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Interaction of High-Density Lipoprotein With Trypanosoma brucei: Effect of Membrane Stabilizers

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The specific lysis of bloodstream trypanosomes by serum from a nonpermissive mammalian host is the result of interaction between the serum trypanocidal factor (high-density lipoprotein) and the trypanosome surface. The studies described in this paper attempt to define further the mode of action of this cytotoxic lipoprotein. The binding of high-density lipoprotein to Trypanosoma brucei was instantaneous at 4°C and readily reversible. Binding was not mediated by the surface glycoprotein as removal of the surface coat enhanced binding at 4°C, and no stable glycoprotein-lipoprotein complex could be detected. Pretreatment of trypanosomes with the cross linker dimethylsuberimidate rendered cells resistant to lysis. Addition of membrane-stabilizing drugs, such as cytochalasins C, D, and E, and local anesthetics (dibucaine, tetracaine, and procaine), also inhibited high-density lipoprotein-induced cell lysis. The data presented support the idea that at 37°C lateral diffusion of the variant surface glycoprotein, an integral membrane protein, allows maximal high-density lipoprotein-cell interaction in serum-sensitive cells, and that altered properties of the plasma membrane induced by low temperature or the addition of cytochalasins, local anesthetics, or zinc inhibit this interaction, possibly by increasing the shielding of the plasma membrane by more rigidly anchored surface glycoprotein molecules.

Key words: local anesthetics, cytochalasins, surface glycoprotein, high-density lipoprotein, Trypanosoma brucei

Host range among trypanosomes of the brucei subgroup of African trypanosomes appears to be dependent in part on cytotoxic properties of normal (nonimmune) serum of the mammalian host. The lytic effect of serum is not dependent on complement and exhibits specificity with regard to both host and parasite. Thus, serum from permissive hosts (eg, rats, rabbits) is not cytotoxic for Trypanosoma brucei whereas serum from nonpermissive hosts (eg, humans, baboons) lyses these trypanosomes. Moreover, T brucei, a trypanosome noninfective to humans, is neutralized by normal human serum whereas T rhodesiense, a human pathogen, is not [1–3].

It has been reported that the sensitivity of trypanosomes to serum lysis may be dependent on the particular variant surface glycoprotein (VSG) present on the surface of the parasite [4]. Since trypanosomes are coated with about 10^7 molecules of this

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single glycoprotein [5], it was of interest to determine 1) whether VSG might be a receptor for the trypanocidal factor, and 2) whether the surface coat might act as a barrier that could modulate the ability of the trypanocidal factor to exert its effect on the cell.

The experiments described in this paper aim to elucidate the role of the trypanosome surface coat in the interaction of purified high-density lipoprotein (HDL), the trypanocidal factor [6], with T brucei. The data presented provide evidence that soluble VSG from serum-sensitive trypanosomes does not interact to a significant extent with cytotoxic HDL. However, treatments which cross-link or immobilize VSG on the surface of the trypanosome render the cell resistant to lysis, presumably by altering the properties of the surface coat glycoprotein.

METHODS

Parasites and Sera

Trypanosoma brucei, derived from a clone of strain EATRO 110 [7], was used in all studies. Male NCS mice (Rockefeller University) weighing 20–25 g were each infected with a capillary stabilate containing approximately 10^6 trypanosomes. Three days after infection, trypanosomes were harvested and purified as described previously [7].

Baboon blood, drawn with EDTA, was obtained from the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP, New York University School of Medicine) and processed on the day of bleeding. Plasma was prepared by centrifugation and $CaCl_2$ (10 mM final concentration) was added to induce clot formation. Aliquots of serum were frozen and stored at −70°C until needed. Rat, rabbit, and mouse (NCS) sera were prepared as described [6].

HDL Preparation

HDL was purified essentially as described by Rudel et al [8]. Briefly, serum was adjusted to a density of 1.225 by adding solid KBr. The equivalent of 10 ml initial serum volume was placed in a SW40 ultracentrifuge tube and overlayered with a NaCl-EDTA-KBr solution of density 1.225. Tubes were centrifuged in a Beckman SW40 rotor for 40 hr at 15°C and 40,000 rpm. After centrifugation the top 1.5 ml was removed using a tube slicer and applied to a Bio-Gel A-5m (Bio-Rad Laboratories) column, 1.7 × 90 cm. The column was eluted with 0.15 M NaCl–0.01% EDTA, pH 7.0. Fractions corresponding to HDL were pooled, avoiding any possible contamination from adjacent low-density lipoprotein (LDL) peaks, and concentrated using Centriflo CF50A filter cones (Amicon Corp). Concentrated HDL solutions were stored at 4°C.

^{125}I-labeled baboon HDL was prepared by the method of Langer et al [9]. The iodinated HDL had a final specific activity of 62-135 cpm/ng protein. In these preparations, less than 1% of the radioactivity was TCA-soluble and less than 3% of the radioactivity was extractable into chloroform:methanol (2:1).

