Persistence of Chlamydia trachomatis infections: Bacterium and host based? letter

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Persistence of Chlamydia trachomatis Infections: Bacterium and Host Based?

To the Editor—We read with interest the publication by Dean et al. [1] and would like to comment on both the reported serovar J variant and the persistence of Chlamydia trachomatis infection. While the cervical persistence of C. trachomatis infection was being studied, a new variant J genotype, termed “Ja” (GenBank accession no. AF202458), was identified. Compared with the prototype J sequence, there were 7 nucleotide substitutions, each of which encoded for a change in amino acid. Four of these amino acids were nonconservative; in addition, 6 silent mutations were found. Of interest, we have published the omp1 sequence of another serovar J variant, termed “Jv” [2]. However, our variant and Ja were not compared in the article by Dean et al. [1], probably because the sequence of Jv had not been submitted to GenBank, although the complete sequence was published [2].

Table 1 compares all nucleotide substitutions in serovars Ja and Jv with those in serovar J. Surprisingly, all mutations reported in Ja are also present in Jv. In addition, 2 more mutations are present in Jv, both resulting in amino acid changes (variable segment 1 [VS1] and constant segment 2 [CS2]). As expected for structural conserved segments, all silent mutations were found in the constant segments, whereas all amino acid changes but 1 were identified in the variable segments. The 2 J variants can also be separated by omp1 restriction fragment—length polymorphism (RFLP) genotyping because of the loss of an AluI and a Hinfl restriction-enzyme site in VS2. Since the differences in the subsequent RFLP pattern are small, careful analysis of the RFLP is warranted. From an epidemiologic point of view, it is important to observe the spread of specific C. trachomatis serovars and variants. The observed variant Jv that was detected in 3 of 93 isolates analyzed [2] in Amsterdam might have evolved from the Ja strain identified by Dean et al. [1] in Seattle.

The persistence of C. trachomatis infections is an important subject for 2 reasons: Persistence of infection is thought to be associated with the development of late complications in women, and persistence of infection could potentially reflect antibiotic resistance. In addition to reducing the prevalence of C. trachomatis infections, the initiated and advocated screening programs for C. trachomatis could potentially result in enhanced antibiotic-resistant C. trachomatis strains. Dean et al. [1] found that 1 of 7 isolates tested had an elevated MIC for doxycycline and azithromycin, compared with 1 control isolate. One could conclude that antimicrobial resistance does not seem to be a likely explanation for persistence of infection in these patients. However, Dreses-Werringloer et al. [3] showed that standard determination of MICs is not always sufficient to verify that the antibiotic will eliminate the organism in vivo, since these assays do not include monitoring of chlamydial persistence. They showed that failure of eradication often induced a state of chlamydial persistence characterized by the presence of nonculturable but fully viable bacteria and the development of aberrant inclusions. Furthermore, Jones et al. [4] showed that resistance to tetracycline, erythromycin, and clindamycin occurred in C. trachomatis and may be a factor in some treatment failures.

During the recent International Congress of Sexually Transmitted Infections (Berlin, 24–27 June 2001), it was shown that heterotypic resistance may be common and is not induced by cotreatment of gonorrhea with tetracyclines. In addition, treatment failure was more likely in women infected with heterotypic resistant strains than in women infected with susceptible isolates [5]. On the other hand, persistence of infection could represent reinfection from an untreated partner, although that seemed unlikely in the study by Dean et al. [1], given that the infections occurred over 5–10 years; however, one cannot exclude the fact that some people had untreated partners. Since a significant association of C class serovars with persistent cervical infections was found, further research to confirm these results are warranted to investigate if biological properties of C class serovars allow for persistence.

Table 1. Comparison of the omp1 gene of Chlamydia trachomatis serovars J, Ja, and Jv.

<table>
<thead>
<tr>
<th>Location, aa</th>
<th>Serovar</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS1</td>
<td>68</td>
<td>ACC/Thr</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>CAA/Gln</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>GTT/Val</td>
</tr>
<tr>
<td>VS2</td>
<td>96</td>
<td>ATG/Met</td>
</tr>
<tr>
<td></td>
<td>141</td>
<td>GCT/Ala</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>AAT/Asp</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>TTT/Phe</td>
</tr>
<tr>
<td>CS1</td>
<td>Identical</td>
<td>Identi-</td>
</tr>
<tr>
<td>CS2</td>
<td>61</td>
<td>GTC/Val</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>GAG/Glu</td>
</tr>
<tr>
<td></td>
<td>204</td>
<td>TTA/Leu</td>
</tr>
<tr>
<td>VS3</td>
<td>Identical</td>
<td>Identical</td>
</tr>
<tr>
<td>CS4</td>
<td>247</td>
<td>TAC/Tyr</td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>GTC/Val</td>
</tr>
<tr>
<td></td>
<td>315</td>
<td>GAC/Asp</td>
</tr>
<tr>
<td>CS5</td>
<td>342</td>
<td>ACC/Thr</td>
</tr>
<tr>
<td></td>
<td>343</td>
<td>ACT/Thr</td>
</tr>
<tr>
<td></td>
<td>345</td>
<td>GTA/Val</td>
</tr>
</tbody>
</table>

