Letter to the Editor

Phylogenetic Analysis of Chlamydia trachomatis

An understanding of the evolution and biological relationships of Chlamydia trachomatis major outer membrane protein (MOMP) and MOMP gene (omp1) sequences is an ongoing process and one that requires complex analyses. In our estimation, the study by Stothard et al. (5) is limited by the lack of sufficient numbers of omp1 genotypes for the analysis presented and by the use of only one method for evaluation of the molecular evolution of chlamydiae. For example, the authors state that what causes lymphogranuloma venereum, trachoma, or urogenital strains to infect certain cell types cannot be explained by MOMP. This conclusion is based solely on phylogenetic reconstructions of 6 trachoma and 22 nontrachoma genotypes. For MOMP, phylogenetic analyses may be more difficult to interpret since diversity is probably generated by means other than simple substitutions and insertions and deletions (indels); current tree topologies may be obscured by recombination events such that a common ancestor for genotypes with similar disease presentations can no longer be identified. We believe that estimates of mutation rates and other more complicated analyses are needed before any firm conclusions can be made. Further, the collection of published sequences does not necessarily represent the extent of omp1 diversity and may not be adequate to describe molecular evolution for chlamydiae. For example, fewer omp1 variants have been described for E than for D and F. As more genotyping has been performed, additional E variants have been identified: Stothard et al. report 3 variants of 19 (16%); we previously found 11 variants of 67 (16%) (2). Thus, additional sequencing of omp1 from multiple trachoma and sexually transmitted disease specimens worldwide is needed to determine a representative distribution of all genotypes for analysis.

The authors correctly mention that our E/Bour sequence is different from that of Peterson et al. (4). They state that “variation in E/Bour reported by Dean and Millman is not easily explained.” Herein, we provide an explanation. E/Bour was first described in the 1950s when serotyping was the only method for identifying chlamydial subtypes. The sequence differences between that of Peterson et al. and ours reside in conserved regions of omp1; variable segments of our sequence are exactly the same as that published by Yuan et al. (6). Since serotyping is based on antibody reactivity to variable segments of MOMP, it is highly probable that different clones of serovar E were labeled similarly as E/Bour. The source of E/Bour may also differ; ours was from Dr. Julius Schachter, San Francisco, Calif. Further, although the authors found no evidence for “culture-induced...mutation,” this is an in vitro system that does not necessarily reflect the selective pressure of an in vivo environment. In fact, there is in vivo evidence for allelic flux. Hayes et al. (3) observed flux in The Gambia, albeit with minimal nonsynonymous mutations over 18 months. Dean et al. (1) found significant flux over 3 years in Tunisia. Thus, some mutational drift may have occurred in vivo before E clones were serotyped and labeled as E/Bour in the 1950s.

REFERENCES


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Authors’ Reply

We appreciate the opportunity to reply to the letter submitted by Drs. Dean and Millman in response to our article “Phylogenetic analysis of the Chlamydia trachomatis major outer membrane protein and examination of potential pathogenic determinants” (4). We agree with Drs. Dean and Millman that understanding the evolution of the Chlamydia trachomatis major outer membrane protein (MOMP) is an ongoing process, one that will change as new omp1 sequences from a variety of clinical and geographic sources are submitted to sequence databases. Indeed, one of the goals of our recent publication was to update the work by W. M. Fitch, E. M. Peterson, and L. M. de la Maza which appeared in 1993 (2). The 1993 publication did not include representatives from serovars Ba, D, Da, G, I, Ia, J, and K because full-length sequences for these serovars were not available at the time. We were able to bridge this gap in our 1998 publication by providing full-length sequences for these serovars. Our conclusions did not differ from those of Fitch, Peterson, and de la Maza, namely, that the MOMP phylogenetic tree is not congruent with strain clustering by cell type infected, organ infected, or disease presentation, and we also concluded that the variability in MOMP appears to be more likely the result of providing for pathogen diversity than of specific and directed evolution of pathogenic epitopes or determinants.

Drs. Dean and Millman state that our analysis was limited by the lack of sufficient numbers of omp1 genotypes. We agree. We could have expanded our data set by including partial omp1 sequences in GenBank, those which consist of only the variable regions of the omp1 gene. However, inspection of complete omp1 sequences clearly shows that substitution in this gene occurs in both conserved and variable regions. Dean and Millman provide an example in that their E/Bour sequence (1) differs from another E/Bour sequence (3) only in conserved regions. Therefore, to include partial sequences in our analysis would have led to the exclusion of phylogenetically informative sites in the conserved regions of complete omp1 sequences. In the end, we felt that the most accurate phylogenetic reconstruction would be produced by using complete omp1 gene
sequences. We also hope that our publication will encourage more investigators to examine the entire omp1 gene when performing epidemiologic studies and surveys. In this way, we can build a larger and more comprehensive collection of omp1 sequences, which will produce a more complete and accurate history of this gene.

Finally, we recognize that there are multiple possible explanations as to why the two E/Bour sequences that exist in GenBank are different. In fact, it was this phenomenon which led us to examine whether long-term expansion in cell culture could induce such mutations (5). While cell culture did not appear to produce mutations, we agree with Dean and Millman that selective pressure from the host immune system in an in vivo environment could.

There is still much work to be done on understanding the evolutionary complexities of the C. trachomatis MOMP. Our recent publication only scratches the surface, but it raises real issues that must be considered if MOMP is going to be a vaccine candidate for chlamydial disease. We hope that concerted efforts by everyone in this area of research will facilitate this process.

REFERENCES


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