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Direct and Sensitive Detection of a Pathogenic Protozoan, *Toxoplasma gondii*, by Polymerase Chain Reaction

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We applied the polymerase chain reaction to detection of the pathogenic protozoan *Toxoplasma gondii* based on our identification of a 35-fold-repetitive gene (the *B1* gene) as a target. Using this procedure, we were able to amplify and detect the DNA of a single organism directly from a crude cell lysate. This level of sensitivity also allowed us to detect the *B1* gene from purified DNA samples containing as few as 10 parasites in the presence of 100,000 human leukocytes. This is representative of the maximal cellular infiltration (10^5/ml) in 1 ml of cerebrospinal fluid obtained from patients with toxoplasmic encephalitis. The *B1* gene is present and conserved in all six *T. gondii* strains tested to date, including two isolates from patients with acquired immunodeficiency syndrome. No signal was detected by using this assay and DNAs from a variety of other organisms, including several which might be found in the central nervous system of an immunocompromised host. This combination of sensitivity and specificity should make detection of the *B1* gene based on polymerase chain reaction amplification a very useful method for diagnosis of toxoplasmosis both in immunocompromised hosts and in congenitally infected fetuses.

There are many instances in the diagnosis of infectious diseases in which it is desirable to detect a pathogen directly. A prominent example is the plethora of opportunistic infections of patients with acquired immunodeficiency syndrome (AIDS; 2, 42) which cannot be reliably diagnosed by serology because of severe immune system dysfunction. The polymerase chain reaction (PCR; 33) has already been used in detection of human immunodeficiency virus types 1 (17, 25, 27) and 2 (30), human papillomavirus (24, 35), human T-cell lymphoma virus (3, 11), and cytomegalovirus (9, 34) from a variety of clinical specimens, including blood, urine, and Formalin-fixed tissue specimens, without prior extraction of DNA. In several instances, PCR has allowed direct diagnosis of viral diseases when serologic methods have failed (13, 19). We describe below the application of PCR to detection of the pathogenic protozoan *Toxoplasma gondii*.

T. gondii can cause severe neurological disease or death in developing human fetuses (22) and in patients immunosuppressed as a result of drug therapy or disease (12, 15, 20). Particularly at risk are patients with AIDS (16, 23, 26), among whom the prevalence of toxoplasmosis is about 10% (14, 36) with the number of such afflicted persons dramatically increasing as the number of AIDS cases rises. In about 5% of AIDS cases, toxoplasmosis is the first indication of human immunodeficiency virus infection (14).

Fetal toxoplasmosis remains a significant disease as a result of acute parasitic infection in mothers not previously infected; consequences of infection are most severe if it occurs during the first trimester. Effective prenatal diagnosis can permit a decision to terminate the pregnancy or initiate treatment of late-term fetuses in utero (8, 10).

Current diagnosis in utero relies on a combination of detection of specific immunoglobulin M, culture of amniotic fluid and fetal blood, and other nonspecific measurements of infection (10). The only definitive diagnosis is by culture, which takes up to 3 weeks. A more rapid method of direct detection could offer justification to start antibiotic therapy immediately.

Current diagnosis of *T. gondii* infection in patients with AIDS cannot rely on serology but rather relies on computerized tomography or magnetic resonance imaging scans and brain biopsies to identify parasites encysted within the brain (6, 26). Such technology is time-consuming, expensive, and associated with considerable risk. New procedures, such as PCR, are therefore desirable for the diagnosis of toxoplasmosis both in developing fetuses and in immunocompromised hosts.

