Mycoplasma fermentans, M. hominis, and M. hyorhinis Inhibit Infectivity and Growth of Chlamydia trachomatis and C. pneumoniae in HEp-2 Cells

We read with great interest the data reported by Castilla and Wadowsky about the elimination of a Mycoplasma hominis-like mycoplasma from the TW-183 strain of Chlamydia pneumoniae and its lack of effect on chlamydial infection of HEp-2 cells (1).

We recently described detection of Mycoplasma fermentans, M. hominis, and M. hyorhinis in several strains of C. trachomatis and C. pneumoniae, the successful elimination of Mycoplasma from chlamydial culture by the antibiotic mupirocin, and the profound effect of mycoplasmal contamination on chlamydial infectivity and growth in HEp-2 cells (2). We would like to bring to the attention of your readership several important different or additional points.

First, in contrast to the detergent Igepal CA-630 suggested by Castilla and Wadowsky for elimination of Mycoplasma, the antibiotic mupirocin enabled the effective elimination of Mycoplasma without affecting the growth of C. trachomatis or C. pneumoniae (2). Second, we would strongly recommend the use of PCR instead of culture for analysis of Mycoplasma contaminations, since we repeatedly observed mycoplasmal contamination as determined by specific PCR in mycoplasmal culture-negative chlamydial preparations (B.K.-O., unpublished observation). In addition, sequencing of the PCR product allows species identification, information which may be important to track down the potential contaminating source (2, 4). Third, the point that we find most intriguing in the paper by Castilla and Wadowsky is the lack of effect of mycoplasmal contamination on chlamydial infectivity. In contrast to their observation, we found a profound inhibitory effect of mycoplasmal contamination on chlamydial infectivity and growth (2). Similar results showing M. hyorhinis contamination of C. psittaci were recently published (3). Of course the differing mycoplasmal species present or differences in the culture media in those chlamydial cultures may explain this lack of effect. However, when we cocultivated M. hominis with chlamydiae, we also observed a strong inhibition of chlamydial growth (B.K.-O., unpublished observation). Another explanation for the observation of Castilla and Wadowsky may be that although the chlamydiae used in their study were Mycoplasma culture negative, those chlamydiae might have still contained Mycoplasma that was detectable only by PCR. When we determined the ratio of chlamydial organisms, i.e., the number of elementary bodies per inclusion-forming unit for both mycoplasma-free and mycoplasma-contaminated chlamydiae, this ratio was 10 times higher for the mycoplasma-contaminated chlamydiae; that is, although the numbers of inclusion-forming units were identical, the infectivity determined per chlamydial organism was much lower for the mycoplasma-contaminated chlamydiae (2). By using chlamydiae standardized for their infectivity, i.e., the same number of inclusion-forming units, an additional inhibitory effect of the mycoplasma added to the already latently infected chlamydiae might have been missed in their experiments, especially since Castilla and Wadowsky analyzed chlamydial growth already after 3 days of culture. In our experience, the inhibitory effect of Mycoplasma on chlamydial growth became most evident after 7 days of cocultivation (B.K.-O., unpublished observation).

REFERENCES

Authors' Reply
We read with interest the letter by Krauje-Opatz et al. on the comparisons of their study and our study on Mycoplasma contamination of C. pneumoniae stock strains. As indicated in their letter, stock suspensions of C. pneumoniae are often contaminated with various Mycoplasma species. Our study demonstrated the presence of a Mycoplasma hominis-like Mycoplasma in the TW-183 strain of C. pneumoniae, obtained from the American Type Culture Collection (ATCC), by microbiologic culture. Similarly, another group (1) reported the isolation of Mycoplasma by culture from two additional and independent sources of the TW-183 strain. These results suggest that Mycoplasma contamination of the TW-183 strain is widespread. In contrast, a report by Krauje-Opatz et al. (2) did not identify any Mycoplasma spp. in the TW-183 strain derived from another source on the basis of testing with a nested PCR-based assay that amplifies targets within ribosomal DNA. One possible explanation for the different findings is that their TW-183 strain is indeed mycoplasma-free. Alternatively, there may be strains of Mycoplasma that are not detected with the PCR-based assay. Collectively, these studies would suggest that it may be prudent to evaluate stock cultures of C. pneumoniae for Mycoplasma contamination using both a sensitive culture assay and a PCR-based assay. However, the application of PCR-based assays that amplify ribosomal DNA targets for detection of Mycoplasma contamination in C. pneumoniae stock cultures should be interpreted with some caution since cross-reacting sequences have been found in the ribosomal DNA of Mycoplasma and C. pneumoniae (3). Although it appears that endonuclease treatment of the ribosomal DNA amplicons and analysis of the digests are helpful in identifying Mycoplasma contamination, in our opinion isolation of the contaminant by culture should be considered as definitive ev-
idence of contamination. Isolation of the Mycoplasma contaminant by culture also permits assessment of the effect of the “actual” contaminant on the infectivity of the C. pneumoniae strain. It is likely that the TW-183 strain of C. pneumoniae used in many laboratories has been contaminated for some time with Mycoplasma. Serial passages of the TW-183 strain would have resulted in the simultaneous passage of the autochthonous Mycoplasma, a practice that may have selected for chlamydiae and mycoplasma with traits of coexistence. In the study by Krauße-Opatz et al. (2), exogenous strains of Mycoplasma were coincubated with a strain of C. pneumoniae, which was a different strain from the one used in our study. These differences could have contributed to the difference observed between the two studies on the effect of the various mycoplasmas on the infectivity of the chlamydiae. We do not agree that 7 days of incubation are necessary to observe an effect with the M. hominis-like Mycoplasma as stated by Krauße-Opatz. During the 72-h incubation period of our cocultures, a dramatic increase in the number of CFU of the M. hominis-like Mycoplasma per milliliter occurred in the cocultures (4). Despite their statement on the importance of 7 days of incubation, the study by Krauße-Opatz et al. (2) enumerated inclusions of C. pneumoniae in HEp-2 cells at day 4. We choose a 72-h incubation period since this incubation period is commonly used in many laboratories. We also did not observe any difference in the size of the C. pneumoniae inclusions resulting from coin-cubation of the chlamydiae and the M. hominis-like Mycoplasma in HEp-2 cells and those resulting from incubation of the Mycoplasma-free chlamydiae in HEp-2 cells, a finding that is consistent with the view that the number of elementary bodies per inclusion was the same under both conditions. The finding of Krauße-Opatz et al. (2) that treatment of C. pneumoniae stock suspensions with mupirocin effectively eradicates Mycoplasma contamination is a valuable contribution especially in view of our experience using Igepal CA-630. We found that Igepal CA-630 has considerable activity against C. pneumoniae and provides a relatively small window of opportunity for eradicating Mycoplasma contamination. On the other hand, our treatment schedule for Igepal CA-630 is relatively simple, requiring a single 10-min exposure, compared to the mupirocin treatment schedule, which utilizes incubation for up to 2 weeks with frequent changes with mupirocin-containing medium.

We thank B. Krauße-Opatz et al. for their thoughts on our study and also acknowledge the importance of their new and important contributions.

REFERENCES


Elias A. Castilla
The Cleveland Clinic Foundation
Department of Anatomic Pathology
Cleveland, Ohio 44195

Robert M. Wadowsky
Department of Pathology
Children’s Hospital of Pittsburgh
Pittsburgh, Pennsylvania 15213