CORRESPONDENCE

Use of polymerase chain reaction to detect Toxoplasma

Burg *et al*[1] and Savva and Holliman[2] recently described the use of the polymerase chain reaction (PCR) to detect *Toxoplasma gondii*. We have also been using amplification reactions to detect the presence of this parasite in clinical specimens since 1989.

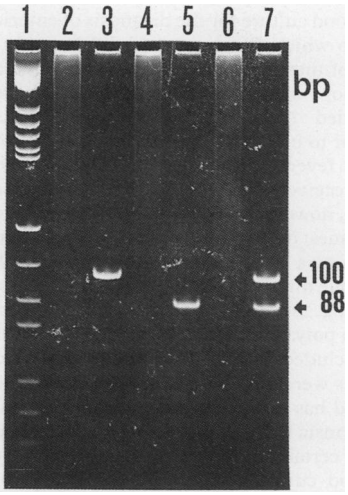
Our method is different from that described by these authors, being based on a different target gene. We use a ribosomal DNA (rDNA) sequence, initially described by Johnson *et al* in phylogenetic studies.[3] Indeed, in eucaryotes the rDNA is frequently repeated and its sequence consists of a patchwork of highly conserved and specific segments. The primers were chosen to match the most specific segment. Due to its property of natural amplification (hundreds of repeats of the gene), the rDNA is an interesting target for very sensitive detection of *T gondii* in biological fluids by the PCR. The natural amplification of ribosomal RNA has recently been used elsewhere to detect and differentiate species of *Plasmodium*.[4] Because RNA is very susceptible to enzyme degradation, a PCR based on rDNA is likely to be much more reliable than one based on cDNA or RNA.

Briefly, an 88 base pair segment of *T gondii* rDNA is amplified with the synthetic oligonucleotide primers 5' -CCTTGGCCGATAGGTCTAGG and 5' -AGGCATTCGGGTTAAAGATT. Thirty five cycles are performed (denaturation step 95°C for 10 seconds, annealing step 64°C for 30 seconds, synthesis step 70°C for 60 seconds) as described.[5] The product can be visualised directly on a polyacrylamide gel and stained with ethidium bromide.

The identity of the amplified molecules can be proved by restriction analysis or Southern blot analysis. Particularly, the 88 base pair PCR product can be controlled by NsiI restriction, providing 35 and 53 base pair fragments. As for hybridisation using a radiolabelled internal probe, we can detect one copy of the rDNA gene (0·01 parasites). The higher sensitivity of this procedure avoids having to use hybridisation to detect the presence of *T gondii* in clinical samples. In fact, we can detect just a single parasite in a buffer solution, and less than five parasites in a mixture of human cells, and the detection is completed within six hours.

We have used this PCR to examine 80 amniotic fluid samples from pregnant women. The results were positive in 10 cases, showing the superiority of this technique over amniotic fluid cultures (four positive) or fetal blood examination (eight positive). For the remaining two, infection was confirmed after birth. There were no false positive results in this prospective trial. Nor were there any false negative results: the PCR tests for the other 70 samples were negative just like the prenatal diagnostic tests and those at one year follow up.

An additional feature is the ability to combine the sensitivity of rDNA detection with the specificity of P30 detection. The single copy P30 gene encodes for the major surface antigen of *T gondii*, a membranous tachyzoite protein that is apparently conserved in most strains. Coamplification is achieved in a 100 µl reaction buffer (50 mM KCl; 10 mM TRIS-HCl, pH 8·3; 20 mM MgCl2; 200 µM each dATP, dCTP, dTTP, dGTP) with 0·5 µM rDNA specific primers and 0·5 µM P30 specific primers: 5' -GCTCCTTGATTCCTGAAGCA 5' -GGGAACTTCTCGATTGGAAC. Two units of *Taq* Polymerase (Perkin Elmer-Cetus) are added for a 35 cycle reaction. The PCR procedure was as already described, except for the annealing temperature, which was optimised and fixed at 55°C. The figure shows the PCR products obtained by an rDNA or P30 reaction and by rDNA/P30 coamplification. No interference is observed in this twin test which remains highly sensitive, and is more specific. The 100 base pair PCR product can be controlled by *HinfI* restriction analysis (providing 42 and 58 base pair fragments), and by Southern blot analysis.



Analysis of amplified products after ethidium bromide staining on 10% acrylmide gel.
Lane 1: PBR 322/HæIII molecular weight marker.
Lanes 2, 4, 6: 200 ng human DNA negative controls with, respectively, rDNA, P30, and rDNA/P30 primers.
Lanes 3, 5, 7: 1 pg T gondii DNA diluted in 200 ng human DNA positive reactions, with respectively, rDNA, P30, and rDNA/P30 primers.

Studies to determine the efficacy of these tests for antenatal diagnosis and other applications, such as cerebrospinal fluid from patients with AIDS, are in progress.

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Giant cell arteritis

The paper by Wawryk, Ayberk, Boyd and Rode[1] provides interesting new data to support the role of adhesion molecules in the development of the inflammatory infiltrate and the granulomatous response in giant cell (temporal) arteritis.

The article began with the premise that the aetiology of giant cell arteritis is unknown and indicates that no antigen (foreign or self) has been defined. These statements do not do justice to what has been written on this subject. Although the authors do allude, in passing, to the studies of Wilkinson and Russell in 1972, they seem to ignore their own observation of a close relation between CAM positive macrophages and the internal elastic lamina in affected vessels (fig 2 of their paper).

We would like to draw attention to the larger body of evidence which supports the view that elastic tissue changes may be the source of the primary antigen in temporal arteritis and that actinic damage may constitute the primary insult responsible for generating or liberating an autoantigen derived from elastic tissue.

The American College of Rheumatology Subcommittee[2] notes that "a granulomatous inflammatory process is seen that is usually focused along the internal elastic lamina". Identification of the elastic lamina as the probable source of the antigen has been promoted by many observers—for instance, by Healey and Wilske,[3] Mowat and Hazleman,[4] and Bengtsson.[5] The concept that the inflammatory reaction is secondary to primary changes within the lamina is supported by the autoimmune studies of Cid *et al*.[6]

That the specific fault in the lamina is actinic elastotic degeneration (solar, basophilic, or "senile" degeneration) was suggested by one of us[7] in 1978, and further evidence has been gathered to support this in later reviews.[8 9] The demonstration of actinic degeneration, which occurs classically in the elastic tissue of "photoageing" skin that has been exposed to the sun, is notoriously uncertain in routine histological studies using standard haematoxylin and eosin stains. In sections stained with Harris's haematoxylin under controlled conditions with buffered eosin, however, elastic tissue that has been damaged by radiation stains a distinct blue colour; normal elastic tissue stains a contrasting red colour.[8 9] Such changes are more advanced in the superficial aspect of the internal elastic lamina of exposed temporal arteries.[8 9] Electron microscopic examination confirms that actinic elastotic degeneration is a prominent feature of affected elastic laminae in temporal arteries exposed to sunshine.[9]