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Radiolabeled Total Parasite DNA Probe Specifically Detects *Trypanosoma cruzi* in Mammalian Blood

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A DNA-DNA hybridization procedure is described in which radiolabeled total parasite DNA is used to detect *Trypanosoma cruzi* spotted directly onto nylon membrane. Under conditions that favor hybridization of repetitive DNA sequences, the radiolabeled total DNA was able to detect as few as five parasites spotted onto nylon membrane. Trypanosomes were detectable in blood from mice with *T. cruzi* parasitemias spotted directly onto membranes. All 10 strains of *T. cruzi* examined, which came from different areas of the United States and South America, were readily detected with a total DNA probe from any 1 strain of the parasite. No signals were detected with up to 20,000 *Trypanosoma rangeli*, *T. brucei*, *Crithidia fasciculata*, or *Leishmania* parasites or with normal mouse blood or mammalian DNA. The hybridization method is sensitive, specific, rapid, and inexpensive and is potentially applicable to the detection of other parasites.

Repetitive DNA sequences occur in many thousands of copies that are dispersed throughout the genomes of eucaryotes (5, 6). Purified DNA fragments that hybridize to repetitive DNA elements have been used as probes for the detection of various parasites, including those that cause malaria (3, 9), leishmaniasis (2, 16, 18, 19), filariasis (15), and South American trypanosomiasis (12). These procedures have involved spotting of intact parasites, detergent-lysed blood containing parasites, or purified parasite DNA onto nitrocellulose or nylon membranes and hybridizing the membranes with radiolabeled repetitive DNA probes. Cloned repetitive DNA probes have been used to detect *Trypanosoma cruzi*, the parasite that causes South American trypanosomiasis (Chagas' disease), and these probes are of potential value for the diagnosis of this disease (10, 12).

We have used a simple alternative procedure for the detection of *T. cruzi* in mammalian blood. The procedure uses total parasite DNA as a probe and is based on the observations that total DNA from a eucaryotic organism hybridizes, under appropriate conditions, preferentially to repetitive DNA sequences (13) and that repetitive DNA sequences are evolutionarily divergent between different eucaryote species and are often specific to one or a group of related species (7). Radiolabeled total human DNA, for example, has been used to specifically detect human DNA sequences introduced into mouse cells under conditions in which mouse DNA showed little or no hybridization to the total human DNA probe (13, 14).

Radiolabeled total DNA from *T. cruzi* was prepared as follows. About 10^9 *T. cruzi* epimastigotes were harvested and washed once with phosphate-buffered saline. The parasite cell pellet was suspended in 1 ml of 1% sodium dodecyl sulfate (SDS)–25 mM EDTA–50 mM Tris hydrochloride (pH 7.5) containing 400 μg of proteinase K ml^{-1} and incubated for 5 h at 37°C. DNA was extracted once with an equal volume of phenol saturated with water, twice with phenol-chloroform (1:1, vol/vol), and once with chloroform and then precipitated at −20°C with 2 volumes of absolute ethanol for 3 h. The DNA was pelleted by centrifugation in a Microfuge at 4°C for 10 min, air dried for 60 min, and dissolved in 1 mM EDTA–10 mM Tris hydrochloride (pH 8.0) at a concentration of 0.5 mg of DNA ml^{-1}. About 2.5 μg of the DNA was radiolabeled by nick translation in the presence of [^{32}P]dCTP as described previously (17).

We examined the specificity of the radiolabeled total *T. cruzi* DNA probe under conditions that favor detection of highly repetitive DNA sequences. Cultured *T. cruzi* epimastigotes, obtained from different areas of South America and the United States (11), and *Trypanosoma brucei*, *Crithidia fasciculata*, *Leishmania*, and *Trypanosoma rangeli* parasites were spotted directly onto nylon membrane (Amersham Corp.) and allowed to air dry for 10 min. The membrane was then soaked in 0.5 M NaOH–1.5 M NaCl for 10 min and then in 3 M NaCl–0.5 M Tris hydrochloride (pH 7.0) for 6 min and was rinsed in 2× SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0). After air drying for 60 min, the DNA was baked onto the membrane by illuminating it with UV light for 5 min. The membrane was prehybridized for 2 h at 45°C in 5× Denhardt solution (0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone)–6× SSC–50% formamide–0.1% SDS–salmon testis DNA (sheared, 1 mg ml^{-1}). Hybridization to ^{32}P-labeled total DNA from *T. cruzi* (about half of the nick-translated probe for each 150 cm^2 of nylon membrane) was carried out at 45°C for 16 h in 5× Denhardt solution–6× SSC–50% formamide–0.1% SDS–salmon testis DNA (sheared, 0.1 mg ml^{-1})–10% dextran sulfate. The membrane was washed for 10 min at room temperature with 2× SSC–0.2% SDS and then at 50°C for 25 min with 0.2× SSC–0.2% SDS. The membrane was then dried briefly between sheets of filter paper and exposed to XAR-5 film (Eastman Kodak Co.) for 4 to 24 h at −70°C with an intensifying screen.

Radiolabeled DNA from any one strain of *T. cruzi* readily detected DNA equivalent to that from 50 trypanosomes from each of the 10 strains of the parasite that were examined, and weak signals were given even with 5 parasites. However, total *T. cruzi* DNA did not give any signals with up to 20,000 *T. brucei*, *T. rangeli*, *C. fasciculata*, or *Leishmania* parasites or with up to 2.5 μg of mammalian DNA (Fig. 1).