MATERIALS AND METHODS

Preparation of *T. gondii* cells and DNA. *T. gondii* C56 and independent strains A1 and A2 isolated from two patients with AIDS were grown in mouse ascites after intraperitoneal injection (strains provided by J. S. Remington, Palo Alto Medical Foundation, Palo Alto, Calif.). *T. gondii* RH tachyzoites were grown in a monolayer of human foreskin fibroblasts in Eagle minimal essential medium (GIBCO Laboratories) supplemented with 3% fetal bovine serum and antibiotics (28). All strains were purified from host cell material by harvesting lysed cultures, passing the cell suspension through a 27-gauge needle, and filtering through a CF-11 cellulose (Whatman, Inc.) column (37) or a 3-μm nitrocellulose filter (Nuclepore Corp.) (41). Individual *T. gondii* organisms were isolated by using a single-laser fluorescence-activated cell sorter (FACS; Becton Dickinson FACSTAR) after labeling by indirect immunofluorescence with a mouse monoclonal antibody (DG-52) to the p30 antigen of *T. gondii*, followed by fluorescein isothiocyanate-conjugated goat anti-mouse antiserum. Cells were sorted under a narrow window directly into 0.5-ml microcentrifuge tubes (Sardstedt) and stored frozen.

Whole parasites were prepared for amplification by being heated in microcentrifuge tubes to 94°C for 10 min in 50 μl of deionized water to completely lyse the cells. Positive and negative mock clinical samples (representative of the cellular contents of cerebrospinal fluid [CSF]) were prepared by mixing uninfected human peripheral blood leukocytes (iso-

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lated over a 6% glucose gradient column, followed by two 30-s hypotonic lyses in deionized water to rupture residual erythrocytes) with or without whole *T. gondii* cells. A mixture of human and parasite DNAs was prepared from these samples by incubation for 2 h at 60°C in 200 μg of proteinase K per ml–0.2% sodium dodecyl sulfate–10 mM Tris hydrochloride (pH 7.4)–10 mM NaCl–10 mM EDTA–10 μg of tRNA per ml, followed by phenol-chloroform extraction and ethanol precipitation.

The *B1* gene. The *B1* gene was isolated as described elsewhere (4). The complete sequence of the *B1* gene as cloned in plasmid vector pAT153 (38; to give pTXgB1) was determined by using the chemical modification method of Maxam and Gilbert (21).

The copy number of the *B1* gene was determined by comparative hybridization using a titration of plasmid DNA. Serially diluted samples of pTXgB1 DNA (5,860 base pairs) were mixed with 1 μg of *Eco*RI-digested calf thymus carrier DNA and electrophoresed on a 0.7% agarose gel. Two dilutions of genomic DNA (1 and 0.25 μg, containing less than 5% host DNA contamination) were digested with *Eco*RI and run in parallel (*T. gondii* is haploid, with a DNA content of about 7×10^7 base pairs [7]). The plasmid amounts, 16.8, 8.4, 4.2, 2.1, 0.84, and 0.42 ng, correspond to 200, 100, 50, 25, 10, and 5 times, respectively, the number of moles of a single-copy gene found in 1 μg of genomic DNA.

Following electrophoresis, the DNA was transferred to nitrocellulose and hybridized with a nick-translated 2.2-kilobase (kb) *B1* fragment in 3× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate)–1× Denhardt solution (1× Denhardt solution contains 0.02% Ficoll 400, 0.02% bovine serum albumin, and 0.02% polyvinylpyrrolidone)–50 μg of carrier DNA (calf thymus) per ml at 65°C. Following autoradiography, the bands were cut out of the nitrocellulose and the radioactivity of each was quantitated in a scintillation counter.

Amplification procedures. Four oligonucleotides (oligos) were synthesized on an Applied Biosystems DNA synthesizer. Two are of the same sense as the sequence presented in Fig. 2; oligo 1 corresponds to *B1* gene nucleotides 694 to 714 (5′-GGAACTGCATCCGTTCATGAG), and oligo 2 (5′-TAATACGACTCACTATAGGGTGCATAGGTTGCAGT CACTG) comprises 40 nucleotides, 20 nucleotides constituting the sense strand promoter sequence for T7 RNA polymerase (underlined), followed by 20 nucleotides of the *B1* gene sequence (757 to 776). Oligos 3 and 4 are of the opposite sense and correspond to nucleotides 853 to 831 (5′-GGC GACCAATCTGCGAATACACC) and 887 to 868 (5′-TCTT TAAAGCGTTCGTGGTC), respectively, on the antisense strand.

