Title

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Journal

Infection and Immunity, 53(2)

ISSN

0019-9567

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Publication Date

1986-08-01

DOI

10.1128/iai.53.2.235-239.1986

INFECTION AND IMMUNITY, Aug. 1986, p. 235–239
0019-9567/86/080235-05$02.00/0

Vol. 53, No. 2

Variation in Antigenic Determinants Specific to the Infective Stage of *Trypanosoma cruzi*

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Received 23 December 1985/Accepted 14 April 1986

Monoclonal antibodies reactive with the surface antigens of the Peru strain of *Trypanosoma cruzi* were analyzed by Western blots and immunofluorescence assays to determine their reactivity with three life cycle stages and five strain isolates of *T. cruzi*. One monoclonal antibody, 7.6, recognized a 68-kilodalton (kDa) polypeptide in Western blots of Peru strain trypomastigotes, epimastigotes, and amastigotes. A 68-kDa polypeptide was also detected by monoclonal antibody 7.6 in trypomastigotes of the CL and Y strains and in the clonal isolates Esmeraldo clone 3 and Silvio X10 clone 1. Positive immunofluorescence results were obtained for all life cycle stages of the five strains that were reacted with monoclonal antibody 7.6, thus indicating that the antigen recognized by monoclonal antibody 7.6 is universally present in all *T. cruzi* strains tested. In contrast, monoclonal antibody 4.2 reacted with a polypeptide doublet of 90 and 105 kDa in Western blots of Peru strain trypomastigotes, but it did not detect these antigens in epimastigotes or amastigotes. The same polypeptide doublet of 90 and 105 kDa was also detected in Western blots of Y strain trypomastigotes; however, no bands were detected in blots of strain CL or isolate Silvio X10 clone 1 trypomastigotes. In blots of Esmeraldo clone 3 trypomastigotes, a single band of 130 kDa was detected by monoclonal antibody 4.2. In immunofluorescence assays of monoclonal antibody 4.2, positive reactions were obtained only with trypomastigotes of Peru, Y, and Esmeraldo clone 3 strains. Thus, monoclonal antibody 4.2 recognizes a trypomastigote-specific antigen which is not universally present on all strains of *T. cruzi*.

The protozoan parasite *Trypanosoma cruzi* causes Chagas' disease in humans and produces a complex spectrum of symptoms among infected individuals. Approximately 20 million people are currently infected with *T. cruzi*, and an estimated 100 million individuals living in endemic areas of Central and South America are at risk of infection. At present there are no completely effective means for treatment or prevention of Chagas' disease or for diagnosis of asymptomatic infections. Results of recent work suggests that distinct subgroups of *T. cruzi* can be identified which may be responsible for the different symptomatic presentations of Chagas' disease (13). Genetic polymorphism as reflected by isoenzyme electrophoretic mobility (14), kinetoplast DNA restriction patterns (15), total DNA content (6), growth rates (7–9), and infectivity for mice and tissue culture cells (4, 17) have been detected among different strains of *T. cruzi*. Because the identification of parasite antigens suitable for use as diagnostic and vaccine reagents has been targeted as a primary goal in Chagas' disease research, an investigation of potential heterogeneity of the surface antigens among *T. cruzi* strains is warranted.

Monoclonal antibodies have proven to be valuable in the identification of distinct strains of herpes simplex virus (16), feline leukemia virus (11), and protozoans such as *Leishmania* sp. (12). The development of monoclonal antibody probes which react specifically with *T. cruzi* subgroups would be beneficial in epidemiological and diagnostic studies. Several anti-*T. cruzi* monoclonal antibodies have been developed (1, 3, 10, 20). However, none of these antibodies characterizes antigens specific to the infective trypomastigote stage. Previously, we derived hybridoma cell lines from mice infected with the Peru strain of *T. cruzi* which produced antibodies reactive with Peru trypomastigote surface antigens in radioimmunoassays, immunofluorescence assays, and Western blots (2). Here, we characterize further these monoclonal antibodies by determining the antigens recognized in the various life cycle stages of *T. cruzi* and in other strain isolates of *T. cruzi*.

