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Reversible Acquisition of a Host Cell Surface Membrane Antigen by *Trypanosoma cruzi*

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The ability of *Trypanosoma cruzi*, the etiological agent of Chagas' disease, to acquire host cell surface antigen was tested. Parasites emerging after intracellular replication in WOS sarcoma monolayers expressed a sarcoma-associated surface antigen. This antigen was deleted from these parasites after replication in the MNS control monolayer, which does not express WOS sarcoma-associated surface antigen, or by replication in cell-free medium. This type of reversible acquisition of host surface antigen has not been previously described in *T. cruzi* or other intracellular protozoan parasites.

Serological cross-reactivity between host and parasite indicates the presence of shared antigenic determinants. Since successful parasitism may result from immunological unresponsiveness of the host, these shared determinants have been attributed to convergent evolution resulting in similar host and parasite gene products (11). Alternatively, host mimicry might result from integration of host molecules into the structural composition of the parasite. For example, there are data which indicate that *Schistosoma mansoni* adsorbs disguising substances from the host (10); however, other data show that similar substances may be synthesized by the schistosoma (A. J. G. Simpson, D. Singer, A. Sher, and T. M. McCutchan, Fed. Proc. **41**:729, 1982). Either process would confer a selective advantage to the parasite.

The mechanisms which permit evasion of host immune attack by *Trypanosoma cruzi*, the etiological agent of Chagas' disease, are not fully understood. Specific antigenic determinants present in striated muscle sarcoplasmic reticulum (7), certain neurons (14), and the basement membrane component laminin (12) have been identified in *T. cruzi* flagellates. It seems unlikely that these shared determinants permit evasion of the host immune response, since, with the possible exception of laminin, they are located in the interior of live *T. cruzi* organisms.

The experiments we report here test the hypothesis that *T. cruzi* acquires host surface membrane antigen during replication in mesodermal cells.

MATERIALS AND METHODS

***T. cruzi* strain.** The Colombia strain was obtained from S. Andrade, Universidade Federal da Bahia, Salvador, Brazil. The strain was isolated from a patient with Chagas' disease by xenodiagnosis, passed in NNN blood agar tubes (6), and grown in liver infusion-tryptose cell-free medium (2) after more than 100 passages. Trypomastigote forms were produced in African green monkey kidney cell cultures (Microbiological Associates, Inc., Walkersville, Md.).

Experimental cell lines. Two mesodermal cell lines were selected from more than 100 isolates being studied as part of an effort to identify tumor-related antigens. These two cell lines (WOS and MNS) are maintained in the laboratory of Y. Hirshaut, Memorial-Sloan Kettering Cancer Center, New York, N.Y. The test mesodermal cell line (WOS) was isolated by primary culture of a human osteogenic sarcoma and passaged more than 50 times. This cell line exhibits stable expression of a sarcoma-related surface antigen (WOS-SA). The control mesodermal spindle cell line (MNS) was isolated by trypsinization of normal skin and does not express WOS-SA. The tissue cultures were grown at 37°C in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10% fetal calf serum (FCS) (Flow Laboratories, Inc., McLean, Va.) and 100 U of penicillin and 100 μg of streptomycin (GIBCO) per ml. Confluent monolayers were passaged after trypsinization by standard techniques (9).

Specificity of anti-WOS-SA activity. Anti-WOS-SA activity was measured by immune adherence assay (IAA) and by indirect immunofluorescence. These assays measured the expression of WOS antigenicity of separate portions of cultured cells harvested from each passage. IAA was done according to the method of Tachibana and Klein (13), modified for monolayer target cells. Briefly, washed trypsinized cells were distributed to the wells of Falcon Microtest II plates (Becton, Dickinson & Co., Oxnard, Calif.) (1,500 to 2,000 cells per well) and incubated in culture medium overnight. A 0.1-ml amount of serial twofold dilutions of serum was added to each well, and the plates were incubated at 4°C for 30 min. Indicator type O human erythrocytes and guinea pig serum complement were

added to each well. The plates were then incubated at 37°C for 30 min. The plates were gently agitated and examined under a light microscope for rosettes.

Negative sera were considered to be those which gave less than 10% rosettes at 1:8 dilutions. About one-third of normal human sera have anti-WOS-SA activity by IAA (n = 30). Serum no. 190 was selected as the positive indicator from this panel. This serum produced 95% rosettes with WOS cells but less than 10% rosettes with control MNS cells. The negative indicator serum, no. 13196, produced less than 10% rosettes with either cell line.

