Detection of Mycobacterium tuberculosis in Cerebrospinal Fluid following Immunomagnetic Enrichment

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Received 20 July 1995/Returned for modification 5 September 1995/Accepted 10 November 1995

The detection of Mycobacterium tuberculosis by culture of cerebrospinal fluid (CSF) is unacceptably slow. Low numbers of organisms and the presence of reaction inhibitors may prevent detection of M. tuberculosis by PCR. We used immunomagnetic enrichment to accelerate and enhance the detection of mycobacteria in CSF after demonstrating the utility of the method with pure suspensions. Growth was detected earlier in Bactec cultures of magnetically recovered mycobacteria than in untreated CSF (7 versus 15 days). We detected M. tuberculosis DNA by PCR in the immunomagnetically enriched sample but not in untreated CSF. PCR fingerprints of the immunomagnetically recovered M. tuberculosis and of the isolate subsequently recovered by culture were identical.

Early diagnosis of and prompt initiation of treatment for tuberculous meningitis improve the outcome of the disease, but the detection of Mycobacterium tuberculosis in cerebrospinal fluid (CSF) by conventional methods remains a challenge (5). Low numbers of M. tuberculosis organisms and their slow growth limit detection by acid-fast stains and culture techniques (9, 11). PCR techniques have been applied but are limited by the small sample size that can be tested, the low number of organisms present in clinical samples, false-positive results due to contamination, and the presence of PCR inhibitors (1, 4, 6–8, 13, 14). To overcome these obstacles, we used an immunomagnetic enrichment technique to concentrate mycobacteria and separate them from debris. Immunomagnetic separation and enrichment have facilitated detection of numerous microbes (12, 17, 18). Additionally, magnetic force has been used to separate Mycobacterium paratuberculosis and Mycobacterium avium DNAs from crude DNA extracts by using streptavidin-coated magnetic particles (10).

Paramagnetic beads coated with an anti-Mycobacterium bovis BCG antibody were added to dilutions of various mycobacteria and to CSF from a patient with meningitis. The beads with adsorbed mycobacteria were recovered by magnetic force. This immunomagnetic enrichment facilitated detection of M. tuberculosis by PCR and by Bactec culture. PCR fingerprinting confirmed that the magnetically extracted M. tuberculosis was identical to that recovered by conventional culture methods.

Preparation of magnetic beads. A polyclonal anti-M. bovis (BCG) antibody (Dako Corporation, Carpinteria, Calif.) was coupled to amine-terminated paramagnetic silanized FeO2 beads (Biomag 4100 Particles; Advanced Magnetics Inc., Cambridge, Mass.) by incubating 7.5 μg of antibody with 5 × 109 particles in 5% gluteraldehyde for 24 h according to the method of Weston and Avrameas (16). After washing, the final concentration was 5 × 109 particles/ml per ml of wash buffer (0.01 M Tris, 0.1% NaN3, 0.1% bovine serum albumin, 0.15 M NaCl, and 0.001 M EDTA), with greater than 80% of the applied antibody bound to the particles.

Studies involving pure cultures. Suspensions containing approximately 104, 105, 106, and 107 CFU of BCG Tice, M. tuberculosis H37Rv, or clinical isolates of M. tuberculosis or M. avium per ml were prepared in Middlebrook 7H9 medium after agitation of each culture with 3-mm-diameter glass beads on a Minibead Beater (Crescent Dental Mfg. Co., Lyons, Ill.) to reduce clumping. Antibody-coated beads (100 μl) were added to 1-ml samples of each suspension, and the samples were incubated with slow shaking for 1 h at room temperature. The beads were recovered magnetically, and the supernatant was retained for culture. The beads from each sample were washed with Middlebrook 7H9 medium, recovered, and inoculated into a Bactec 12B bottle (Becton-Dickinson Laboratories, Cockeysville, Md.) labeled “enriched sample.” Samples (100 μl) of each supernatant and of each untreated mycobacterial suspension were inoculated into Bactec 12B bottles. Growth at 37°C in the Bactec bottles was monitored on a Bactec TB 460 system (Becton-Dickinson), and growth curves were plotted. The radiometric culture method provided a more accurate estimate of the number of mycobacteria than did counting of colonies on solid medium, because each antibody-coated particle produces only one colony even when multiple mycobacteria are attached.

