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Letter to the Editor

Is *Chlamydia pneumoniae* Present in Cerebrospinal Fluid of Multiple Sclerosis Patients?

In a recent article, Sriram and collaborators (8) described a comparative study designed to detect *Chlamydia pneumoniae* DNA in the cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS) or other neurological diseases (OND). Two PCR assays were performed on a common set of CSF samples at Vanderbilt University Medical Center (VUMC) and the University of South Florida (USF). The PCR assays, which targeted the *ompA* gene (or major outer membrane protein [MOMP] gene) of *C. pneumoniae*, were called VU-MOMP and USF-MOMP and were developed at the respective laboratories. A third assay, a nested-touchdown PCR targeting the same gene (10), was also performed at VUMC. Sriram et al. concluded that the results showed a high prevalence of *C. pneumoniae* in the CSF of MS patients. When the results of all the assays were combined, the overall detection rate was 83% in the clinically definite MS group, 57% in monosymptomatic MS patients, and significantly lower (16%; $P < 0.01$) in OND patients.

In July 2002, the Centers for Disease Control and Prevention (CDC; Atlanta, Ga.) was contacted by the USF collaborators of the comparative study who were interested in testing DNA from peripheral blood mononuclear cells (PBMC) by their USF-MOMP PCR assay for *C. pneumoniae*. The investigators stated that this new USF-MOMP assay detected a high prevalence (more than 80%) of *C. pneumoniae* DNA in PBMC obtained from healthy donors. A total of 65 DNA samples extracted from human PBMC, rabbit PBMC, human placental DNA, human throat swabs, various bacterial species, and water were sent to the USF. All DNA samples had been previously assayed at the CDC, and no *C. pneumoniae* DNA was detected, except in one positive control (9). Surprisingly, 23 (35%) of the DNA extracts were reported to be PCR positive at the USF; 21 (91%) of the positive samples were human PBMC. The samples were then assayed at the CDC using the USF-MOMP protocol. A PCR product was present in all samples considered positive at the USF, including the human placental negative-control DNA. However, the PCR product was clearly different in size than the product obtained by the amplification of *C. pneumoniae* DNA (ATCC VR1360) and was not considered positive. These facts indicate a probability that the primer set of the USF-MOMP protocol is nonspecific.

As mentioned by Sriram et al. (8) in the discussion section of the article, another collaborative study involving four laboratories had been conducted for the detection of *C. pneumoniae* DNA in the CSF of MS patients (5). VUMC found PCR evidence of *C. pneumoniae* in the CSF from 22 (73%) of 30 patients and 5 (23%) of 22 controls ($P < 0.001$), whereas the laboratories at the John's Hopkins University, Baltimore, Md., University Hospital of Umeå, Umeå, Sweden, and the CDC found no evidence for *C. pneumoniae* in any of the 52 CSF samples submitted. In a continuing effort to review current diagnostic tests for *C. pneumoniae* (2), the CDC analyzed 19 of these 52 CSF specimens by using the VUMC protocols for DNA extraction and the VUMC PCR. VUMC claimed that the use of phenol-chloroform and dithiothreitol is critical for DNA extraction to ensure the solubilization of the cysteine-rich outer membrane proteins of *C. pneumoniae.* None of the 19 CSF samples were positive for the presence of *C. pneumoniae* DNA when they were tested at the CDC. Instead, the agarose gel electrophoresis of the respective PCRs showed a large smear of DNA on the gel for 14 (73.6%) CSF samples. Thirteen of those samples had been previously reported to be PCR positive by the laboratory at VUMC. More importantly, amplification of human placental-control DNA (catalog no. D-3160; Sigma, St. Louis, Mo.) was detected as a band with a size similar to the VUMC's *C. pneumoniae* amplified product size. Although the set of primers used by Sriram et al. (8) is different than the one published previously (5, 7), both sets have a high sequence similarity to human DNA, as determined by BLAST search. These findings demonstrate the requirement for validation of *C. pneumoniae* PCR assays for specificity before their use on clinical samples.

There is considerable controversy concerning the evidence from PCR analysis for the presence of *C. pneumoniae* in the CSF of MS patients (1, 3–7). Although other investigators have reported *C. pneumoniae* DNA in the CSF of MS and OND patients (4), we consider that the possibility of false-positive PCRs due to the amplification of human DNA still needs to be further investigated. In conclusion, large, multicenter collaborative studies are still necessary to confirm the possible involvement of *C. pneumoniae* in the pathogenesis of MS.

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Authors' Reply

Dr. Tondella et al. in response to our paper raise an important issue regarding the specificity of PCR assays used for the detection of *C. pneumoniae* in the CSF and are concerned that the primers used in Dr. Yamamoto's assay (6) may not be specific for *C. pneumoniae* MOMP.

It has been noted that placental DNA sent from Dr. Tondella's laboratory and tested in Dr. Yamamoto's laboratory was positive by his MOMP PCR assay. In order to evaluate a possible cross-reaction with human DNA, Dr. Yamamoto's laboratory obtained human placental DNA from Sigma (human placental DNA, D-4642, lot 072K9135, and DNA type XIII from human placenta, D-7011, lot 21K3787) and repeated the studies. At concentrations of 0.01, 0.1, and 1 μg/PCR mixture for both DNA samples, there was no amplification of the MOMP gene. The reason for the initial amplification of placental DNA from Dr. Tondella's laboratory is not clear and may involve the contamination of blood products during the extraction procedure. We agree that the presence of *C. pneumoniae* in clinical samples needs to be validated by the use of more than one set of PCR primers.

We disagree with the implication of the respondents that the PCR results represent cross-contamination of human DNA. Our experimental design used three different sets of MOMP primers in two different laboratories; one of these primers (C. Y. Tong and M. Sillis) is identical to that used by J. Boman and C. A. Gaydos in their published studies (1). There has been no suggestion from prior studies that these primers amplify human DNA. Eight of 18 MS patients were positive by all three PCR assays, while all 14 control patients were negative, a result that makes unlikely the amplification of human DNA in MS patients alone. Finally, we have sequenced the PCR product of the VU-MOMP primers and found them to be consistent with *C. pneumoniae* MOMP genes.

The respondents also refer to an earlier collaborative study in which 19 of the 52 samples were analyzed using the VUMC protocol of extraction and PCR analysis. The respondents were unable to detect a PCR product using our extraction method and PCR running conditions. Recent collaborative studies of samples with known amounts of *C. pneumoniae* DNA demonstrate considerable variability in the detection of *C. pneumoniae*, particularly at low copy numbers. Interlaboratory positive rates varied from 8 to 63% (2). Inherent technical difficulties in PCR running conditions that are independent of DNA extraction are an important aspect of PCR methodologies that cannot be ignored.

We agree that additional collaborative studies are needed, and we are planning to implement them. The amount of CSF that can be obtained from each patient limits the number of laboratories that can be included for such studies. Since we published these observations, two additional studies using a different set of PCR primers and showing the increased presence of *C. pneumoniae* in the CSF of MS patients over that in controls have been reported (3, 5). Moreover, seroepidemiological studies suggest that *C. pneumoniae* may play a role in the progression of MS (4). These separate and independent studies suggest that the association between MS and *C. pneumoniae* needs to be further explored and that standardized PCR assays are needed.

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