When indirect immunofluorescence was used, portions of WOS cells were detached with a rubber policeman, incubated in twofold dilutions of negative or positive indicator serum (1 h at 4°C), and washed three times with culture medium. These cells were then suspended in an optimal dilution of fluorescein-labeled goat anti-human immunoglobulin M (IgM) (FAIgM) (Miles Laboratories, Inc., Elkhart, Ind.) for 1 h at 4°C, washed three times in culture medium without FCS, and mounted in glycerol (80% in phosphate-buffered saline [PBS], pH 7.4). A polyvalent label may also be used. We used FAIgM to specifically identify only those antibodies in the indicator sera which might also contribute to our IAA. (IAAs are known to be mediated by IgM-class antibodies.) All WOS cells showed IgM-specific immunofluorescence of the plasma membrane at dilutions of 1:40 with serum 190, whereas the negative indicator, serum 13196, did not react at any titer. MNS cells did not show fluorescence with either serum.

Selective harvesting of experimental trypanosomes. All test and control tissue culture flasks (75 cm^2) were inoculated in duplicate with 10^5 trypomastigotes. With inoculation, the tissue culture medium was changed from 10 to 1% FCS. This diminished parasite multiplication in the supernatant and favored infection of the monolayers. Inoculated cultures were incubated at 35°C for 48 h. At this time, intracellular amastigotes and free-swimming trypomastigotes could be seen by phase-contrast microscopy. The supernatant was then discarded, and the monolayer was washed twice with warm (37°C) Hanks balanced salt solution to remove any flagellates derived from the original inoculum which had not penetrated the monolayer. Fresh medium containing 1% FCS was added, and the cultures were incubated at 37°C for 48 to 72 h. Trypanosomes in the supernatant were harvested by centrifugation (500 × g for 10 min).

Localization of WOS antigen in trypanosomes. Harvested trypanosomes were vigorously washed twice in PBS. A 10-μl sample containing 2×10^4 parasites was transferred to sections of a previously scored glass slide. These cells were killed by acetone fixation and stored at −20°C.

Twofold dilutions in Hanks balanced salt solution of heat-inactivated (30 min at 56°C) indicator serum absorbed against human blood group antigens [AB Rh(+)] were incubated with the flagellates (30 min at 28°C). This treatment was followed by two 15-min washes in PBS and incubation with FAIgM (30 min at 28°C). After two more PBS washes, the slides were examined for specific fluorescence with a UV light source (Ortholux UV microscope; E. Leitz, Inc., Rockleigh, N.J.). All titers were read in a blind study.

In parallel experiments, WOS-SA was localized in living trypomastigotes. This was accomplished by incubation (1 h at 25°C) in positive indicator serum (1:20) before killing by acetone fixation.

Uptake of any WOS-SA which might be shed into the supernatant was tested by incubation (12 h at 37°C) of trypanosomes in the overlay from a growing sarcoma monolayer.

WOS-SA localization tests were controlled by incubation of trypanosomes with FAIgM but without indicator sera and by absorption of specific fluorescence with WOS cells. In addition, serum from a patient with chronic Chagas' disease and strongly positive *T. cruzi* serology was used to detect *T. cruzi* antigens. Serum from a normal subject from a nonendemic area without anti-WOS-SA was also used for control of each indirect immunofluorescence test.

Deletion of WOS-SA. To test the permanence of any WOS-SA activity which might be detected on trypanosomes harvested from the WOS monolayers, portions of these parasites were used to inoculate the WOS-SA-negative MNS monolayers. A similar inoculum was used for conversion of trypomastigotes to epimastigotes in cell-free liver infusion tryptose medium (25 ml). After measured passages and replications (doublings) in these substrates, trypanosomes were harvested and tested for WOS-SA.

TABLE 1. Acquisition of WOS-SA by *T. cruzi*

Cell line[a]	WOS-SA reactivity of cell line	Serum	WOS-SA reactivity of serum	Anti-WOS-SA titer (IgM)
WOS	+	13196	−	0
WOS	+	190	+	1:40
MNS	−	13196	−	0
MNS	−	190	+	0
WOS	+	Absorbed 190[b]	ND[c]	1:10

[a] The *T. cruzi* harvests from WOS and MNS yielded FAIgM titers of 1:160 with control anti-*T. cruzi* serum.
[b] Serum absorbed by incubation (24 h at 4°C) with WOS monolayer.
[c] ND, Not done.