MATERIALS AND METHODS

Parasites. *T. cruzi* Peru, Y, and CL strains were obtained from Stuart M. Krassner, University of California, Irvine. The cloned *T. cruzi* strains Esmeraldo clone 3 and Silvio X10 clone 1 were obtained from James Dvorak, National Institutes of Health, Bethesda, Md. *T. cruzi* Peru has been maintained in our laboratory by passaging through mice and in tissue culture (2). The Y, CL, Esmeraldo, and Silvio strains were maintained as epimastigote cultures.

Epimastigotes were grown in brain heart infusion medium (Difco Laboratories, Detroit, Mich.) (5). Tissue culture-derived trypomastigotes and amastigotes were obtained from infected WI-38/Va-13 subline 2RA cells (American Type Culture Collection, Rockville, Md.) by the procedure of Sanderson et al. (18). To establish continuous trypomastigote cultures from the epimastigote stocks, late stationary phase epimastigote cultures containing metacyclic trypomastigotes were added to monolayers of WI-38/Va-13 subline 2RA cells. Trypomastigotes obtained from the initial flasks were used for subsequent passes of the trypomastigote cultures.

Monoclonal antibodies. Hybridoma cell lines producing anti-*T. cruzi* monoclonal antibodies were derived from fusions with spleen cells from B6D2 F_1 mice (Jackson Laboratories, Bar Harbor, Maine)infected with the *T. cruzi* Peru (2). Monoclonal antibodies were purified from tissue culture supernatants by ammonium sulfate precipitation and DE-52 chromatography. The immunoglobulin isotype of monoclonal antibodies 7.6 and 4.2 is immunoglobulin G3 (IgG3), as

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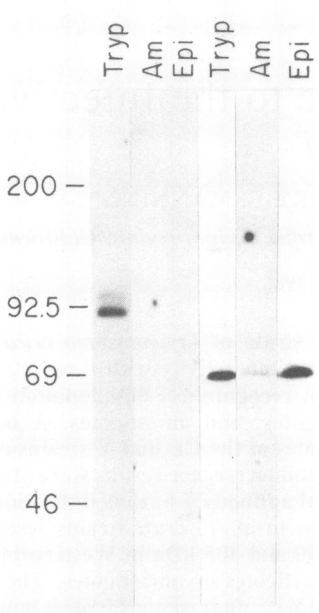

FIG. 1. Western blot analysis of antigens recognized by monoclonal antibodies 4.2 and 7.6 in the various life cycle stages of *T. cruzi* Peru. The first three lanes show blots of trypomastigote (Tryp), amastigote (Am), and epimastigote (Epi) lysates reacted with monoclonal antibody 4.2; lanes 4 to 6, lysates of trypomastigotes (Tryp), amastigotes (Am), and epimastigotes (Epi) reacted with monoclonal antibody 7.6. Molecular weight markers indicated to the left of the gels are as follows: myosin, 200,000 (200); phosphorylase *b*, 92,500 (92.5); bovine serum albumin, 69,000 (69); ovalbumin, 46,000 (46).

determined by Ouchterlony diffusion with subclass-specific antisera (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.).

Western blot analysis. Cells were pelleted by centrifugation and washed twice with cold phosphate-buffered saline (0.02 M sodium phosphate [pH 7.4], 0.9% sodium chloride) containing 0.1% glucose. Cell pellets were solubilized in lysis buffer containing 1% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; Sigma Chemical Company, St. Louis, Mo.), 20 mM Tris hydrochloride (pH 7.6), 25 mM sodium chloride, 30 mM magnesium chloride, 1 mM phenylmethylsulfonyl fluoride, and 1 mM *N*-ethylmaleimide at concentrations of 5×10^8 cells ml^{-1}. Lysates were adjusted to 62.5 mM Tris, 10% glycerol, 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate, and 0.001% bromphenol blue and were applied to one-dimensional polyacrylamide slab gels (2). Following electrophoresis, samples were transferred to nitrocellulose by electroblotting in a Trans-blot cell (Bio-Rad Laboratories, Richmond, Calif.) and probed with anti-*T. cruzi* monoclonal antibodies and ^{125}I-labeled sheep-anti-mouse serum (Amersham Corp., Arlington Heights, Ill.) (2).

Indirect immunofluorescence assay. Binding of monoclonal antibodies in indirect immunofluorescence assays (IFAs) with acetone-fixed parasites and fluorescein isothiocyanate-labeled goat anti-mouse serum (Miles) was performed as described previously (2). Myeloma antibody subclass IgG3 (Litton Bionetics, Kensington, Md.) was used as a negative control in all experiments.

