J Clin Pathol 1986;**39**:1313–1316

Class specific immunoglobulin response to individual polypeptides of *Chlamydia trachomatis*, elementary bodies, and reticulate bodies in patients with chlamydial infection

R CEVENINI, F RUMPIANESI, M DONATI, A MORONI, V SAMBRI, M LA PLACA

From the Institute of Microbiology, University of Bologna, Ospedale San Orsola, Bologna, Italy

SUMMARY Sera from 10 women with *Chlamydia trachomatis* culture positive cervicitis and sera from six men with *C trachomatis* positive non-gonococcal urethritis were studied for the presence of IgG, IgM, and IgA antibodies to polypeptides of *C trachomatis* elementary bodies and reticulate bodies using immunoblotting techniques. All the sera with IgG, IgM, or IgA immunoglobulins specific to *C trachomatis* recognised the major outer membrane protein (MOMP) of elementary bodies. IgG antibodies also detected several other proteins, whereas IgM immunoglobulins recognised only MOMP and proteins of 60 kD, 62 kD, and 66 kD. The IgA reacted with MOMP and the 60 kD and 62 kD proteins in elementary bodies. Class specific antibody response against the proteins of reticulate bodies was similar to that observed for elementary body antigens—with one substantial difference: no reaction was observed in the 60 kD and 62 kD positions. This suggests that 60 kD and 62 kD proteins are deficient in reticulate bodies.

Chlamydiae are pathogenic bacteria that multiply within the cytoplasm of eukaryotic cells, causing these infectious elementary bodies to alternate with non-infectious reticulate bodies.[1]

Genital tract infections caused by *Chlamydia trachomatis* are a source of major interest in sexually transmitted diseases. Consistent experimental evidence has already been obtained about the aetiological role of *C trachomatis* in non-gonococcal urethritis in men and cervicitis in women.[2] The host humoral response to chlamydial antigens in both natural and experimental infections is constant against the major outer membrane protein (MOMP), and often recurring against 60 kD and 62 kD proteins of elementary bodies.[3 4] Some sera also react with 45 kD protein and sometimes with other less immunogenic proteins.[3 4]

This investigation aimed to define the antigenic specificity of class antibody response of patients with *C trachomatis* cervicitis and urethritis. Sera from patients and controls were tested by SDS-PAGE immunoblotting using purified elementary bodies and reticulate bodies of *C trachomatis* LGV2 serotype as the antigen.

Accepted for publication 30 June 1986

Material and methods

Twelve women with *C trachomatis* culture positive cervicitis and six men with *C trachomatis* culture positive non-gonococcal urethritis (NGU) were included in this study. The patients were selected on the basis of culture positivity, and the presence in their serum of IgG specific to *C trachomatis* (titre $\geqslant$ 1:16) as determined by immunofluorescence. Sera from 10 *C trachomatis* negative healthy subjects were also studied as controls.

C trachomatis PURIFICATION

LGV2 serotype (434/BU strain) was grown in LLC-MK2 cells, and elementary bodies were purified 48 hours after infection according to the technique of Caldwell *et al*,[5] as described previously.[6] Reticulate bodies were purified 20 hours after infection using 30,

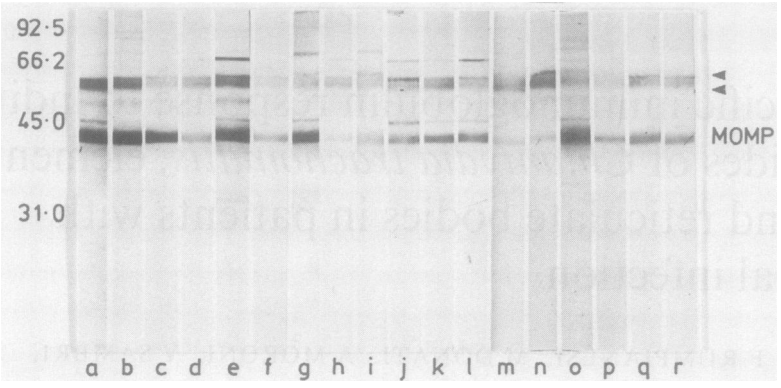

Fig 1 *Immunoblot pattern of sera from 12 patients with cervicitis (lanes a–l) and from six patients with urethritis (lanes m–r). Purified C trachomatis (LGV2 serotype) elementary bodies were applied. Rabbit immunoglobulins to human IgG was used as second antibody. Position and molecular weight of markers are indicated on the left. Arrows indicate 60 kD and 62 kD proteins.*