The amplification reactions were performed with the thermostable DNA polymerase of *Thermus aquaticus* (*Taq*; Cetus Corp.) as previously described (32). Briefly, 2.5 U of *Taq* polymerase was used in a 100-μl reaction volume with 10 mM Tris (pH 8.3)–2.5 mM $MgCl_2$–100 μM deoxynucleoside triphosphates (Pharmacia)–each oligo at 1.0 μM–0.01% gelatin–whole-cell lysates or purified DNA, as indicated. Samples were overlaid with 100 μl of paraffin oil (J. T. Baker Chemical Co.) in 0.6-ml tubes (Sarstedt) and amplified for 25 to 60 cycles in an automated PCR machine (Perkin-Elmer–Cetus, Ericomp, or a machine built in our laboratory). Each cycle consisted of 1 min of denaturation at 93°C, 1 to 2 min at the annealing temperature of 55°C, and 1.5 to 3.0 min of extension at 72°C. The final extension step continued for an additional 5 min.

Hybridization assays. Quantitation of amplified products was performed as follows. Various amounts of reaction products, as indicated in the figure legends, were denatured by treatment with 7.4% formaldehyde at 65°C for 15 min. A portion of each sample was then applied to nitrocellulose (0.22- or 0.45-μm pore size; Schleicher & Schuell, Inc.) with a slot blot apparatus (Bio-Rad Laboratories). The filters were prehybridized in prehybridization buffer (6× SSC, 5× Denhardt solution, 0.1% sodium dodecyl sulfate, 50 μg of tRNA per ml) at 42°C for 1 h and hybridized in hybridization buffer (6× SSC, 1× Denhardt solution, 0.1% sodium dodecyl sulfate, 50 μg of tRNA per ml) at 42°C for 6 to 15 h with oligo 3 (5′ ^{32}P labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase [United States Biochemicals]). Oligo 3 was chosen as the probe because it is wholly within the region being amplified and does not overlap the amplification primers. The filters were subsequently washed in 6× SSC–0.1% sodium dodecyl sulfate twice at 55°C for 30 min each time. Following several rinses in 6× SSC at room temperature, the filters were autoradiographed at −70°C with Kodak XRP or XAR film and a single Du Pont Cronex Lightning-Plus intensifying screen for the times indicated.

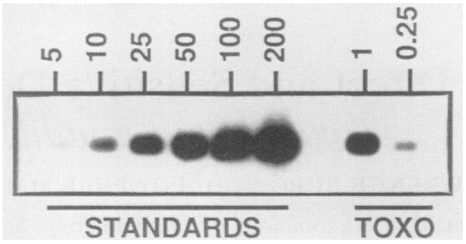

FIG. 1. Characterization of the *B1* gene as a target for amplification. Various amounts of *T. gondii* RH genomic DNA (TOXO) or plasmid DNA containing a cloned *B1* gene (STANDARDS; 4) were digested with *Eco*RI and analyzed by agarose gel electrophoresis and Southern blot hybridization with a nick-translated ^{32}P-labeled *B1* probe. The autoradiograph is shown here. The numbers above the standards represent molar equivalents of plasmid DNA relative to the number of moles of a single-copy gene in 1 μg of toxoplasma DNA. The numbers above the TOXO lanes indicate the amounts (micrograms) of genomic DNA loaded.