RESULTS

Acquisition of WOS-SA by *T. cruzi*. Amastigotes and trypomastigotes produced in WOS sarcoma monolayers acquired WOS-SA, detected at a titer of 1:40 (Table 1). Acquired WOS-SA was localized in the cell membrane of acetone-fixed and live organisms (Fig. 1). Reactivity of sera to trypomastigotes produced in WOS sarcoma monolayers could be quenched by absorption of sera with WOS sarcoma cells. No appreciable loss of trypanosomal antigens was detected in the WOS-SA-positive flagellates when these organisms were tested with the control anti-*T. cruzi* serum. Trypomastigotes produced in the control MNS monolayers remained WOS-SA negative. Incubation of WOS-SA-negative epimastigotes and trypomastigotes in the overlay from a growing WOS monolayer

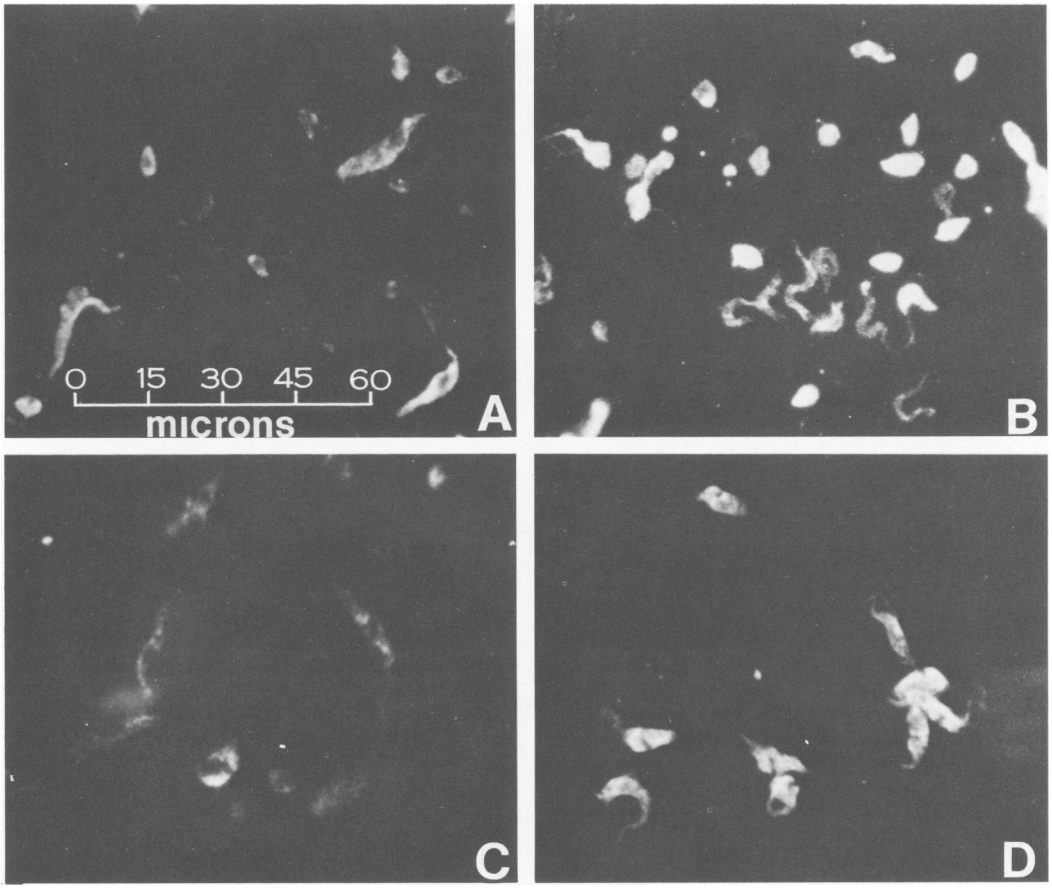

FIG. 1. Acquired WOS-SA was localized in the cell membrane of acetone-fixed (A) and live (B) organisms (serum 190; 1:40). A single passage of WOS-SA-positive trypomastigotes through a WOS-SA-negative monolayer caused marked reduction of anti-WOS-SA titer (C) (serum 190; 1:10). Control serum obtained from a patient with Chagas' disease showed no loss of reactivity to native trypanosomal antigens in these flagellates (D) (1:160).

did not result in detectable WOS-SA acquisition.