35, and 40% Renografin (ER, Squibb, Princeton, New Jersey) step gradients.[7] The SDS-PAGE was performed according to the procedure of Laemmli.[8]

IMMUNOBLOTTING

The immunoblot procedure of Towbin *et al*[9] and of Bittner *et al*,[10] as modified by Batteiger *et al*,[11] was used, as previously described.[4]

Electrophoretic transfer was done in 25 mM sodium phosphate (pH 7·5) at 27 volts (0·9 to 1·0 A) for two hours at 20°C to HAHY millipore nitrocellulose paper with transblot cell (Bio-Rad Laboratories, Richmond, California). After transfer the nitrocellulose paper was incubated in 50 mM sodium phosphate, 0·15M sodium chloride, 0·02% sodium nitrate, containing 3% bovine serum albumin (BSA), for 30 minutes. The nitrocellulose paper was then divided into strips and these were incubated with the patient's serum 1/100 diluted in phosphate buffered saline containing 3% BSA for 16 hours on a rocker platform. The nitrocellulose paper was washed three times for 10 minutes with phosphate buffered saline followed by incubation with peroxidase labelled rabbit immunoglobulins to human IgG, IgM, or IgA (Dako, Copenhagen, Denmark), respectively, diluted 1/1000 (IgG) and 1/400 (IgM and IgA) in phosphate buffered saline for two hours at room temperature. The nitrocellulose strips were then washed three times for 10 minutes with phosphate buffered saline and transferred to distilled water. The immunoblots were developed with a solution of 40 mg benzidine (Fluka AG, Buchs, Switzerland) dissolved in 4 ml acetone, 96 ml phosphate buffered saline, and 0·1 ml hydrogen peroxide from 33% stock solution. After development the blots were repeatedly rinsed with distilled water.

CHLAMYDIAL SEROLOGY

The elementary bodies and the reticulate bodies of *C trachomatis* LGV2 serotype, purified as described above, were also used as antigen in a microimmunofluorescence assay (MIF) to assess serum titres specific for IgG, IgM, or IgA immunoglobulins.

Results

Fig 1 shows the SDS-PAGE pattern of *C trachomatis* elementary bodies antigens by immunoblotting with sera from 12 women with cervicitis and six men with urethritis, using rabbit immunoglobulins to human IgG as the second antibody. All the sera (with MIF titres for IgG ranging from 1/16 to 1/4096) reacted with the major outer membrane protein (40 kD) and with at least two other polypeptides of 60 kD and 62 kD. Some sera with MIF titres ranging from 1/64 to 1/4096 also reacted with 66 kD (lanes e, h, j, k, l), with 45 kD protein (lanes a, e, g, j, k, l, o), and with other minor proteins. Fig 2 shows the pattern obtained with the same sera tested with the same procedure against purified *C trachomatis* reticulate bodies. All the sera reacted with MOMP, some (lanes c, e, j, k, l, n, o) also reacted with the 66 kD protein, but no reaction was observed in the 60 kD and 62 kD positions. When the elementary body immunoblots were developed with the sera of two women exhibiting positive (≥ 1/32) MIF IgM titres and rabbit immunoglobulins specific to human IgM, only a slight reac-

