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# Shift of Excretory-Secretory Immunogens of *Trypanosoma cruzi* during Human Chagas' Disease

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**We studied secreted-excreted immunogens in human patients infected with *Trypanosoma cruzi*. A pair of 45- to 55-kDa antigens and a family of shed acute-phase antigens characterized the acute phase, while antibodies against a 160- to 170-kDa immunogen appeared at the chronic phase of the disease.**

*Trypanosoma cruzi*, the agent of Chagas' disease, causes a long-lasting infection with very low parasitemia in the blood and resistance to new acute infections. During the acute phase of the disease, the parasites resist complement attack, and large numbers of organisms are found in the blood (4, 11). Conversely, during the chronic phase, a very low parasitemia is found (14). Studies in animals showed that resistance to new acute infections could be generated by living trypanosomes (5, 13). Excreted or secreted antigens might be involved in the mechanisms of parasite dissemination and resistance to new acute infections (9). We have previously cloned and sequenced a family of shed acute-phase antigens (SAPA) (1, 10). In this study we performed longitudinal studies of secreted-excreted immunogens in human patients and found markers of the acute and chronic phases of the disease.

We searched for exoantigens by reacting immunoblots of excretory-secretory *T. cruzi* proteins with sera from chagasic patients. The parasites were trypomastigotes and epimastigotes of the RA and CAI strains (3). Blots were prepared after electrophoresis. Each line contained 30 μg of supernatant proteins or lysates from $3 \times 10^7$ trypomastigotes. The filters were saturated in 3% skim dried milk and 2% glycine (blocking solution), incubated for 90 min with sera diluted 600-fold in blocking solution, and extensively washed in 50 mM Tris (pH 7.5)–150 mM NaCl. Bound antibodies were detected by autoradiography after incubation with protein A labeled with $^{125}I$ by the Iodogen procedure (Pierce Chemical Co.). The films were exposed for 1 to 2 days; in control experiments with normal human sera or with uninfected supernatants, the films were exposed for up to 14 days. Figure 1 shows the reactivity of sera from two chagasic patients and two rabbits infected with $2 \times 10^6$ live parasites of the RA and AWP strains (3). The sera selected were highly reactive against many antigens in the parasite lysates. However, only a few exoantigens were detected in the supernatants. Six normal human serum samples were used for control immunoblots; results were negative after a 14-day exposure of the films (not shown).

The presence of exoantigens in two different developmental forms of the parasite and in the acute and chronic phases of the disease were investigated next. Blots containing 15 to 30 μg of proteins from the infective (trypomastigotes), noninfective (epimastigotes), and control supernatants were prepared. Figure 2 shows that the immunogens detected by acute-phase sera were different from those detected by chronic sera. The sera taken from patients with acute infections showed a family of 150- to 200-kDa bands and a very strong pair of 45- to 55-kDa bands in the trypomastigote supernatants. We identified the heavier bands as a family of antigens with repetitive sequences that we call SAPA and that we have previously cloned and partially sequenced (1). As a control, Fig. 2 shows an immunoblot reacted with anti-SAPA antibodies. On the other hand, the chronic-phase sera detected a 160- or 170-kDa band in trypomastigote supernatants.

