

SHORT REPORT

Chlamydia trachomatis serovar E isolates from patients with different clinical manifestations have similar courses of infection in a murine model: host factors as major determinants of *C trachomatis* mediated pathogenesis

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Background: Some investigators have proposed an association between certain *Chlamydia trachomatis* serovars and the clinical course of infection in humans. A recent study of over 1100 patients with culture confirmed and serotyped *C trachomatis* urogenital infection detected no such association.

Aims: To corroborate these results using a murine model of female genital tract infection.

Methods: Various parameters of infection were assessed in mice intravaginally infected with human genital isolates of *C trachomatis* serovar E from four cases with either a clear symptomatic or asymptomatic clinical course in both the patient and their partner.

Results: No differences were seen among the strains in the incidence or duration of infection, polymorphonuclear granulocyte responses, or upper genital tract progression.

Conclusions: An investigation to determine the correlation between the clinical manifestations of different isolates of *C trachomatis* serovar E in humans and certain parameters of microbial pathogenesis in a mouse model failed to reveal an association between the measured parameters and the tendency of serovar E to produce symptomatic versus asymptomatic infections in humans. These findings suggest that differences in the clinical course of infection in humans seen with these strains may be more related to host factors than to genetic variation among strains.

Some studies have shown an association of certain *Chlamydia trachomatis* serovars with specific clinical symptoms and upper genital tract progression, whereas others have found no such association. These conflicting results are partially the result of small study groups, potentially confounding factors, and inconsistent clinical characterisation. Recently, a very large study provided evidence that the role of specific serovars in defined clinical outcomes may be limited. Geisler *et al* studied over 1100 patients with culture confirmed and serotyped *C trachomatis* infection and concluded that there was no association between clinical manifestations and the infecting serovar.¹

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Analysis of the course of infection in an animal model using clinically well characterised human isolates might be

helpful in addressing this issue, by allowing the controlled assessment of the role of host versus pathogen factors that influence the course of genital tract infection. Therefore, using a murine model of female genital tract infection that can reproducibly distinguish between different human isolates,² we investigated the course of *C trachomatis* infection in mice using four serovar E isolates from cases in which a clear symptomatic or clear asymptomatic course was observed in both the index patients and their partners. The incidence and duration of infection, the polymorphonuclear granulocyte (PMN) response, and upper genital tract progression were assessed after lower genital tract infection.

MATERIAL AND METHODS

Bacterial strains and culture

Four *C trachomatis* serovar E strains isolated from female patients with either a clear symptomatic (S1, S2) or asymptomatic (AS1, AS2) clinical course in both the patient and their partner³ (table 1) were propagated, titrated, and isolated in cyclohexamide treated HeLa cell monolayers using standard techniques.

Murine model, inoculation, and specimen collection

Using a standard model of female genital tract infection,^{4 5} four groups of 12 progesterone pretreated CF-1 female mice were inoculated intravaginally by direct instillation of 10 µl of bacterial suspension containing approximately 1 × 10⁵ inclusion forming units. Four mice from each strain were euthanised at day 14, the remaining mice at day 56. Eight mice served as non-infected controls. Lower genital tract specimens were obtained by swabbing the vaginal vault and ectocervix with a calcium alginate swab every two to seven days up until day 56. Swabs were placed in transport medium (2-SP) and immediately tested for PMN content before being frozen. Genital tract tissues and local lymph nodes were aseptically isolated, divided into specimens, and stored in 2-SP for culture and polymerase chain reaction (PCR) analysis. All specimens were frozen at –70°C until tested.

Culture, PCR, and PMN responses

The presence of *C trachomatis* in both the material obtained by swabbing the lower genital tract, and the thawed, homogenised, and centrifugation clarified supernatants from the upper genital tract was determined using previously described culture and PCR techniques.^{3 5 6} Finally, lower genital tract inflammation was monitored during the first two weeks of infection by assaying for PMN associated

Abbreviations: IL, interleukin; PCR, polymerase chain reaction; PMN, polymorphonuclear granulocyte

Table 1 Clinical characteristics associated with *Chlamydia trachomatis* serovar E isolates

Strain	Self reported complaints				Clinical signs of infection	
	Dysuria	Abn Vag Dis	Discharge	LAP	Discharge*	Leucocytes†
	Men/Women	Women	Men	Women	Men/Women	Men/Women
S1	+/-	+	+	+	m/mp	10/9
S2	+/-	+	+	+	p/mp	10/9
AS1	-/-	-	-	-	-/-	10/0
AS2	-/-	-	-	-	-/m	10/9

*Discharge: -, none; m, mucous; mp, mucopurulent; p, purulent; †leucocytes, number of leucocytes/field, 0-10 or >10.

Abn Vag Dis, abnormal vaginal discharge; LAP, lower abdominal pain.

esterase activity (Multistix® 2 reagent strips; Bayer Corporation, Diagnostic Division, Elkhart, Indiana, USA).

STATISTICAL ANALYSIS

Duration of infection data were analysed using the Wilcoxon rank sum test and the frequency data by χ^2 or Fisher exact tests where appropriate.

