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Research Note

REPETITIVE DNA SEQUENCES OF *TOXOPLASMA GONDII* FOR DEVELOPMENT OF DIAGNOSTIC PROBES

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Accurate and rapid diagnosis of congenital and reactivated toxoplasmosis infection is essential for patient management and prognosis. In these cases direct detection of parasite is desirable. The detection of *Toxoplasma gondii* utilizing DNA techniques was aproached with the novel polymerase chain reaction (PCR) assay, involving low number copy genes (C. Grover et al., 1990, *J. Clin. Microbiol., 28:* 2297-2301; R. Holliman et al., 1990, *Scand J. Infect. Dis., 22:* 243-244; L. Weiss et al., 1991, *J. Infec. Dis., 163:* 180-186). In the last years several groups have been searching for repetitive DNA sequences in order to use them as probes for the diagnosis of parasite diseases (R. Baker Jr, 1990, *Exp. Parasitol., 70:* 494-499). Recently we isolated and cloned three repetitive DNA sequences of *T. gondii* (J. Blanco et al., 1990, *J. Protozool., 37, 1:* abstract 105). One of these DNA sequences cloned (named Tg4) is repeated up to 1,000 times in the genome of *T. gondii* (S. Angel et al., 1991, *J. Protozool., 38, 1:* abstract 225), being the most sensitive in the detection of total parasite DNA.

In this work we report the entire Tg4 sequence (GenBANK Acc# 59, 905, *Toxoplasma gondii* ABGTg4 DNA) which is 427 bp long with a G + C percentage of 56.9. Furthermore, we found a 37 bp sequence repeated twice within the fragment in a direct repeat (DR) fashion, separated by a 302 bp sequence with an internal open reading frame (Fig. 1). In a comparative study of this sequence utilizing the GenEMBL bank, we found that it has a high percentage of homology (94%) with the terminal portion (the last 87 bp) of the 353 bp $TGR1_E$ cloned by N. Cristina et al. (1991, *Exp. Parasitol., 73:* 73-81). The segment in which these two sequences are overlaped, included the 37 bp DR mentioned.

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On the other hand, the potenciality of Tg4 as a DNA probe for diagnosis of acute toxoplasmosis was studied. We have previously demonstrated that Tg4-^{32}P radiolabelled is able to detect parasites in tissues from mice with experimental acute toxoplasmosis (S. Angel et al., 1991, *Medicina, Buenos Aires, 51:* 185-186). In this work we used a non radioactive digoxigenin-dUTP labelling of Tg4 (DIG DNA labelling kit from Boehringer-Mannheim). Eight weeks CF1 mice were infected intraperitonealy with 10^4 tachyzoites of the RH strain of *T. gondii.* Eight days after infection, they were sacrificed to obtain their organs (liver, spleen and brain) and ascitic fluid, which were DNA extracted. When this material was analyzed in dot blot experiments, strong positive signals were found in all samples that belonged to infected mice (Fig. 2-A). Control DNA obtained from tissues of non infected mice and from human leukocytes did not show any signals.

Utilizing this system we were able to detect parasites in blood samples (leukocytes preparation) of infected mice as soon as the first day after infection (Fig. 2-B). No signals were evidenced in blood samples from uninfected mice.

With these results we are the first laboratory in demonstrate the utility of high repetitive DNA sequence in the detection of *T. gondii* in experimentaly infected mice. Other workers (D. Savva et al., 1990, *J. Med. Microbiol., 32:* 25-31; L. Weiss et al., 1991, *J. Infec. Dis., 163:* 180-186) applying the PCR technique in the detection of *T. gondii* obtained higher levels of sensitivity. However, when they carried out PCR experiments to the detection of parasites in tissues from infected animal models, their results were similar to those reported here. Although we think that the high levels of sensitivity of this sequence is

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                10                  20                  30                  40                  50                  60
TGGCATGTTT CATTTGCGGC ACAGGTGCTC CCGCAGGTCC GACATCACTC TGGCACCGAA

                70                  80                  90*                 100                 110                 120
TTGTCGACGT GCTGGGTGTG GCGGCGCTCA TGGGTCGGCT GGTACGTGAA AGGAGAATCG

                130                 140                 150                 160                 170                 180
TCGCTGTACC TGCGCGGATG GGTCAGAGGT TATTGGTTGT GTGTCGTGTG CAGTCTGTCT

                190                 200                 210                 220                 230                 240
CCGATATTGT TGAGCGTTTT TTTCAGGGCC AGACGGTAAG AGCAGAAAGG TTGCTGGGCC

