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SIGNIFICANTLY HIGHER SEROLOGIC RESPONSES OF *CHLAMYDIA TRACHOMATIS* B GROUP SEROVARS VERSUS C AND I SEROGROUPS

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SUMMARY

Chlamydia trachomatis *serovars are divided into three serogroups, namely serogroup B, serogroup I (Intermediate) and serogroup C, and subsequently into 19 different serovars. Worldwide, serogroup B is the most prevalent followed by serogroup I. Clear differences have been observed in the duration of infection and growth kinetics between serovars from different serogroups in murine and cell culture models. Reasons for these observed differences are bacterial and host related, and are not well understood. The aim of this study was to determine the differences in immunoglobulin (Ig) G responses between the three serogroups in a group of patients infected with different serovars. Serovars were assessed from 235* C. trachomatis*-positive patients and quantitative IgG responses were determined. Analyses of variance were used to compare the IgG responses between the three serogroups. Of the serovars, 46% were B group (with serovar E the most prevalent: 35.3%), 39.6% were I group and 14.3% were C group. A highly significant difference in serologic response was shown when comparing the mean IgG concentrations (AU/mL) of patients having serovars in the most prevalent serogroup compared to the other serogroups: B = 135, C = 46 and I = 60 (B vs. C and B vs. I, P < 0.001). In conclusion, the most prevalent serovars generate the highest serologic responses.*

INTRODUCTION

Chlamydia trachomatis is one of the most common bacterial sexually transmitted diseases (STDs) worldwide. In most cases, infected patients undergo an asymptomatic and uneventful course of infection, and are thus likely to remain untreated. Untreated *C. trachomatis* can give rise to late complications, including pelvic inflammatory disease (PID), ectopic pregnancy, and tubal pathology resulting in tubal infertility (1).

Research devoted to the bacterium has provided insight into the structural components of *C. trachomatis*. Strains of *C. trachomatis* are classified into serovars based on nucleotide sequence differences in the *omp*1 gene, encoding the major outer membrane protein (MOMP) (2). To date, 19 serovars of *C. trachomatis* are known, generally causing conjunctival and urogenital infections (3). The different serovars are divided into serogroups based on phylogenetic mapping (Table I).

Conventional serotyping involves *C. trachomatis* culture and both monoclonal and polyclonal antibodies against the MOMP protein. Currently, polymerase chain reaction (PCR)-based techniques allow rapid identification of different serovars (4-6). Geographical distributions of serovars/serotypes are very similar worldwide, except in small core groups (e.g., men having sex with men [MSM] and small communities [7, 8]).

Relations between specific serovars and the clinical course of infection have been observed (9-11), although conflicting data have been reported (12-15). Ito et al. demonstrated that serovars D and E (belonging to serogroup B) cause the longest duration of infection in a murine model. Furthermore, a comparison of the invasiveness of strains D and H demonstrated a much higher frequency of uterine horn infection with serovar D (16). In addition, they studied the in vitro growth and elementary body (EB)-associated cytotoxicity of *C. trachomatis* serovars D and H in order to identify the above mentioned differences. These differences correlate with virulence variations between these strains in the mouse model of human female genital tract infection, and with phenotypic characteristics that could explain human epidemiological data on serovar prevalence and levels of shedding during serovar D and H infection. They showed that serovar H elementary bodies (EBs) were significantly more cytotoxic compared with serovar D EBs, which have a longer duration of infection in the murine model and are much more prevalent in humans (17). The data suggest a relation between the different serovars and the serologic responses in the murine model. This relation has not yet been studied in humans, but the murine and in vitro data suggest different serologic responses could be observed.

Different commercial serologic assays to detect IgG against *C. trachomatis* are currently available (18). Medac Diagnostika has recently developed a new *C. trachomatis* IgG ELISA kit (*Chlamydia trachomatis*-IgG-ELISA plus) which allows quantitative measurement of IgG levels in serum, enabling study of the relation between host serologic responses and specific serovars.

The aim of this study was to elucidate serologic IgG responses in patients infected with different serogroups.

Table I. *Serovar distribution into serogroups.*

Serogroup	Serovar
B	B, Ba, D, Da, E, L1, L2, L2a
C	A, C, H, I, Ia, J, K, L3
I (Intermediate)	F, G, Ga

PATIENTS AND METHODS

Patient populations

The study included 235 *C. trachomatis*-infected patients, who visited either the outpatient department (OPD) of the Department of Obstetrics and Gynecology at the MC Haaglanden Clinic, or the STD outpatient clinic in the Hague, the Netherlands from January to October 2008. In the Department of Obstetrics and Gynecology clinical samples were obtained from patients visiting the OPD for various reasons including pregnancy, discharge, menstrual disorders, subfertility and contraception. At the STD outpatient clinic reasons for visiting were ST-related complaints, partner notification or STD check-up at the client's request. Information was collected about age, gender (STD clinic only, OPD all female), ethnicity and symptoms (i.e., asymptomatic, symptoms, or upper genital tract infection). Serum samples were collected from all patients.

C. trachomatis detection and genotyping

For the detection of *C. trachomatis* we used a probe hybridization assay from urethral and endocervical swabs (PACE 2 assay, Gen-Probe). Swabs were analyzed within 24 h according to Gen-Probe's package insert instructions.