RESULTS

Reactivity of monoclonal antibodies with developmental stages of *T. cruzi* Peru strain. Monoclonal antibodies derived from spleen cells of mice infected with the *T. cruzi* Peru have previously been shown by Western blot analysis and IFAs with both acetone-fixed and live cells to react with the trypomastigote stage of *T. cruzi* (2). We wished to determine whether the antigenic determinants recognized by these monoclonal antibodies were present on other developmental stages of *T. cruzi*. Two monoclonal antibodies, 7.6 and 4.2, were selected for these studies. Monoclonal antibody 7.6 recognizes a 68-kilodalton (kDa) polypeptide in Western blots of trypomastigote lysates, while monoclonal antibody 4.2 reacts with a protein doublet of 90 and 105 kDa. Lysates of tissue culture-derived trypomastigotes, amastigotes, and epimastigotes were subjected to one-dimensional polyacrylamide gel electrophoresis and electroblotted to nitrocellulose. Blots of the various stages were individually probed with monoclonal antibodies 4.2 and 7.6. The 68-kDa polypeptide previously identified in trypomastigotes by monoclonal antibody 7.6 was also observed in the amastigote and epimastigote stages of the parasite (Fig. 1). This protein appears to be constitutively synthesized throughout the different developmental stages of the parasite. In contrast, the protein doublet of 90 and 105 kDa identified by monoclonal antibody 4.2 in trypomastigotes was not detected in either the amastigote or the epimastigote stages.

The reactivity of monoclonal antibodies 4.2 and 7.6 with the developmental stages of *T. cruzi* was further tested by IFA. The pattern of reactivity observed in the IFA was similar to that observed in Western blots. In IFAs with monoclonal antibody 7.6, all of the *T. cruzi* developmental stages tested gave positive fluorescence patterns (Fig. 2). However, only the trypomastigote stage was positive in IFAs with monoclonal antibody 4.2. Fluorescence was not observed in amastigotes or epimastigotes that were reacted with this monoclonal antibody. Sera from *T. cruzi* Peru-infected mice was positive by IFA with all of the *T. cruzi* stages tested. Control myeloma antibody (subclass IgG3) was negative for all stages tested (data not shown).

Reactivity of monoclonal antibodies with selected strains of *T. cruzi*. The reactivity of monoclonal antibodies 4.2 and 7.6, which were derived from mice infected with the Peru strain, was examined in Western blots and IFAs with other *T. cruzi* strains isolated from various geographical locations. *T. cruzi* Y and CL and the clonal-isolate strains Silvio X10 clone 1 and Esmeraldo clone 3, which differ in their zymodeme profiles, growth rates, and infectivity for mice (8, 9), were selected for these experiments. Trypomastigote lysates from each of the strains were tested for reactivity with monoclonal antibodies 4.2 and 7.6 in Western blots. Monoclonal antibody 4.2 identified a polypeptide doublet in the Y strain with the same molecular weights as the polypeptide doublet observed in the Peru strain (Fig. 3). Interestingly, the relative intensity of the bands detected by monoclonal antibody 4.2 differed in the two strains. In blots of Peru strain trypomastigotes, monoclonal antibody 4.2 recognized a protein doublet of 90 and 105 kDa, with the 90-kDa band having a relatively greater intensity. In blots of Y strain trypomastigotes, the same bands were observed, but a relatively greater intensity of the 105-kDa band was observed.

An entirely different pattern was observed in blots of Esmeraldo clone 3 trypomastigotes. Monoclonal antibody 4.2 detected a single polypeptide of 130 kDa, and no bands of 90 or 105 kDa were observed. When blots of CL or Silvio X10 clone 1 trypomastigotes were probed with monoclonal antibody 4.2, no polypeptides were detected.