We next examined the ability of radiolabeled total *T. cruzi*

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DNA to detect parasites in mammalian blood spotted onto nylon membrane. Mice were injected intraperitoneally with about 10^5 trypanosomes. After 7 days, blood samples were examined microscopically for parasites, and parasite-containing blood was mixed with half its volume of heparin (5,000 U ml^{-1}). Heparinized blood was spotted onto nylon membrane and hybridized with radiolabeled total *T. cruzi* DNA. Trypanosomes were clearly detectable in mammalian blood (Fig. 2). The procedure allowed a maximum of 50 μl of blood to be spotted; volumes greater than 50 μl produced weak but detectable signals. Similar, nonspecific binding of radiolabeled DNA probes to components of mammalian blood has been described previously (4) and can be reduced by detergent lysis and proteolysis of blood samples before they are spotted onto the membrane (3).

Total *T. cruzi* DNA is, therefore, a specific and sensitive probe for the detection of trypanosomes in material spotted directly onto nylon membranes. However, the procedure described here may be useful only for the diagnosis of the acute phase of Chagas' disease, in which significant numbers of trypanosomes are present in the blood. It remains to be seen whether the method will allow detection of parasites in the chronic phase of the disease, in which levels of trypanosomes are frequently too low to be detectable by direct microscopic examination of the blood but are often detectable by xenodiagnosis. In a preliminary trial with blood from only three individuals, a patient with acute Chagas' disease gave a positive result, but two patients with chronic disease tested negative (data not shown). The use of total *T. cruzi* DNA as a probe for blood parasites is much less time-consuming than direct microscopy or xenodiagnosis, as several hundred samples can easily be spotted onto a filter and the whole procedure can be completed within 24 h. This method also readily discriminates between *T. cruzi* and *T. rangeli*, which has a geographical distribution similar to that of *T. cruzi* but infects humans nonpathologically, and between *T. cruzi* and *Leishmania* spp., which are also prevalent in South America.

The method described here may be useful for epidemiological or xenodiagnostic surveys in which large numbers of triatomine bugs or other arthropod vectors are examined for protozoan infections. We have, indeed, successfully used total *T. cruzi* and *T. rangeli* DNA probes to detect these parasites differentially in samples of feces from triatomine bugs spotted directly onto nylon membrane (12b). Another application might be the screening of thousands of sand flies differentially for *Trypanosoma*, *Endotrypanum*, and *Leishmania* spp. in a search for novel parasite-transmission cycles (1).

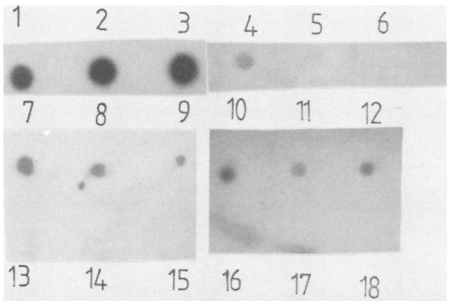

FIG. 1. Species specificity of total *T. cruzi* DNA probe. Between 100 and 2,000 epimastigotes from 10 strains of *T. cruzi* (spots 1 and 4: strains X10, Tc050, Esmeraldo, and 92/80, respectively; spots 7 to 12: strains Opossum, Armadillo, C8, CAN III, California, and Y, respectively); about 20,000 *T. rangeli* (spot 5), *Leishmania donovani* (spot 6), *L. mexicana* (spot 13), *L. braziliensis* (spot 14), *T. brucei* (spot 15), and *C. fasciculata* (spot 16) parasites; and about 2.5 μg of mouse (spot 17) and human (spot 18) DNA were spotted onto nylon membrane and probed with radiolabeled total *T. cruzi* DNA (strain Tc050). The autoradiographs shown were exposed for 16 h.

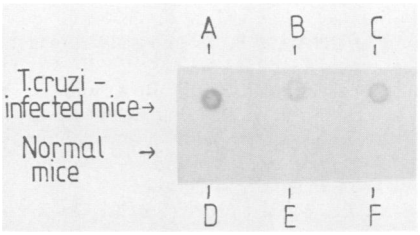

FIG. 2. Detection of *T. cruzi* in blood from infected mice. About 20 μl of blood from three normal mice and from three mice infected with *T. cruzi* were spotted onto nylon membrane and probed with radiolabeled total *T. cruzi* DNA (strain Tc050). The parasitemias of the infected mice were determined microscopically to be about 10, 4, and 7 trypanosomes μl of $blood^{-1}$, respectively. The autoradiograph is a 16-h exposure.

Preparation of total parasite DNA has the obvious advantage that no recombinant DNA technology is required; all that is needed is a source of pure parasite DNA. With the exception of an extremely sensitive total DNA probe for *Plasmodium falciparum* (16), it seems that the potential of such probes has been overlooked. We suggest that the method described here might be useful for detection of a range of parasites and in particular for detection of those parasite infections for which existing methods of diagnosis are unsatisfactory. The procedure is presumably applicable only to eucaryotic parasites, which contain highly repetitive DNA sequences in their genomes. The development of DNA detection systems based on chromogenic reactions (8) may circumvent the use of radiolabeled DNA probes and allow the method to be applied in the field.

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