Deletion of WOS-SA from *T. cruzi*. A single passage of WOS-SA-positive trypomastigotes through control MNS monolayers (nine doublings of flagellates) caused marked reduction of anti-WOS-SA titer. All specific fluorescence was gone from trypomastigotes, but it was detectable at a titer of 1:10 on amastigotes derived from prematurely ruptured pseudocysts (Table 2). Epimastigotes recovered from cell-free medium after seven doublings showed complete loss of WOS-SA titer (Table 2).

DISCUSSION

The data reported here demonstrate reversible acquisition of WOS-SA by replicating *T. cruzi*. WOS-SA was found on the outer membrane of trypomastigotes which emerged from parasitized sarcoma cells in a density similar to that of the sarcoma itself. No similar antigen was present on organisms produced in the control monolayers. The antigen was demonstrated on live *T. cruzi* and was not detached by vigorous washing with Hanks balanced saline. However, the acquired WOS-SA could be deleted from the progeny of these organisms by replication in a cell line that did not express WOS-SA or by replication in cell-free medium.

Molecular exchanges between other species of trypanosomes and their mammalian hosts have been observed (5). These exchanges may involve substances of low molecular weight, such as vitamins. There may also be nonspecific adsorption of host serum proteins (1, 4) or specific attachment of host antibody (3) during parasitemia. These events differ from WOS-SA acquisition, which occurs during the intracellular (amastigote) stage of parasitism.

The mechanism of WOS-SA acquisition may be a nonspecific physical attachment between

TABLE 2. Deletion of WOS-SA from *T. cruzi*

WOS-SA-positive *T. cruzi* inoculum passed in:	Replication (no. of doublings)	Serum	WOS-SA reactivity in serum	Anti-WOS-SA titer (IgM)
WOS-SA-negative	9	13196	−	0
MNS cell line		190	+	1:10[a]
Cell-free medium	7	13196	−	0
		190	+	0

[a] The remaining fluorescence was only on amastigotes from disrupted pseudocysts.

replicating amastigotes and host membrane components. There may also be direct incorporation of host components into the structure of newly formed surface membrane of replicating amastigotes. Trypanosomal receptors for mesodermal surface membrane targets may have a role in this process. Receptors of forming amastigotes may bind to nascent host targets in the cytoplasm and in so doing may also recruit adjacent host antigens by nonspecific carryover. Finally, one may postulate some form of inductive synthesis of WOS-SA by *T. cruzi*; however, there are no studies to suggest that protozoan parasites are capable of DNA-directed synthesis of host antigens. Whichever mechanism actually occurs, deletion of WOS-SA is probably a process of antigen substitution which occurs when the parasite replicates in a WOS-SA-free environment. We are currently performing experiments with other antigen systems from different cell lines to establish whether antigens other than WOS-SA can be acquired.

Reversible acquisition of host cell surface membrane antigen, if it occurs in nature, may provide *T. cruzi* with a mechanism to evade immune attack. The presence of host surface antigens on emerging trypomastigotes would confer an obvious selective advantage during the extracellular stage of their life cycle. Bloodborne *T. cruzi* trypomastigotes bearing host surface antigen on their outer membranes might be better capable of evading immune attack by masquerading as self.

Several previous studies have shown that there are antigenic determinants common to mammalian cells and *T. cruzi*. These determinants include specialized components of striated muscle (7), neurons (14), and connective tissue (12). Unlike WOS-SA, these antigens (with the possible exception of laminin) are expressed in the interior of the parasite, and they are not deleted by multiple passages. It has been suggested that such "shared" host antigens play a role in the immunopathogenesis of the lesions seen in patients with *T. cruzi* infections (8). It is also possible that "acquired" host antigens induce autoreactive phenomena due to their juxtaposition to parasite antigens as presented on the surface of circulating *T. cruzi*. In this context, the possibility that the acquisition of tumor antigens by *T. cruzi* might effect immune surveillance is not excluded.

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