NOTE. aa, Amino acid location; CS, constant segment of the omp1 gene; NA, sequence information not available; VS, variable segment of the omp1 gene.
Besides the investigation of *C. trachomatis* bacterium, serovars, and antibiotic resistance, an additional variable influencing the course of *C. trachomatis* infection must be taken into account: the host genetic background. Recent studies of twins and adoptees have indicated that host genetic factors are major determinants of susceptibility to infectious diseases in humans [6]. The immunogenetics of human infectious diseases has been reviewed recently by Hill [7]. No cytokine gene polymorphism studies have been published for urogenital *C. trachomatis* infections. However, studies of patients with trachoma, the leading cause of blindness worldwide that is caused by specific ocular *C. trachomatis* strains, showed that up-regulated local production of interleukin-1 and tumor necrosis factor-α might contribute to conjunctival damage and scarring in trachoma [8]. Murine-model studies of urogenital *C. trachomatis* infections showed an important role for interferon-γ knockout mice [9]. Stable variations in the production rates of cytokines between individuals as well as significant increases within an individual, in response to infections in general or other proinflammatory stimuli, have been observed. It is now known that these interindividual differences in cytokine production are associated with polymorphisms or mutations in regions of cytokine genes that regulate transcription or translation. Therefore, further studies are needed on the genetic properties of *C. trachomatis*, with emphasis on persistence of infection and on the host genetic background.

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**Reply**

To the Editor—We thank Morré et al. [1] for pointing out a variant of prototype serovar J, serovar Jv, that was dissimilar to serovar Ja, which we reported [2], by 2 amino acid substitutions [3]. There may have been additional differences between Ja and Jv, but no sequence data were available for the constant segment 5 region of *ompA* of Jv where Ja had 3 additional nucleotide substitutions encoding for an amino acid change. Although the Jv strain was found in 3 of 93 isolates, there is insufficient molecular and epidemiologic information available to understand the evolutionary relationship of the Jv and Ja strains at the present time.

Jones et al. [4] were the first to report resistance of *Chlamydia trachomatis* to tetracycline and erythromycin. We found no significant difference in MICs for doxycycline and azithromycin when the persistent and control strains were tested; yet, subsequent patient samples were culture negative and ligase chain reaction (LCR) positive. This evidence suggested that the strains were persistent, as opposed to resistant to the antibiotics used for treatment.

Although we found a significant association between C class serovars and persistent genital infections, it is unlikely that these infections represented reinfection within a core group, because (1) the isolates came from women seen at many different clinics in the Seattle area and during different years; (2) chlamydial persistence occurred over many years; (3) there was a significantly higher rate of culture-negative, LCR-positive samples in the persistence group than in the reinfection or control groups; and (4) variant genotypes only arose after treatment and were very similar to the parent genotype. However, our sample size was small, and we concluded that “this raises the question of whether there are particular biologic properties of C class serovars that allow for persistence” and that “it will be important to do additional in vitro and animal studies comparing selected persistent strains, such as those described here, with B and intermediate class serovars, to determine relative rates of persistence, differential effects on infectivity, and influence of antibiotics on rates of gene mutation” [2, p. 915].

The focus of our study was to determine whether there was
evidence for the persistence of cervical *C. trachomatis* infections, given the high rates of recurrent chlamydial sexually transmitted diseases that significantly contribute to ectopic pregnancy and infertility. Thus, we concentrated on the molecular properties of the pathogen instead of host genetic susceptibility. We certainly agree that host factors are likely to play an important role in persistence.