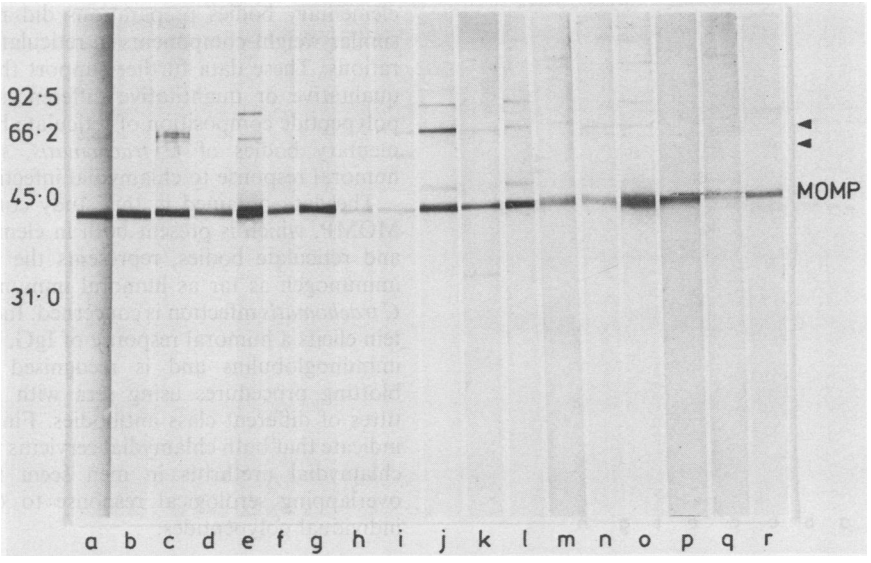

Fig 2 *Immunoblot pattern of same sera as described in fig 1, but reticulate bodies were applied for purified C trachomatis. Arrows indicate 60 kD and 62 kD positions.*

tion with the MOMP and with the 60 kD, 62 kD, and 66 kD proteins was observed (data not shown). Negative results were obtained with reticulate body preparations (data not shown). The reactivity of IgA antibodies to *C trachomatis* elementary bodies and reticulate body individual polypeptides was also studied, using as second antibody, rabbit immunoglobulins specific to human IgA. The sera with a MIF IgA titre of $\leqslant$ 1/16 gave negative results in the blot both with elementary bodies and reticulate bodies. All the IgA positive sera with a MIF IgA titre of $\geqslant$ 1/32 recognised MOMP, and most of these sera also identified 60 kD and 62 kD proteins in the blot performed with elementary bodies (fig 3). When tested with reticulate body preparations (fig 4) some sera (lanes a, b, d, f, and h) detected MOMP and 66 kD protein, and two sera (lanes c and g) detected only MOMP. In the 60 kD and 62 kD positions no reaction was observed. Sera from healthy subjects, which were negative by the various MIF tests, did not react against any *C trachomatis* protein.[4]

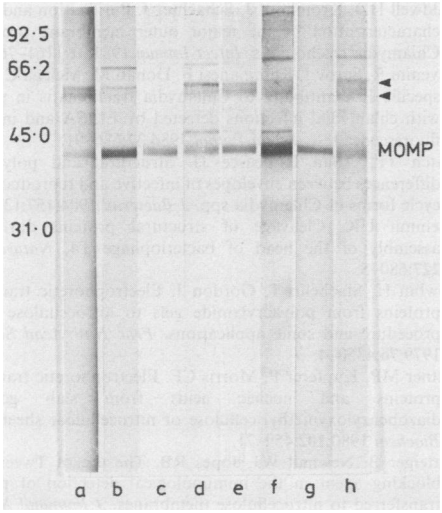

Fig 3 *Immunoblot pattern of sera from six patients with cervicitis (lanes a–f) and two patients with urethritis (lanes g and h). Purified C trachomatis elementary bodies were applied and rabbit immunoglobulins to human IgA were used as second antibody. Arrows indicate 60 kD and 62 kD proteins.*

Discussion

The outer membrane protein of *C trachomatis* was shown to be one of the primary antigens recognised after animal immunisation with chlamydial elementary bodies.[5] Other data suggest that the MOMP of *C trachomatis* elementary bodies is also of primary importance in the IgG humoral response to *C trachomatis* in patients with chlamydial urethritis.[3 4]