We next investigated whether the differences in the acute- and chronic-phase sera tested were differences between patients or changes occurring in the same patient at different stages of the disease. Six patients infected with *T. cruzi* were diagnosed by conventional serology early after the appearance of acute symptoms (2). All six patients were treated for 60 days with benznidazole (10 mg/kg of body weight per day). Four patients remained positive by conventional serology or by xenodiagnosis (parasite detection after amplification in the insect vector) and were considered resistant to treatment, while two patients had negative serology and xenodiagnosis after treatment and were considered cured. Serum samples from all patients were collected several times for up to 8 years after the appearance of acute symptoms. Figure 3 shows the results obtained with sera from four resistant patients. There was a progressive change in the immunogens throughout the disease. In panels A and B, the 150- to 200-kDa SAPA family of bands was very faint because we preabsorbed the sera with SAPA. The 45- to 55-kDa bands were characteristic of the acute phase, while the 160- to 170-kDa bands appeared around 480 days after the appearance of acute-phase symptoms and remained until the last time tested (8 years). The same bands were revealed when two different strains of *T. cruzi* were used (Fig. 3). Similar results were obtained in two follow-up studies (panels C and D). We obtained negative results with patient D at 480 days. This patient was also negative at 480 days by conventional serology. However, he was not considered cured because he showed positive xenodiagnoses during the follow-up. The cured individuals showed similar patterns of bands during the acute phase. On the other hand, no band or only a faint band was observed after the patients were cured (Fig. 4).

Secretory-excretory immunogens present at the acute phase of the disease have not been studied, but chronic-

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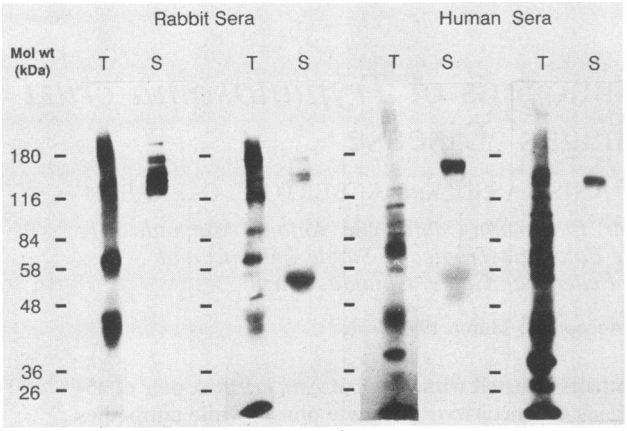


FIG. 1. Detection of trypomastigote antigens with sera from infected humans and rabbits. Immunoblots were prepared after electrophoresis of lysates from $3 \times 10^7$ trypomastigotes (lanes T) and 30 μg of protein from supernatants of trypomastigote-infected cultures (lanes S). The blots were probed with sera from infected humans and rabbits. Autoradiograms after incubation with $^{125}$I-protein A are shown. The positions of prestained molecular weight markers are indicated.

phase immunogens have been recently described by Ouaissi et al. (9). In their experiments, two chronic-phase serum samples had antibodies to exoantigens of 160 and 85 kDa. We did not find the 85-kDa exoantigen in our pool of sera randomly selected from a bank of sera from Argentina and Brazil. The differences might be due to the sera used or the sensitivity of their immunoprecipitation experiments. In any event, the 160-kDa exoantigen was always present in all sera tested by both groups.

A 160-kDa antigen attached to the membranes of the parasites was also found by several groups (7, 8, 14, 15). The authors related this antigen to the production of lytic antibodies against the parasite during the chronic phase of the disease. The lytic antibodies should completely clear the

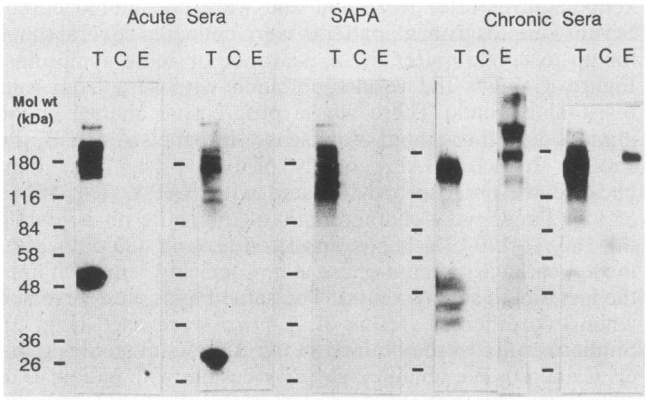


FIG. 2. Detection of trypomastigote and epimastigote secreted-excreted immunogens with human sera from acute- or chronic-phase infections. Immunoblots were prepared with 15 (lanes E) or 30 (lanes T and C) μg of protein from supernatants of epimastigotes (E), trypomastigotes (T), or uninfected control cultures (C). The blots were processed with sera from acute- or chronic-phase cases of Chagas' disease. A blot was incubated with rabbit serum raised against a SAPA. Autoradiograms after incubation with $^{125}$I-protein A are shown.