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**Plasmodium falciparum: pfcr and DHFR Mutations Are Associated with Failure of Chloroquine plus Proguanil Prophylaxis in Travelers**

To the Editor—French clinicians still support the combination of chloroquine and proguanil for antimalarial chemoprophylaxis in most of West Africa, where falciparum chloroquine resistance (FCR) remains moderate. Recently, a polymorphism in the *pfcr* gene was reported as strongly associated with in vitro FCR in parasite lines and in natural isolates [1–3]. In vivo studies demonstrated absolute selection of the *pfcr* K76T mutant allele in therapeutic failure [4]. However, in areas where FCR rates are high, the *pfcr* mutant allele was ubiquitous and thus was not predictive of clinical outcomes in immune patients [5, 6]. As has been shown previously in many studies, point mutations in *Plasmodium falciparum* DHFR are linked to antifolate resistance. The aim of the present work was to determine the DHFR and *pfcr* genotypes of isolates obtained from travelers who had used combination chloroquine-proguanil prophylaxis.

From 1997 to 2000, 62 isolates of *P. falciparum* were obtained, at the Bichat–Claude Bernard Hospital (Paris), from symptomatic malaria-infected travelers who had correctly taken chloroquine-proguanil prophylaxis. The countries visited were categorized into 2 groups, according to the classification adopted by French experts: group 2 countries are those where the recommended chemoprophylaxis regimen is chloroquine-proguanil, and group 3 countries are those where the recommended chemoprophylaxis regimen is mefloquine. Isolates came from 15 countries in sub-Saharan Africa, Southeast Asia, and South America; 1 isolate was from an undetermined African country. Twenty-eight isolates were from group 2 countries, and 33 were from group 3 countries. The presence of chloroquine, monodesethyl-chloroquine, proguanil, and cycloguanil in plasma was determined by high performance liquid chromatography [7]. Drugs or metabolites in plasma were detected for at least 3 of the 4 drugs or metabolites tested in 48 of the 62 cases. In 14 cases, only chloroquine and monodesethyl-chloroquine were detected.

DNA was extracted from clinical isolates, and DHFR 108, 51, and 59 and *pfcr* 76 genotypes were determined by polymerase chain reaction followed by DNA sequencing (see table 1, published only in the electronic edition of the Journal [http://www.journals.uchicago.edu/JID/home.html]). All isolates had the *pfcr* K76T mutation. Sixty-one isolates (98%) presented the DHFR S108N mutation. Fifty isolates (81%) had the triple DHFR S108N, N51I, C59R. Eleven isolates (18%) had double DHFR mutations, either S108N, N51I (2 isolates) or S108N, C59R (9 isolates). One isolate had a wild-type DHFR genotype. There was no significant association between the number of mutations in DHFR and the classification of the country. There also was no significant association between the number of mutations in DHFR and drug concentrations in plasma.

The interpretation of the efficacy of a prophylactic regimen depends on drug compliance and may be controversial. In this regard, well-conducted interrogations were crucial to validate the prophylaxis failures included in this series. Measurements of proguanil and its metabolite cycloguanil were not always useful because their short half-life limited interpretation of drug ingestion. In particular, most patients who did not show a protective cycloguanil level in plasma developed malaria >4 weeks after returning to France and discontinuing prophylaxis. In addition, a large interindividual variability of drug or metabolite levels in plasma has often been reported. However, the only patient who presented an isolate having a DHFR wild-type genotype had no detectable cycloguanil in plasma.

The mutant allele *pfcr* K76T was found in all isolates of this series. This could reflect a very high frequency of this allele in countries visited by the patients, but results of previous studies did not confirm this hypothesis [2–4, 8]. The more likely explanation is a selection of the mutant allele by the chloroquine...
included in the prophylactic regimen of patients. These results, obtained with prophylactic doses given mostly to nonimmune subjects, confirmed the association of the mutation pfcrf K76T with chloroquine resistance.

Mutations observed in the DHFR gene were dramatically frequent among isolates of the series. All isolates but 1 showed the S108N mutation, and most had a triple mutation. In a comparable series of isolates from travelers who had not taken antifolate prophylaxis, the proportion of wild-type (S108) isolates was reported as near 50% [9]. The rapid selection of DHFR point mutations in positions 108, 51, and 59 has already been described under antifolate pressure.

Among isolates with double mutations in the present series, a mutant allele was selected more frequently in codon 59 than in codon 51, as reported by Dourno et al. [10] in a study using prophylaxis by pyrimethamine alone. More than one-half of isolates in the present series came from group 3 countries, where chloroquine-proguanil prophylaxis is not recommended by French clinicians. Authentic prophylaxis failures with this combination remain rare in group 2 countries. To continue to recommend combination chloroquine-proguanil prophylaxis to travelers, it is important to regularly monitor the susceptibility of parasites in areas where FCR is not already extensively present. It may be stressed that prophylaxis failures in travelers may detect resistance earlier and at weaker levels than treatment failures within local populations, because the doses used are lower and because travelers usually are nonimmune subjects.

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