Growth curves (Fig. 1) show that mycobacteria were detected earlier in Bactec vials inoculated with immunomagnetically enriched samples of BCG than in those with untreated BCG suspensions. In fact, the rate of growth from the enriched BCG samples approached that from untreated samples that contained 10 times as many mycobacteria. Growth from supernatants was significantly lower than that from untreated BCG suspensions, indicating a decreased bacterial content. The antibody-coated beads also effectively captured M. tuberculosis and M. avium. Figure 2 demonstrates that the rate of growth from enriched samples of M. tuberculosis approached that from untreated samples with 10 times as many mycobacteria. A 10-fold enrichment in M. avium was observed as well when antibody-coated beads were used (Fig. 3). Similar degrees of enrichment were obtained with the reference strain H37Rv and when experiments were repeated with different shipments of beads and antibody (data not shown).

Recovery of M. tuberculosis from CSF. CSF specimens from a 26-year-old female with fever, hydrocephalus, and a positive purified protein derivative skin test were withdrawn for diag-
nostic and therapeutic reasons at 3 and 10 days after antituberculous therapy was initiated. A portion (500 μl) of each CSF sample was added directly to a Bactec 12B bottle labeled "untreated CSF," while an additional portion (100 μl) was retained for PCR studies. The mycobacteria in the remaining CSF (3 ml of the first CSF sample and 6 ml of the second sample) were recovered immunomagnetically, a process which also helped to concentrate and purify the organisms. Antibody-coated beads (100 μl) were incubated with CSF for 1 h at room temperature. Studies with pure cultures had demonstrated that 100 μl of beads could capture at least one million organisms, far more than are expected to be in 3 or 6 ml of CSF. The beads and any adherent mycobacteria were recovered magnetically and resuspended in 600 μl of Middlebrook 7H9 medium. Five hundred microliters of the bead suspension was inoculated into a Bactec 12B bottle labeled "enriched CSF," and the remainder (100 μl) was retained for PCR studies. Bactec bottles were incubated at 37°C, and the growth index was monitored with a Bactec TB 460 system. A growth index of greater than 10 was considered to be positive (15).

Immunomagnetic enrichment facilitated the detection of M. tuberculosis in CSF. The enriched CSF sample obtained on the third day of treatment showed significant growth (growth index > 10) after 7 days of incubation, while the untreated CSF

FIG. 1. Growth curves of BCG measured from Bactec 12B bottles containing untreated mycobacterial suspensions (×), enriched samples (●), or supernatants of enriched samples (○) when the test suspension contained approximately 10⁷ (dashed line), 10⁶ (solid lines), or 10⁵ (dotted lines) organisms per ml.

FIG. 2. Growth curves of M. tuberculosis measured from Bactec 12B bottles. Symbols are as described in the legend to Fig. 1.
Immunomagnetic enrichment facilitated the detection of \textit{M. tuberculosis} by PCR. We were unable to amplify or detect \textit{M. tuberculosis} DNA in untreated or uncultured CSF samples but successfully amplified \textit{M. tuberculosis} DNA from the enriched sample. The PCR fingerprints of immunomagnetically recovered \textit{M. tuberculosis} were identical to the PCR fingerprints of organisms subsequently isolated by Bactec culture of untreated CSF (Fig. 4).

Two characteristics of immunomagnetic enrichment may improve the sensitivity of PCR testing of enriched samples. First, it concentrates the target into a very small volume. The bead-mycobacterial pellet was less than 50 \textmu l in our experiments. Second, it appears to eliminate inhibitors of the PCR. The increased manipulation of clinical samples may increase the chance of contamination and false-positive PCR results. However, previously described methods of avoiding PCR contamination (11) are applicable to enriched samples such that enrichment need not adversely affect specificity. We used PCR fingerprinting to demonstrate that the immunomagnetically recovered \textit{M. tuberculosis} was not a contaminant. The immunomagnetically recovered \textit{M. tuberculosis} and the organisms recovered by conventional and radiometric culture techniques had identical PCR fingerprints. This fingerprint comparison illustrates a second benefit of enrichment. PCR fingerprinting of immunomagnetically recovered \textit{M. tuberculosis} can assist in rapid strain identification; this information can be important to the clinician when disease due to drug-resistant organisms is suspected.

We conclude that immunomagnetic enrichment of mycobacteria can be clinically useful in diagnosing tuberculous meningitis. In addition, the technique can assist in rapid strain identification.

We thank Alice Johnson for her assistance in preparing the manuscript and Phillip Rumley for his photographic assistance.

This study was supported by a grant from the Lizzonell and Colbert Coldwell Foundation.

REFERENCES