RESULTS

Identification and characterization of a repetitive target (*B1*) in *T. gondii*. The *B1* gene was isolated as described elsewhere (4). Briefly, a *T. gondii* RH genomic library in λgt11 was screened by using mouse polyclonal antiserum to a lysate of *T. gondii* P tachyzoites. One of the recombinant bacteriophage thus identified contained a 2.2-kb *Eco*RI genomic fragment which was subsequently found to be tandemly repeated in the genome (4; see below). This gene is arbitrarily referred to as the *B1* gene. The insert from this phage was subcloned into the *Eco*RI site of plasmid vector pAT153 (38) to give pTXgB1 and further characterized.

Because of its repetitive nature, the *B1* gene is an attractive target for detecting *T. gondii* parasites through amplification of *B1*-specific DNA. As the first step in exploiting this gene as a target, we sought to precisely quantitate the number of *B1* repeats in the parasite genome. To do this, quantitative Southern blot analysis was performed by using defined amounts of *B1* plasmid (mixed with carrier DNA) and total genomic DNAs. The autoradiograph presented in Fig. 1 shows that the *B1* gene is between 25- and 50-fold repetitive; quantitation (data not shown) of the radioactivity present in each band more precisely indicated that there are about 35 copies of the *B1* gene in the *T. gondii* genome.

