IgA antibody, one showed a fourfold decrease in titre, and one had a constant serum IgA antibody titre. Urethral IgA antibodies were detected in five out of eight patients in the specimens taken 2–4 years after chlamydial urethritis. The GMT of these positive specimens was 512, compared with 1123 for the patients with actual isolation-positive chlamydial urethritis (table I).

Chlamydial IgM antibodies could not be detected with acceptable accuracy in sera of patients in different study groups. This was despite the use of the same batches of antihuman IgM immunoglobulins that have worked well, e.g., in measuring rubella IgM antibodies (Meurman, 1978).

**Determination of local chlamydial IgA RIA antibody as a screening test**

The use of local chlamydial IgA antibody determination as a screening test was evaluated by testing 737 male urethral specimens and 909 cervical specimens simultaneously by isolation and by IgA RIA (tables II and III). When the IgA antibody results were expressed as cpm values at the dilution 1 in 100, 52.6% of the isolation-negative male urethral specimens had a value of 200 cpm or less, whereas only 4% (6 out of 151) of the isolation-positive specimens belonged to this group. This difference is highly significant ($\chi^2 = 113.9, p < 0.001$). A very high cpm was observed in 189 (25.6%) of male urethral specimens and 98 (51.9%) were isolation positive (table II).

RIA on female cervical specimens showed a similar distribution of cpm for IgA (table III). Nearly half (423 out 872) of the isolation-negative specimens belonged to the IgA-negative group. Out of the isolation-positive specimens 5.4% (2 out of 37) had 200 cpm or less. This figure differs highly significantly from the isolation-negative cervical specimens ($\chi^2 = 24.8, p < 0.001$). Only 7% (63 out of 909) of cervical specimens had more than 1000 cpm and one fifth of these (13 out of 63) were isolation positive.

**Chlamydial RIA serology in patients with psittacosis**

Fourteen sera from seven patients with pulmonary psittacosis were tested by CF, and IgG and IgA RIA (table IV).

All the four significant titre changes in the CF test were also detected by RIA. This change was observed in IgG (one case), in IgA (one case) and in
TABLE II
*Distribution of chlamydial IgA RIA cpm from male urethral specimens at an approximate dilution of 1 in 100*

<table>
<thead>
<tr>
<th>IgA (RIA cpm)</th>
<th>Number of specimens</th>
<th>Number of chlamydia isolation positive</th>
<th>Percentage isolation positive of the group</th>
<th>Percentage isolation positive of all positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤200</td>
<td>314</td>
<td>6</td>
<td>1.9</td>
<td>4.0</td>
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<tr>
<td>201–1000</td>
<td>234</td>
<td>47</td>
<td>20.1</td>
<td>31.1</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>189</td>
<td>98</td>
<td>51.9</td>
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</tr>
<tr>
<td>Total</td>
<td>737</td>
<td>151</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

RIA = radioimmunoassay.

TABLE III
*Distribution of chlamydial IgA RIA cpm from female specimens at an approximate dilution of 1 in 100*

<table>
<thead>
<tr>
<th>IgA (RIA cpm)</th>
<th>Number of specimens</th>
<th>Number of chlamydia isolation positive</th>
<th>Percentage isolation positive of the group</th>
<th>Percentage isolation positive of all positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤200</td>
<td>423</td>
<td>2</td>
<td>0.5</td>
<td>5.4</td>
</tr>
<tr>
<td>201–1000</td>
<td>421</td>
<td>22</td>
<td>5.2</td>
<td>59.5</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>63</td>
<td>13</td>
<td>20.6</td>
<td>35.1</td>
</tr>
<tr>
<td>Total</td>
<td>909</td>
<td>37</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

RIA = radioimmunoassay.