                250                 260                 270                 280                 290                 300
CTCTTCTGTG TCTTGAGGCA ACTGCCGAGC ACGAGAGATT AGTGCTGATG GGTCACGCGC

                310                 320                 330                 340                 350                 360
TGAGGCGTAC GTAGCTGCCG CGAGGCACTG TGCGGCGGAC GACATGTTTC ATTTGCGGCA

                370                 380                 390                 400                 410                 420
CAGGTGCTCC CGCAGGTCGA GCATACTGGC ACAGCTTGTT CGACGTGTGG TTGCGCGCCA

                430
TGGTCCG
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Fig. 1: nucleotide sequence of Tg4 *Toxoplasma gondii* fragments. The 37 bp DR element are boxed. The * indicates the putative transcription start and the underlined sequence represents the possible coding region.

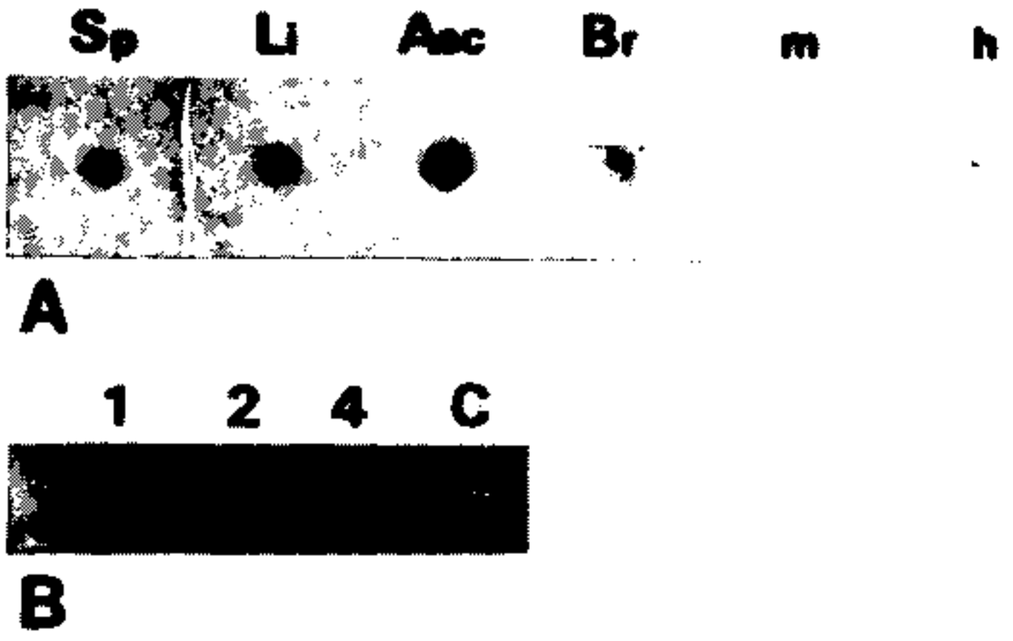

Fig. 2: tissue analysis using non radioactive labelling of Tg4. A: dot blot containing 2 μg of DNA from spleen (Sp), liver (Li), brain (Br) and 1 μg from ascitic fluid (Asc) from experimentally infected mice, were hybridized with Tg4 probe. Five μg of DNA material from non infected mice (m) and 5 μg of human DNA (h) were spotted as control. B: dot blot of 2 μg of DNA material obtained from periphereal leukocytes from infected mice extracted at different days after infection (1, 2 and 4). (m) is a negative control of white blood cells from normal mice. Hybridization and washes were performed in BLOTTO at 65 °C, in 6 X SSC and 0.1 X SSC buffer respectively. 300 ng of Tg4 were digoxigenin-dUTP labelled according the manufacture recomendation.

due to its high number of repetition into the parasite genome (about 1,000 copies), at this moment we unknow the sharp sequences into Tg4 that is repeated this number of times. However, the fact that the whole 37 pb DR are present into two repetitive fragments cloned separately by two different laboratories, would suggest that this sequence could be the repetitive element. In order to elucidated this point, we are conducting experiments with a synthetic oligonucleotides containing this sequence. On the other hand, it is noteworthy that Tg4 labelled with digoxigenin-dUTP has similar levels of detection than the radioactive labelling of this probe (data not shown), an it does not show cross hybridization with human DNA material (Fig. 2-A). For these reasons, the utilization of the dot blot system with this non radioactive probe may be an interesting approach toward the diagnosis of acute human toxoplasmosis in clinical laboratories.

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