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   1 GAATTCGTTC GACAGAAAGG GAGCAAGAGT TGGGACTAAA TCGAAGCTGA GATGCTCAAA
     EcoRI
  61 GTCGACCGCG AGATGCACCC GCAGAAGAAG GGCTGACTCG AACCAGATGT GCTAAAGGCG
 121 TCATTGCTGT TCTGTCCTAT CGCAACGGAG TTCTTCCCAG ACGTGGATTT CCGTTGGTTC
 181 CGCCTCCTTC GTCCGTCGTA ATATCAGGCC TTCTGTTCTG TTCGCTGTCT GTCTAGGGCA
 241 CCCTTACTGC AAGAGAAGTA TTTGAGGTCA TATCGTCCCA TGAAGTCGAC CACCTGTTTC
 301 CTCTCTTCAC TGTCACGTAC GACATCGCAT TCAAGGGAAG AGATCCAGCA GATCTCGTTC
 361 GTGTATTCGA GACAAGAGAG GTCCGCCCCC ACAAGACGGC TGAAGAATGC AACATTCTTG
 421 TGCTGCCTCC TCTCATGGCA AATGCCAGAA GAAGGGTACG TGTTGCATCA TAACAAGAGC
                                 intron start
 481 TGTATTTCCC GCTGGCAAAT ACAGGTGAAA TGTACCTCCA GAAAAGCCAC CTAGTATCGT
 541 GCGGCAATGT GCCACCTCGC CTCTTGGGAG AAAAAGAGGA AGAGACGCTG CCGCTGTTTT
 601 GCAAATGAAA AGGATTCATT TTCGCAGTAC ACCAGGAGTT GGATTTTGTA GAGCGTCTCT
 661 CTTCAAGCAG CGTATTGTCG AGTAGATCAG AAAGGAACTG CATCCGTTCA TGAGTATAAG
                                                 oligo #1
 721 AAAAAAATGT GGGAATGAAA GAGACGCTAA TGTGTTTGCA TAGGTTGCAG TCACTGACGA
                                                 oligo #2
 781 GCTCCCCTCT GCTGGCGAAA AGTGAAATTC ATGAGTATCT GTGCAACTTT GGTGTATTCG
                                                 oligo #3
 841 CAGATTGGTC GCCTGCAATC GATAGTTGAC CACGAACGCT TTAAAGAACA GGAGAAGAAG
  intron end                      oligo #4
 901 ATCGTGAAAG AATACGAGAA GAGGTACACA GAGATAGAAG TCGCTGCGGA GACAGCGAAG
 961 ACTGCGGATG ACTTCACTCC CGTCGCACCA GCAGCAGAGG AGTGCCGGGC AAGAAAATGA
1021 GATGCCTAGA GGAGACACAG CGTGTTATGA ACAAATCTAT TGAGGTTTCG CGAAGAGGAG
1081 GGAACATATT ATATACAGAA GAAGAACAAG AGACGTGCCG CATGTCGCTA AGCCATCGGA
1141 AGGGATGCTC AGAAAATGGC ACAGTATCAC ATTACAGTTC CGTTGATTCG TCTGATGGTG
1201 ACGAAAGGGG AAGAATAGTT GTCGCACCAA AACTGGCTAG TTGTTATTTT GAAGAAGACG
1261 AGAGATGGAG TGAACCACCA AAAATCGGAG AAAATCGATG GTGTCACGTT TTTTGTCAGA
1321 CTTCACTTTG TGCAGAAGCA TTGCCCGTCC AAACTGCAAC AACTGCTCTA GCGTGTTCGT
1381 CTCCATTCCG TACAGTCTTC AAAAATACAA AAGAGAACAT TCCAGCAACT TCTGCCTTTG
      poly A site
1441 TTCTTTTAGC CTCAATAGCA GGATGACGCC TCCCTCCTAT CTTTCAGCCA ACCCAGCAAA
1501 CACCGACGAA CTCTCTGTAG AGTAACAAAG AGAAGGCAAA ACGCGCCATC ACGAACACTC
1561 GCAGAGATGA TACAGAGACG TGTCATCAGG ACAAGGTTGG TCGCTTAATT TTCTGTATAT
1621 AGCATTTTTA GAATGCACCT TTCGGACCTC AACAACCGTG CAAAAGGATC GCCACCTGGT
1681 GTCTCTTCAA GCGTCAAAAC GAACTATCTG TATATCTCTC AAGGAGGACT GGCAACCTGG
1741 TGTCGACAAC AGAACAGCTG CAGTCCGGAA ATAGAAAGCC ATGAGGCACT CCAACGGGCG
1801 AGTAGCACCT GAGGAGATAC AAACTGCTAA ACGGTCCGGG TGAAACAATA GAGAGTACTG
1861 GAACGTCGCC GCTACTGCCC AGTTGTCATG CCATCGACGT AGACCCAGAA ATGAGGCGAG
1921 AAATTAATAT TGTTAGTAAA GCATTCAAAA AGTTCCGGTC GAGAGGCTAA ACCACAAAAG
1981 TGCAAACCAT GCGCAGCCAT CAGCTTAACA AAAGCAGTTG GTGATGGTTG CCTCGAGTTC
2041 CTTCTGAAAA TGGATTACTT CATCAACGAG CCCACCACGC AGAATCATGC TTTCCCAGTG
2101 CTAAAGCGTT TCTAAAGTAG CCGCACAATG CGGAATGCTA AGGGGATCGC CTACGTAGCA
2161 CATGTTGTGC CTCACCCCCC AGCTCGTGCG CTCATTCTCC TTTCGTGCGC GGCT(GAATTC)
                                                              EcoRI
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FIG. 2. Complete sequence of a *B1* gene repeat (sense strand only), beginning at an arbitrary position (*Eco*RI site) and extending to the same site in the next repeat. Indicated are locations of the one intron mapped (▼), the polyadenylation site (▲), the oligo primers, and the *Eco*RI sites. Oligos 1 and 2 (continuous underlining) are upstream primers consisting of sense sequences. Oligo 4 is an antisense sequence and serves as the downstream primer for amplification. Oligo 3 (also antisense) is internal to the amplified regions and serves as a detection probe. Antisense oligos are indicated by broken underlining.

Figure 2 depicts the complete sequence of one *B1* gene repeat. The sequence has no long open reading frame, suggesting that there are introns within this protein-coding gene. We were unable to clone a full-length cDNA to deduce the complete open reading frame. However, one intron was mapped, on the basis of sequence analysis of a partial cDNA, to the region spanning nucleotides 456 to 843 (i.e., this region is missing in the cDNA). Consistent with its being an intron, this sequence begins and ends with the canonical 5′ and 3′ splice site sequences (5). The identity of the *B1*-encoded polypeptide which is reactive to serum was not determined.