TABLE IV
*Chlamydia1 antibodies in sera of patients with psittacosis determined by CF and by RIA tests*

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Serum no.</th>
<th>CF titre</th>
<th>RIA IgG titre</th>
<th>RIA IgA titre</th>
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</thead>
<tbody>
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<td>1</td>
<td>I</td>
<td>32</td>
<td>1024</td>
<td>1024</td>
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<td></td>
<td>II</td>
<td>128</td>
<td>4096</td>
<td>2048</td>
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<td>2</td>
<td>I</td>
<td>8</td>
<td>&lt;64</td>
<td>256</td>
</tr>
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<td></td>
<td>II</td>
<td>128</td>
<td>512</td>
<td>1024</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>4</td>
<td>&lt;64</td>
<td>64</td>
</tr>
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<td>II</td>
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<td>512</td>
<td>256</td>
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</tr>
<tr>
<td>4</td>
<td>I</td>
<td>256</td>
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<td>256</td>
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</tr>
<tr>
<td>7</td>
<td>I</td>
<td>128</td>
<td>16000</td>
<td>8192</td>
</tr>
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</table>

CF = complement fixation; RIA = radioimmunoassay.
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both classes (two cases) of RIA antibodies. Two patients with elevated CF antibodies had IgG and IgA RIA antibodies in a high titre, while one patient having high titres of CF and RIA IgG antibodies lacked RIA IgA antibodies.

DISCUSSION

The results of this study indicate that the RIA test developed is practical and reliable for measuring chlamydial IgG and IgA antibodies. The commonly used CF test for chlamydial serology measures antibodies against group antigen shared by *C. trachomatis* and *C. psittaci*. The present RIA test probably also detects group-specific antibodies as can be seen in table IV. It is not yet clear whether this RIA test measures species-specific or type-specific antibodies. Studies on sera from infected humans and experimental animals are in progress in our laboratory.

Serotype L2 was selected as an antigen because of its broad cross reactivity with genital *C. trachomatis* serotypes (Wang et al., 1977). The use of combined LGV serotypes (L1, L2 and L3) or paratrachoma (Treharne et al., 1977) might, however, enhance the sensitivity of the test, particularly in the measurement of antibodies against types H, I, J and K.

A significant practical problem in the serological diagnosis of chlamydial urethritis is the difficulty of obtaining appropriately timed specimens to demonstrate titre increases (Schachter, 1978b). Despite the high sensitivity of our RIA test, we could detect titre increases in only 10% of chlamydia isolation-positive urethritis patients. The RIA test thus has no substantial advantage over IF methods for the serological diagnosis of acute NSU.

Chlamydial urethritis in men was found, almost invariably, to induce a local IgA antibody response. Because the urethral IgA titres were usually higher than the corresponding serum IgA antibody titres, and because urethral IgA antibodies were often detected in the absence of serum IgA antibodies, it is evident that the urethral antibodies are produced locally and not passively diffused from the serum. This also seems logical because chlamydial infections are mostly local infections of secretory mucosal surfaces. Similar local antibody responses have been detected in chlamydial eye infections, e.g., trachoma (Grayston et al., 1977).

Because urethral IgA antibodies were often detectable even 3 years after infection, the presence of these antibodies cannot be used as definitive evidence of a recent infection. Similarly, the frequent presence of local IgA antibodies in the cervical secretions of isolation-negative women questions the diagnostic significance of a positive cervical IgA antibody finding (Schachter et al., 1979). However, with regard to the data obtained, the absence of local IgA antibodies is a strong indication that there is no actual chlamydial infection. Here our results differ from those presented by Ng et al. (1978) and by Schachter et al. (1979), who could not detect local antibodies in 36% and local IgA antibodies in 37% of their isolation-positive cases, respectively. On the contrary, results that agree with ours were reported by McComb et al. (1979), who detected
cervical antibodies almost invariably in isolation-positive women but in less than 40% of sexually experienced isolation-negative women.

By the use of the local IgA antibody determination as a screening test and using 200 cpm at the dilution of 1 in 100 as a negativity limit, we would have been able to avoid the isolation procedure in 50% of the cases with a loss of less than 5% of our isolation-positive cases. Thus the use of local IgA antibody determination as a screening test in routine diagnosis would mean a considerable saving in laboratory work and expenses especially when populations with a low prevalence of chlamydial infections are studied. Because the antibody determination is made from the same specimen as the isolation, it would not increase the clinician's work.

Unlike the isolation procedure, the determination of local IgA antibodies is not sensitive to transport delays and storage conditions. Some of the isolation-negative specimens could have been initially positive but, on arrival at the laboratory, might already have lost their infectivity. They could, of course, also have been from patients who had had chlamydial infection in the past, because in some cases local IgA antibodies were detected up to 3 years after chlamydial urethritis. The reason for such a prolonged local IgA response could be a latent local infection analogous to that documented in trachoma (Hanna et al., 1968) and suggested also in the case of genital infections (Jones, 1977; Schachter, 1978a).

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