The reactivity of monoclonal antibody 4.2 with trypomas-

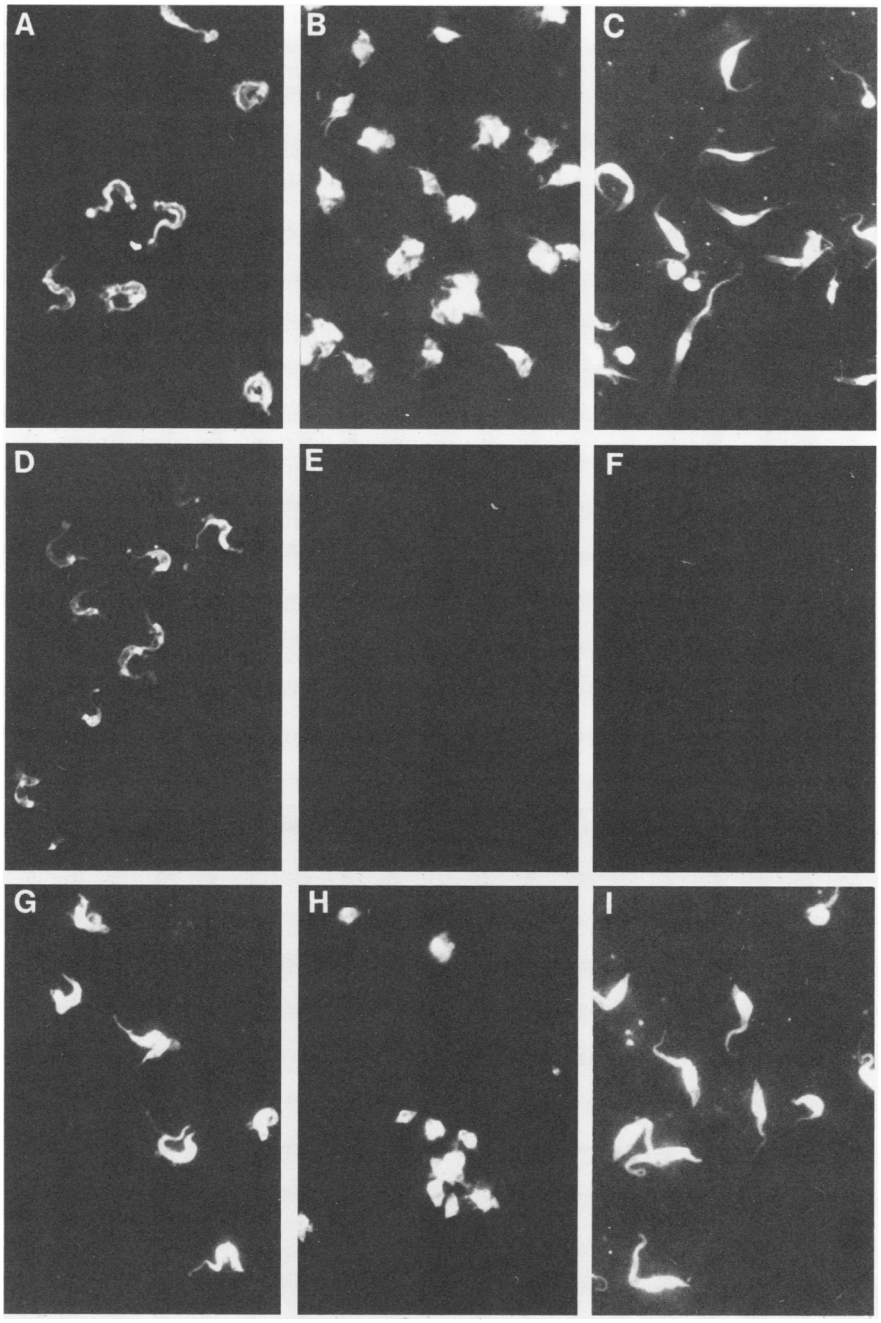

FIG. 2. IFA analysis of reactivity of monoclonal antibodies 7.6 and 4.2 with various life cycle stages of *T. cruzi* Peru. (A to C) Monoclonal antibody 7.6 reacted with trypomastigotes (A), amastigotes (B), and epimastigotes (C). (D to F) Monoclonal antibody 4.2 reacted with trypomastigotes (D), amastigotes (E), and epimastigotes (F). (G to I) *T. cruzi*-immune mouse sera reacted with trypomastigotes (G), amastigotes (H), and epimastigotes (I).

tigotes of the various strains was further tested by IFA. A positive IFA was observed with trypomastigotes from both Y and Esmeraldo clone 3 strains (Fig. 4). No fluorescence was observed when trypomastigotes of CL or Silvio X10 clone 1 strain were tested.