The mRNA for the *B1* gene is about 1.6 kb, as judged by Northern (RNA) blot analysis (data not shown). The poly(A)

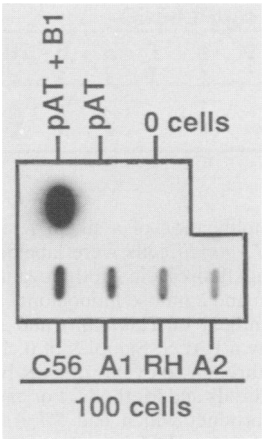

FIG. 3. PCR amplification of the *B1* gene directly from whole-cell lysates of four different strains of *T. gondii*. Cells from RH and C56 strains and two isolates from patients with AIDS (A1 and A2) were obtained from mice or culture (courtesy of J. S. Remington), counted, diluted to 100 organisms, and amplified through 25 cycles of PCR by using oligos 2 and 4 as described in Materials and Methods. We denatured 20% of the product by formaldehyde treatment and detected it by slot blot hybridization with radiolabeled oligo 3. One sample without DNA or cells was also amplified as a negative control (0 cells). The filter also contained 65 ng of pAT153 plasmid DNA (pAT) and 65 ng of pAT153 plus a 2.2-kb *B1* gene repeat (pAT plus *B1*) as positive and negative controls for hybridization, respectively. The autoradiograph shown was exposed for 42 h.

addition site for this gene (position 1384) was deduced from a cDNA clone which contains a poly(A) track as the 3′ end of the insert (Fig. 2). There is no clear consensus sequence for such sites in *T. gondii*. (Note that the sequence presented in Fig. 2 arbitrarily begins at the *Eco*RI site; the mapping data described above indicate that the *Eco*RI site is within the transcribed region.)

Figure 2 also indicates the locations of the oligos used for amplification and detection. Oligos 1 and 2 were used as upstream primers for amplification, while oligo 4 corresponds to the opposite strand of the *B1* gene and was used as the downstream primer for PCR. Oligo 3 was used as a radiolabeled probe internal to the amplified region.

Amplification of cell lysates of different strains. In a first experiment, we used PCR to amplify a segment of the *B1* gene from whole-cell lysates of several strains of *T. gondii*. In one such experiment (Fig. 3), the *B1* target was directly amplified from about 100 *T. gondii* organisms from each of four strains, including two recent isolates from patients with AIDS (A1 and A2) and the RH and C56 strains. After 25 cycles of PCR, the *B1* gene was easily detected in all four strains and yielded roughly equivalent signals. The *B1* gene has also been amplified from two additional strains, P (39) and C (29) (data not shown). It thus appears to be well conserved among the six strains tested to date, at least over the three regions represented by the oligos used for amplification and detection. Although we did not directly determine the copy number of the *B1* gene in each of these strains, these data suggest that it is of similar magnitudes in all of the strains. (Use of 10-fold greater and lesser amounts of cells gave proportionately more or less signal for each strain [data not shown], indicating that amplification did not reach a plateau and that the results were roughly quantitative.) Most importantly, the data show that the *B1* gene is sufficiently

