**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  

J M Lyons, J I Ito Jr, S A Morré

**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.

**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 partner 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, 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 x 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. 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
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 strains within each group or between the combined data for each group (table 2).

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Self reported complaints</th>
<th>Clinical signs of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dysuria</td>
<td>Abn Vag Dis</td>
</tr>
<tr>
<td></td>
<td>Men/Women</td>
<td>Women</td>
</tr>
<tr>
<td>S1</td>
<td>+/−</td>
<td>+</td>
</tr>
<tr>
<td>S2</td>
<td>+/−</td>
<td>+</td>
</tr>
<tr>
<td>AS1</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>AS2</td>
<td>−/−</td>
<td>−</td>
</tr>
</tbody>
</table>

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

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).

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Incidence of infection†</th>
<th>Median duration of infection‡</th>
<th>Vaginal PMN content during the acute phase of infection§</th>
<th>Upper genital tract infection††</th>
<th>Ovaries and tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neg Trace /++</td>
<td>Upper cervix Uterine horns</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>6/8</td>
<td>31.5</td>
<td>22% 58% 20%</td>
<td>3/4 2/4</td>
<td>0/4</td>
</tr>
<tr>
<td>S2</td>
<td>6/8</td>
<td>31.5</td>
<td>20% 62% 18%</td>
<td>2/4 3/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Combined</td>
<td>12/16</td>
<td>31.5</td>
<td>21% 60% 19%</td>
<td>5/8 5/8</td>
<td>0/8</td>
</tr>
<tr>
<td>AS1</td>
<td>8/8</td>
<td>35</td>
<td>15% 60% 25%</td>
<td>2/4 3/4</td>
<td>0/4</td>
</tr>
<tr>
<td>AS2</td>
<td>5/8</td>
<td>35</td>
<td>20% 65% 22%</td>
<td>2/4 2/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Combined</td>
<td>13/16</td>
<td>35</td>
<td>18% 57% 25%</td>
<td>4/8 5/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

††Upper genital tract infection: Data for each group (table 2).
§§PMN responses were determined as those described in the tryptophan synthase operon, 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, 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...
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 ocugenital 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, 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 4 in susceptibility to and severity of C. trachomatis genital tract infection.

ACKNOWLEDGEMENTS

The authors thank J A R van den Hoek (Municipal Health Service, Amsterdam, The Netherlands) for selection of the patients with clearly symptomatic or asymptomatic Chlamydia trachomatis infections in both the index patients and their partners, and J M Ossewaarde (National Institute for Public Health and the Environment, Research Laboratory for Infectious Diseases, Bilthoven, The Netherlands) for the high titre stock cultures of the clinical C. trachomatis isolates used for the infection of the mice.

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Accepted for publication 3 December 2003

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J Clin Pathol 2004 57: 657-659
doi: 10.1136/jcp.2003.013086

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