Monoclonal antibody 7.6 reacted with a 68-kDa polypeptide in all of the *T. cruzi* strains tested (data not shown). Positive IFA reactions were observed with all strains when they were reacted with monoclonal antibody 7.6 (Fig. 4).

DISCUSSION

Studies of biochemical, physiological, and immunological characteristics of *T. cruzi* showed heterogeneity among and within strain isolates from various host sources and geographical locations. Several of these characteristics have been used in attempts to classify strains of *T. cruzi* and to correlate the traits listed above with various clinical symptoms observed in *T. cruzi* infections (10). Interestingly, studies of the surface proteins of several strains of *T. cruzi*

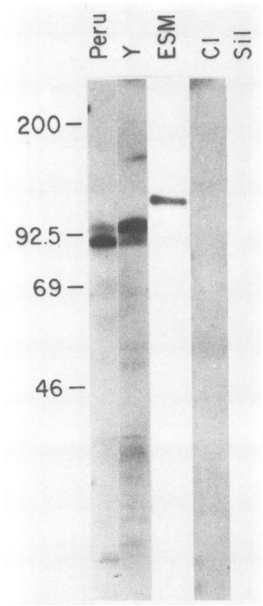

FIG. 3. Western blot analysis of reactivity of monoclonal antibody 4.2 with various *T. cruzi* isolates. Monoclonal antibody 4.2 was reacted with lysates of trypomastigotes of the Peru, Y, Esmeraldo (ESM), CL, and Silvio (Sil) strains.

have shown that these proteins are essentially homogeneous among the different strains (19, 21). Work by Zingales et al. (21) has shown that the surface iodinatable polypeptides of several different *T. cruzi* strains representing different zymodeme groups are remarkably constant. Also, *T. cruzi* (surface) polypeptides immunoprecipitated by sera from patients with Chagas' disease infected with different *T. cruzi* strains all presented nearly the same electrophoretic distribution pattern. This apparent lack of heterogeneity in the surface proteins is somewhat surprising given the widely heterogeneous patterns of isoenzymes, kDNA restriction patterns, and other characteristics found among *T. cruzi* strains. One possible explanation for the observed homogeneity of *T. cruzi* surface proteins is that the methods used to analyze the surface proteins lacked the sensitivity necessary to detect antigenic heterogeneity of these polypeptides among different *T. cruzi* strains. Monoclonal antibodies, however, provide a means for the assessment of the presence or absence of individual antigenic determinants and thus may represent better probes for assessing antigenic heterogeneity within closely related proteins than polyclonal antisera.

The results of Western blots of trypomastigote lysates of five different strains of *T. cruzi* probed with surface protein-specific monoclonal antibody 4.2 indicate that heterogeneity exists among the antigens recognized by this antibody. Of the five strains tested, three (Peru, Y, and Esmeraldo clone 3) contained polypeptides that were recognized by antibody