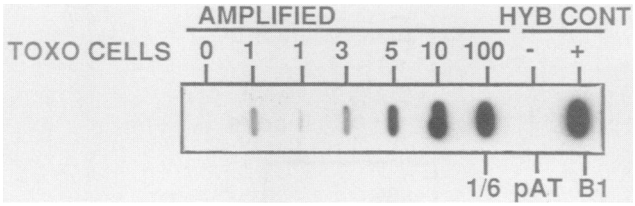

FIG. 4. Direct amplification of a single *T. gondii* cell lysate as selected by FACS. *T. gondii* cells were labeled by indirect immunofluorescence (using fluorescein isothiocyanate-conjugated goat anti-mouse antiserum and a mouse monoclonal antibody [DG-52] to the major surface antigen of *T. gondii*) and sorted directly into amplification tubes by a FACS. Samples of 0, 1, 3, 5, and 100 cells were then amplified through 60 cycles of PCR by using oligos 1 and 4 as described in Materials and Methods. For each reaction product, except the 100-cell product which had 5% (i.e., one-sixth of the material in the other slots), 30% was denatured by formaldehyde treatment and detected by slot blot hybridization with oligo 3 as the probe. The filter also contained 65 ng of plasmid pAT153 (−) and 65 ng of pAT153 plus a 2.2-kb *B1* gene repeat (+) as negative and positive controls for hybridization (HYB CONT), respectively. The autoradiograph shown was exposed for 17 h.

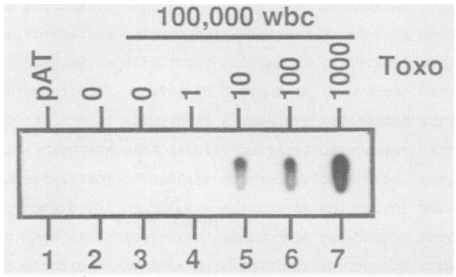

FIG. 5. Amplification of DNA prepared from 0, 1, 10, 100, and 1,000 FACS-selected *T. gondii* organisms (Toxo) in the presence of 100,000 peripheral blood leukocytes (wbc). DNA was prepared from these samples, suspended in water, and amplified through 55 cycles of PCR with oligos 1 and 4 as described in Materials and Methods. Half of the amplified product is shown in a slot blot hybridization format along with 65 ng of unamplified pAT plasmid plus 1 μg of oligos 1 and 4 (pAT lane). Lane 2 was not extracted; lane 3 was extracted in the presence of 100,000 uninfected peripheral blood leukocytes. Lanes 2 and 3 contained no *T. gondii* parasites.

repetitive in each isolate to allow PCR detection with roughly similar sensitivity for each.

To determine the specificity of this assay, amplification and probing using the same set of oligos were attempted with DNAs from a variety of closely related organisms, as well as those which might also be found in the central nervous system of an immunocompromised host. These included *Sarcocystis*, *Neospora*, *Plasmodium*, *Aspergillus*, *Candida*, *Crytpococcus*, and *Absidia* spp. In no case was a signal detectable (data not shown), indicating that this combination of oligos is specific for *T. gondii*.

Detection of amplified product from a single organism. To accurately assess the sensitivity of PCR for detection of the *B1* gene, we used a FACS to place 1, 3, 5, 10, and 100 *T. gondii* parasites directly into tubes for amplification as cell lysates. In one such experiment (Fig. 4), 30% of the reaction products were detected by slot blot hybridization after 60 cycles of PCR amplification. The signal from a single cell was easily detected in this autoradiograph in one replicate sample (lane 2) but only barely seen in the other (lane 3) after 17 h of exposure. We attribute the weakness of the signal in lane 3 to the kinetics of amplification (any delay in amplification during the initial cycles has dramatic effects on the final level of the product). In subsequent experiments, we found detection of a single cell to be highly reproducible, both within a single amplification experiment and throughout a series of experiments (data not shown).

To more closely approximate a clinical sample of CSF or blood which would contain not only *Toxoplasma* cells but also human cells, we isolated human leukocytes from peripheral blood and combined them with our FACS-selected samples of *T. gondii*. Since we are particularly interested in the possibility of direct detection in CSF, we used levels of human cells that represent the maximum generally observed in CSF from patients with AIDS. In studies with a combined total of 42 patients, Koppel et al. (16) and Snider et al. (36) reported a median of 3 to 4 leukocytes per mm^3 (or 3×10^3 to 4×10^3/ml). We used 100,000 human cells, therefore, to represent an approximate upper limit of the concentration likely to be encountered in such samples.