RESULTS

Lower genital tract infection

No significant differences were noted in the incidence or duration of infection either among strains or between the symptomatic and asymptomatic *C trachomatis* serovar E isolates (table 2). All culture positive samples were also PCR positive, and PCR detected additional positive specimens, both for culture negative time points between two culture positive time points (n = 14) and after the last culture positive time points (n = 28). All samples were both culture and PCR negative by day 56. PMN responses in the lower genital tract were also not significantly different either between strains within each group or between the combined data for each group (table 2).

Upper genital tract infection

Progression of the infection into the upper cervix and uterine horns was detected both by culture and, at greater frequency, by PCR, but no differences were noted at day 14 between mice infected with *C trachomatis* from either symptomatically or asymptotically infected women, and no signs of gross pathology were visible. All upper genital tract material was negative for *C trachomatis* on day 56, which was also the case for all lymph nodes and ovaries from both time points. All control mice were negative for *C trachomatis* by either PCR or culture.

DISCUSSION

Using a well established murine model of *C trachomatis* female genital tract infection, we showed that there were no differences in the incidence and duration of infection, the lower genital tract PMN response, or the upper genital tract progression, after lower genital tract infection with clinical serovar E isolates from female patients with either a clear symptomatic or asymptomatic course of infection. These experimental data are in agreement with the findings from the most recent human epidemiological study of Geisler *et al.*,¹ suggesting that, at least within a given oculogenital serovar, genetic variation among strains may not strongly contribute to the course of infection. However, it is important to note that our study was not intended specifically to identify possible host or chlamydial factors that might contribute to diverse clinical outcomes. Of interest within the context of our report are the possible roles that newly described polymorphisms within the chlamydial plasticity zone, such as those described in the tryptophan synthase operon,⁷ might play a role in subtle host-pathogen interactions that affect the course of infection, in addition to the stable pattern of serovar prevalence that exists worldwide among clinical oculogenital isolates.

"It seems reasonable to suggest that the focus of future studies to elucidate the basis for differences in clinical course should include analyses of host genetic factors"

Although our study was limited to a single serovar, serovar E is the most prevalent one isolated from genital tract infections, and the lack of variation in the course of infection among these four strains in the murine model suggests that the bacterial background may not have played an important role in the observed clinical differences in the women from

Table 2 Comparative summary of course of murine genital tract infection with different clinically characterised *Chlamydia trachomatis* serovar E isolates

Strain	Incidence of infection*	Median duration of infection†	Vaginal PMN content during the acute phase of infection‡			Upper genital tract infection§		
			Neg	Trace	+ / ++	Upper cervix	Uterine horns	Ovaries and tubes
S1	6/8	31.5	22%	58%	20%	3/4	2/4	0/4
S2	6/8	31.5	20%	62%	18%	2/4	3/4	0/4
Combined	12/16	31.5	21%	60%	19%	5/8	5/8	0/8
AS1	8/8	35	15%	60%	25%	2/4	3/4	0/4
AS2	5/8	35	20%	55%	25%	2/4	2/4	0/4
Combined	13/16	35	18%	57%	25%	4/8	5/8	0/8

No significant differences were obtained for the parameters evaluated.

*An animal was considered infected if one or more vaginal samples was positive either by culture or PCR; †the duration of infection for a given animal was assigned as the last positive vaginal sample, with the median duration determined from only the infected animals in a group; ‡PMN responses were determined during the first two weeks of infection as instructed by the manufacturer; the percentage of the total number of specimens tested during this period within each category of response is shown; §a tissue was considered infected if positive either by culture or PCR.

PCR, polymerase chain reaction; PMN, polymorphonuclear granulocytes.

Take home messages

- Specific *Chlamydia trachomatis* serovars, which are based on the *omp1* gene encoding the major outer membrane protein, have been suggested to be associated with specific clinical manifestations in humans
- Using a murine model of female genital tract infection, no difference was seen in the course of infection among human genital isolates of the most prevalent *C trachomatis* serovar associated with human genital tract disease (serovar E) obtained from cases with either a clear symptomatic or asymptomatic clinical course in both women and their partners
- Because the serovar determining genetic background of oculogenital serovars of *C trachomatis* may not play an important role in the course of infection, future studies should also be directed at an analysis of host genetic factors that might influence the course of infection; that is, immunogenetic approaches

whom these strains were isolated. Thus, it seems reasonable to suggest that the focus of future studies to elucidate the basis for differences in clinical course should include analyses of host genetic factors. The study of the host genetic background in relation to disease and infection, called immunogenetics, is a new and rapidly developing field. By determining immune mediators that are important in the susceptibility to infection and the severity of subsequent disease, the assessment of functional mutations in the corresponding genes will potentially lead to the identification of the relevant host factors that contribute to an increased risk for severe disease. For *C trachomatis* infections important mediators such as interferon γ , interleukin 12 (IL-12), IL-10, and tumour necrosis factor α have been identified using knockout mouse strains and antibody depletion techniques. Based on these and related findings, studies have already been conducted that link an increased risk of pelvic inflammatory disease and tubal infertility with functional polymorphisms in the IL-10 gene and with certain major histocompatibility complex class I alleles and HLA types,^{8,9} and studies are in progress to determine the possible role of interferon γ , IL-12, and the lipopolysaccharide and heat shock protein responsive Toll-like receptor 4¹⁰ in susceptibility to and severity of *C trachomatis* genital tract infection.

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