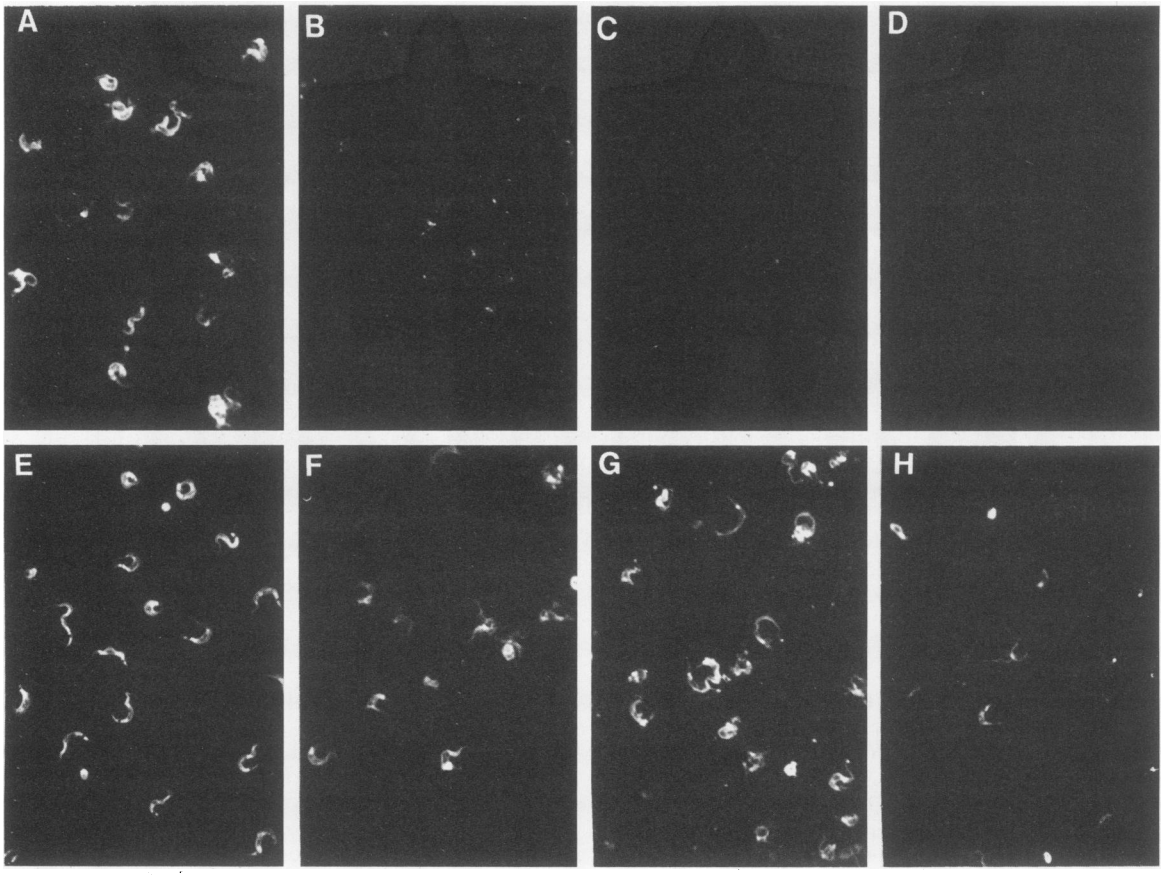

FIG. 4. IFA analysis of reactivity of monoclonal antibodies 4.2 and 7.6 with various strain isolates of *T. cruzi*. (A to D) Monoclonal antibody 4.2 was reacted with trypomastigotes of Y (A), Esmeraldo (B), CL (C), and Silvio (D) strains. (E to H) Monoclonal antibody 7.6 was reacted with trypomastigotes of Y (E), Esmeraldo (F), CL (G), and Silvio (H) strains.

4.2, while two strains (CL and Silvio X10/1) showed no recognition. Of particular interest are the differences in the molecular weights of antigens detected by this antibody. Inspection of the blots of Peru and Y trypomastigotes revealed that monoclonal antibody 4.2 recognizes identical protein doublets of 90 and 105 kDa in the two strains. However, the two protein bands show a clear difference in intensity. Blots of Peru strain trypomastigotes showed a relatively more intense band at 90 kDa, while blots of the Y strain showed a heavier band at 105 kDa. In blots with Esmeraldo clone 3, a single protein of 130 kDa was detected by the monoclonal antibody. It is apparent, therefore, that the antigens recognized by this monoclonal antibody can differ in both molecular weight and abundance among different strains of the parasite.

In summary, results of this study show that the surface antigen recognized by monoclonal antibody 4.2 is present in the trypomastigote but absent in the epimastigote and amastigote stages of the parasite. Also the polypeptides recognized by this antibody exhibit heterogeneity in both molecular weight and abundance among different *T. cruzi* strains. The epitope recognized by monoclonal antibody 4.2 is also not universally present in all strains of the parasite. In contrast, the 68-kDa antigen recognized by monoclonal 7.6 is present in the three stages of the parasite and is present in all of the five strains tested.

ACKNOWLEDGMENTS

We thank James Dvorak for the generous gift of the Silvio X10 clone 1 and Esmeraldo clone 3 stocks, Stuart Krassner and Barbara Granger for assistance in maintenance and growth of parasite cultures, and Jim Massey for assistance.

This work was supported by Public Health Service grant AI18873 from the National Institutes of Health, the United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases, and a gift from Codon Corp., Brisbane, Calif.

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