Initially, attempts were made to directly amplify mixtures of human and *T. gondii* cells following heating to 94°C in distilled water. With this procedure, little or no amplification was observed (data not shown). To obtain amplification in the presence of this number of cells, therefore, we tried extracting the DNA from the samples before amplification. Hence, 100,000 leukocytes were added to samples of 1, 10, 100, and 1,000 *T. gondii* organisms, and the DNA was extracted as described in Materials and Methods. After 55 cycles of PCR amplification, a signal from as few as 10 toxoplasma organisms was easily detected in the presence of the DNA of 100,000 human leukocytes (Fig. 5, lane 5) after 6 h of exposure, while the signal from a single *T. gondii* cell under the same conditions could not be detected in this or in other similar experiments, even after longer exposure of the autoradiograph (lane 4). Lane 3 (0 *T. gondii* organisms and 100,000 human leukocytes) indicates that the *B1* target could not be detected by amplification in the human genome; this result was confirmed by repeated experiments.

DISCUSSION

Our results show that a single *T. gondii* parasite can be directly detected from cell lysate materials by PCR using the 35-fold-repetitive *B1* gene as a target for amplification. With 100,000 human leukocytes and DNA extraction, as few as 10 organisms were detected by using the *B1* gene as a target for amplification. Our results also show that the *B1* gene is highly specific for *T. gondii* and is well conserved among all of the strains tested to date, including several isolates from patients with AIDS.

Further optimization of extraction, amplification, and detection procedures may enhance the reaction sensitivity to permit detection of a single parasite in the presence of 100,000 human cells. Detection of the β-globin gene after amplification from single human sperm cells (unextracted) in a background of 100 ng of *Escherichia coli* DNA (18) suggests that the 35-fold-repetitive *B1* target in a single *T. gondii* parasite should be detectable within a background of 660 ng of human DNA (the amount of DNA in 100,000 cells). Other investigators, however, have documented decreased amplification of low-titer targets from samples with high levels of background DNA or after extraction. For example, Abbott et al. have shown that 1 μg of negative DNA can have a 50-fold inhibitory effect on amplification of low-titer targets (1), while Shibata et al. found that extraction of cytomegalovirus-positive culture supernatants was variable

and less sensitive than direct amplification after cell lysis by boiling (34). Our results confirm that some sensitivity is lost in the transition to mock clinical samples, which require extraction and contain high levels of background human DNA. However, most CSF samples contain less than 10% of the human background DNA of our mock clinical samples (16, 26, 36). Thus, we expect that it may be possible to amplify many samples without prior extraction and with minimal preparation.

Our interest in CSF is because of anecdotal reports that tachyzoites of *T. gondii* are occasionally seen in pelleted material from patients with AIDS who have toxoplasma encephalitis (J. S. Remington, personal communication). However, our results also suggest that the DNA of the parasite may be directly detected in the buffy coat of peripheral blood smears, since *T. gondii* invades monocytes (40) and can be isolated from blood by inoculation into mice (31). PCR amplification of the *B1* gene should also be applicable to the diagnosis of congenitally acquired toxoplasmosis by detection of the parasite in amniotic fluid samples. One study demonstrated that 52% of fetal infections can be identified by xenodiagnosis from amniotic fluid cells (8). An assay based on amplification of the *B1* target would be faster than xenodiagnosis and offer the potential for enhanced sensitivity by detection of nonviable as well as viable parasites.

These results indicate that PCR amplification of the *B1* gene of *T. gondii* may be a viable alternative to the more time-consuming and less direct procedures currently used. Assessment of this approach in a carefully controlled analysis of clinical samples is now necessary.

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ADDENDUM IN PROOF

Six additional isolates (provided by J. S. Remington) from patients with AIDS were all positive by PCR amplification of the *B1* gene. Also positive were strains from Japan (pig), England (sheep), and Australia (human; strains provided by J. K. Frenkel, Kansas University Medical Center, Kansas City).

LITERATURE CITED

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