Our investigation on women with cervicitis and men with urethritis confirmed these findings, showing the relevance of the MOMP as immunogen. In fact, all the sera positive by the MIF test, with titres $\geqslant$ 1/16 for IgG or $\geqslant$ 1/32 for IgA recognised the MOMP both of elementary bodies and reticulate bodies. Sera with an IgM MIF titre of $\geqslant$ 1/32 recognised at least the MOMP of elementary bodies. Sera

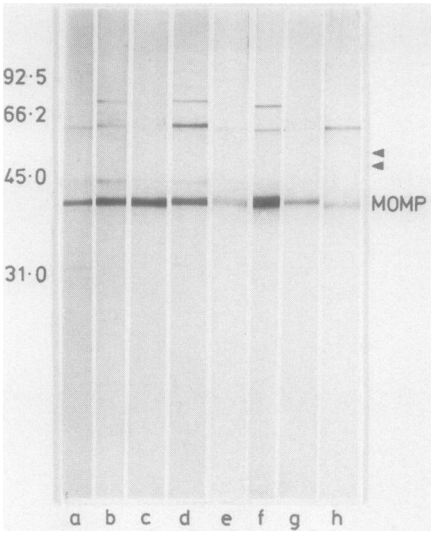

Fig 4 *Immunoblot pattern of sera as described in fig 3, but reticulate bodies were applied. Arrows indicate 60 kD and 62 kD positions.*

with MIF IgM or IgA titres lower than 1/32 failed in our experimental conditions to detect this antigen in reticulate bodies preparations by immunoblotting. The IgG response against the 60 kD and 62 kD proteins of elementary bodies was quite constant and paralleled that observed against the MOMP. Most of the sera with MIF IgA titres $\geqslant$ 1/32 also recognised these proteins. Other elementary bodies proteins, in addition to the MOMP, the 60 kD, and the 62 kD proteins, were detected by IgG specific immunoglobulins, and in particular the 45 kD and the 66 kD proteins. The IgM immunoglobulins could identify only the 60 kD, 62 kD, and 66 kD proteins besides the MOMP.

The class specific antibody response against reticulate body preparations showed some differences compared with that of the elementary bodies. Most notable was the absence of any reaction in the 60 kD and 62 kD positions when the blot of reticulate bodies were tested with immune sera.

In a previous study Hatch *et al*[7] observed that *C trachomatis* elementary bodies harvested 48 hours after infection possess three cysteine rich outer membrane proteins of 62, 59, and 12 kD, whereas reticulate bodies harvested intracellularly 21 hours after infection were severely deficient in cysteine rich proteins but possessed almost as much MOMP as the elementary bodies. Our results seem to confirm indirectly the deficiency of the 60 kD (this protein may correspond to the 59 kD protein reported by Hatch *et al*[7]) and the 62 kD proteins in reticulate bodies as all the sera reacting with the 60 kD and 62 kD proteins of elementary bodies preparations did not react with similar weight components in reticulate body preparations. These data further support the existence of qualitative or quantitative differences between the polypeptide composition of reticulate bodies and elementary bodies of *C trachomatis*, shown by the humoral response to chlamydial infection.

The data obtained in this study confirm that the MOMP, which is present both in elementary bodies and reticulate bodies, represents the most relevant immunogen as far as humoral immune response to *C trachomatis* infection is concerned. Indeed, this protein elicits a humoral response of IgG, IgM, and IgA immunoglobulins and is recognised by immunoblotting procedures using sera with relatively low titres of different class antibodies. Finally, our data indicate that both chlamydial cervicitis in women and chlamydial urethritis in men seem to induce an overlapping serological response to *C trachomatis* individual polypeptides.

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Requests for reprints to: Dr R Cevenini, Institute of Microbiology, University of Bologna, Ospedale S. Orsola, 9 Via Massarenti, 40138 Bologna, Italy.