Amplification, detection and genotyping using the C. trachomatis *DT assay*

C. trachomatis detection and genotyping were determined on all samples positive for *C. trachomatis*, using the *C. trachomatis* (CT)-DT detection and genotyping assay (8). The CT-DT amplification, detection and genotyping steps were performed according to the manufacturer's instructions (Labo Biomedical Products BV, the Netherlands). Briefly, first the CT-DT amplification step was performed on extracted DNA to amplify all serovars available in GeneBank. Second, the *C. trachomatis* detection step was performed to confirm the results detected with the PACE2 assay. The *C. trachomatis* detection step detects all serovars, subserovars and genovariants in GeneBank, but cannot differentiate between serovars. Borderline samples were considered positive. Finally, all PCR products that were positive with the *C. trachomatis* detection step were further analyzed with the CT-DT genotyping assay. The CT-DT genotyping assay is a reverse hybridization probe line blot (RHA) with a probe for the detection of the cryptic plasmid, and probes to detect the three different *C. trachomatis* serogroups (B, C and Intermediate) and the different serovars (A, B/Ba, C, D/Da, E, F, G/Ga, H, I/Ia, J, K, L1, L2/L2a and L3).

Serology

Determination of IgG levels in serum of all patients was done with ELISA, as described below. The ELISA procedure using the *Chlamydia trachomatis*-IgG-ELISA plus kit (Medac Diagnostika), was performed according to the manufacturer's protocols. The assay employed was a quantitative IgG serology kit. Calculations to determine concentrations of IgG (arbitrary units [AU]/mL) in the serum were performed according to the protocols provided. Concentrations below 22 AU/mL were considered negative, concentrations 22–28 AU/mL were considered equivocal, and concentrations above 28 AU/mL were considered positive, as per the manufacturer's instructions.

Statistical analyses

Analysis of variance (ANOVA) statistics were used to compare the IgG serum levels of the three *C. trachomatis* serogroups. Equivocal values were excluded from these calculations. Analyses were performed with GraphPad Instat 3. P values ≤ 0.05 were considered significant: P values between 0.05 and 0.1 were considered a statistical trend.

RESULTS

Serovars

The serovar distribution in the cohort is shown in Table II. Serovar E (35.3%; serogroup B) was the most prevalent, followed by serovar F (25.1%) and serovar G/Ga (14.5%; serogroup I) (Table II). It was found that 108 of the samples (46.0%) contained serovars belonging to serogroup B, 93 samples (39.6%) belonged to serogroup I and 34 samples belonged to serogroup C (14.5%). The serovars A, C (both ocular serovars) and L1–L3 were not observed in the cohort studied.

Serological IgG responses

Serum *C. trachomatis* IgG concentrations were measured for all patients using the Medac *Chlamydia trachomatis*-IgG-ELISA plus assay and mean *C. trachomatis* IgG levels (AU/mL) were calculated per serogroup, as shown in Table II. Serogroup B, containing the most prevalent serovar E, had the highest mean IgG concen-

Table II. *Serovar distribution and mean concentrations of IgG per serogroup.*

Serogroup	Serovar	No. of patients	%	Total patients per serogroup	%	Mean [IgG]*
B	B/Ba	2	0.9	108	46.0	134.9
	D/Da	23	9.8			
	E	83	35.3			
C	H	3	1.3	34	14.5	45.9
	I/Ia	4	1.7			
	J	21	8.9			
	K	6	2.6			
I	F	59	25.1	93	39.6	60.2
	G/Ga	34	14.5			
		235	100%	235	100%	

*Mean IgG concentrations in arbitrary units per mL (AU/mL), as per manufacturer's instructions.

tration. Highly significant differences were observed comparing serogroup B to the other serogroups (B vs. C: $P < 0.001$; B vs. I: $P < 0.001$), while no statistically significant differences were observed between serogroups C and I (C vs. I: $P > 0.05$).

DISCUSSION

This is the first study to compare serologic IgG responses in urogenital *C. trachomatis* infections based on serogroup. As expected, serovar E was the most prevalent serovar in our study, followed by serovars F and G/Ga. The mean *C. trachomatis* IgG response was significantly the highest in serogroup B (including the most prevalent serovar E), compared with the other serogroups. No differences were observed in the mean *C. trachomatis* IgG concentrations between serogroups C and I.

This study clearly shows that serovar E (in the B serogroup) results in the highest serologic responses. This result is partly in line with a previous study showing that variable segments of the serovar E *MOMP* induce high serologic responses (19). A similar increase in serologic responses to a specific serovar (D) has recently been described in an Indian population (20). Furthermore, Morré et al. have shown that serovar E is more frequent in persistent infections compared with resolving infections (21). In the study by van der Snoek et al. it was shown that rectally L2-infected men had significantly higher serologic titers compared with men not infected rectally with lymphogranuloma venereum (LGV) (22). The authors concluded that these significantly increased titers, which can slowly diminish over time, probably represent the severe, more invasive and more often chronic inflammation of the rectum caused by LGV serovars, compared with other serovars. Murine studies have shown that serovar D results in a longer and more invasive infection compared to serovar H, but resulted in less cytotoxicity (17). Combining the results from this study with the published literature clearly shows that specific serovars may elicit stronger serologic responses compared with other serovars, and that this might be due to a more aggressive, or invasive course of infection. Specifically, serovars in the B serogroup (i.e., serovars D, E and L2) seem to result in more severe infections.

These results and those on the toxicity of *C. trachomatis* serovars (17, 23) can be combined with data on development of complications and symptoms in order to gain more insight into the immunological responses underlying *Chlamydia* pathogenesis.

CONCLUSIONS

In conclusion, the present study demonstrates that serovars of serogroup B induce higher serologic responses than serovars of serogroups C and I. The results of this study will be included in a much larger European Union Framework study to confirm these results, to further enhance the power of the study, and enable multivariate analyses to obtain further insight into the immunopathogenesis of *C. trachomatis* infections. This will enable risk profiling of at-risk patients to prevent development of long-term complications.

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DISCLOSURE

The authors have nothing to disclose.

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