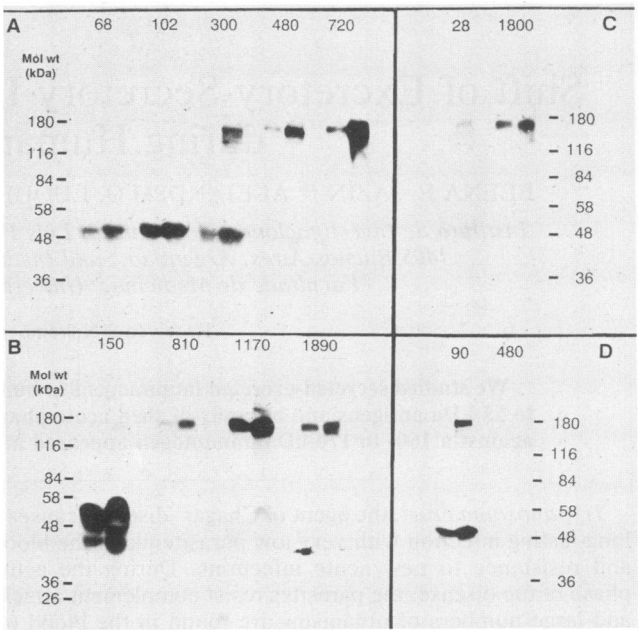


FIG. 3. Follow-up studies of secreted-excreted immunogens in human patients resistant to benznidazole treatment. Immunoblots were prepared with 15 μg of proteins from supernatants of two strains of trypomastigotes, CAI and RA (left and right lanes, respectively, for each indicated day). The immunoblots were incubated with sera extracted from four patients (each panel corresponds to one patient). Numbers at the tops of lanes indicate numbers of days after the appearance of acute symptoms of Chagas' disease.

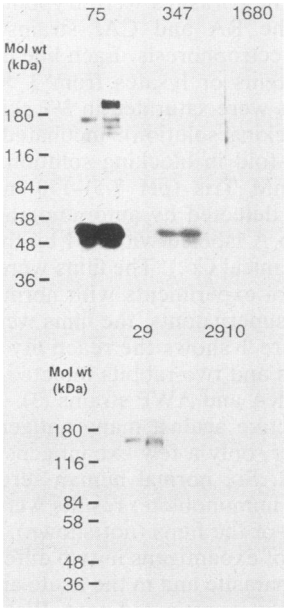


FIG. 4. Follow-up studies of excreted-secreted immunogens in human patients cured after benznidazole treatment. Immunoblots were prepared as described in the legend to Fig. 3 and processed with serum samples extracted from two different patients (top and bottom). Numbers at the tops of lanes indicate numbers of days after the appearance of acute symptoms of Chagas' disease.

parasites from the blood. However, few trypanosomes can be found in the blood of chronic-phase patients. The researchers did not investigate whether the 160-kDa antigen was shed. We speculate that the 160- to 170-kDa exoantigen might be the same antigen they found in the membranes. If such is the case, the shedding of antigens bound to lytic antibodies would consume complement factors and allow a few parasites to escape from the complement attack, as other parasites have been shown to do (6). Alternatively, the 160-kDa exoantigen might be similar to the one described by Schenkman et al. (12). They suggested that a 160-kDa protein might be involved in attachment to host cell receptors. The cloning and characterization of the 45- to 55- and the 160- to 170-kDa exoantigens could show what their role is in the establishment of a chronic infection and the resistance to reinfection.

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