HDL Binding Experiments

^{125}I-HDL was dialized against phosphate-buffered saline containing 0.01 M glucose, 0.01% EDTA, pH 7.4, immediately prior to use in a given experiment. ^{125}I-HDL binding was assayed by incubating trypanosomes at a cell concentration of 3–5 × 10^7/ml under conditions described in the text. At desired time points duplicate

aliquots of the incubation mixture were centrifuged for 2 min in 400 μl Microfuge tubes containing 100 μl 10% TCA overlayered with 50 μl silicon oil (Versilube GE F-50, R.H. Carlson Co). The tubes were sliced just above the trypanosome pellet and the pellets were counted in a Packard gamma counter.

VSG Preparation

VSG was prepared by the method of Cross [10]. The trailing edge of the unretarded peak off the DEAE column was avoided in pooling VSG fractions. Pooled VSG was dialyzed against several changes of water, lyophilized, and resuspended in Eagle's Minimum Essential Medium (MEM, GIBCO). SDS-PAGE indicated the presence of only one band, having a molecular weight of approximately 60,000 after Coomassie Blue staining or fluorography of the gel.

^{3}H-leucine metabolically labeled VSG was similarly prepared after preincubating trypanosomes in ^{3}H-leucine (L-(4,5-^{3}H)-leucine, 60 Ci/mmole, New England Nuclear). Incubation was carried out at 37° for 30–45 min in MEM containing 10% mouse serum, 35 μC ^{3}H-leucine/ml, and 3–5 $\times$ 10^7 trypanosomes/ml. Radioactive cells were centrifuged and resuspended in MEM. An excess of unlabeled trypanosomes was added and the combined cells centrifuged and resuspended two more times before VSG isolation.

Surface Coat Cross Linking

Trypanosomes were suspended in phosphate-buffered saline-glucose solution (PSG: 28 mM Na_2HPO_4, 1.5 mM $NaH_2PO_4{:}H_2O$, 23 mM NaCl, 3% glucose, pH 8.0). Dimethylsuberimidate (DMS) in PSG was made freshly before use, using a procedure similar to that described by Strickler and Patton [11]. Cross linking was performed at room temperature for 45 min at a cell concentration of 2 $\times$ 10^7 and 100–200 μg DMS/ml. The reaction was terminated by rapidly chilling the cells and centrifuging at 4°C. The addition of NH_4 acetate to stop the reaction was avoided as this was found to lead to nonspecific cell lysis in subsequent 37°C incubations. The cell pellet was washed twice with cold MEM before final resuspension in cold MEM at 4 $\times$ 10^7 cells/ml. After this treatment trypanosome morphology appeared normal by phase-contrast microscopy. However, motility of cells treated with the higher DMS concentration was impaired; only the tips of the flagella appeared to twitch. Infectivity of cross-linked trypanosomes was estimated to be about 5% (100 μg DMS/ml treated cells) and 1-5% (200 μg DMS treated cells) of control trypanosomes incubated and washed in parallel.

Assays

Protein concentrations were determined using the Bio-Rad assay and bovine plasma albumin as a standard.

Cell lysis assays were performed as described [7]. Baboon serum (or HDL) was used as the cytotoxic serum (or HDL); control tubes contained noncytotoxic rat or rabbit serum (or HDL). Cell lysis was monitored either by phase-contrast microscopy of fixed cells [3] or by release of ^{3}H-leucine-labeled macromolecules from lysed cells [7].

Infectivity of trypanosomes was estimated by monitoring the length of the prepatent period in mice as described by Hawking [12]. Briefly, 10^6, 10^4, and 10^2 control trypanosomes and 10^6 experimental trypanosomes are injected intraperitoneally and tail blood monitored daily. This method, although rather insensitive, can readily measure the number of infective trypanosomes to within one log unit.

Materials

Cytochalasins A, B, C, D, and E, aldolase (type X), ferritin (type I), bovine liver catalase, procaine-HCl, tetracaine-HCl, dibucaine-HCl, and dimethylsuberimidate were obtained from Sigma. Crystallized bovine albumin was obtained from Miles.

RESULTS

HDL Binding to Trypanosomes

In an attempt to demonstrate the existence of HDL receptors on the trypanosome surface, ^{125}I-HDL binding experiments were performed at 4°C and 37°C. ^{125}I-HDL was indistinguishable from control HDL when tested for trypanocidal activity. All 37°C binding experiments were done under conditions that did not induce any morphological changes—ie, low HDL concentrations (ca 100 μg/ml) and/or short times (45 min)—but that did reduce infectivity of trypanosomes for mice at least fourfold.

At 4°C there was an immediate, specific association of ^{125}I-HDL with trypanosomes. Aliquots were removed immediately after addition of trypanosomes to the incubation tubes and centrifuged. Quantitation of the radioactivity associated with trypanosome pellets from these earliest time points revealed the presence of 10^3–2.2×10^4 molecules HDL/trypanosome when the HDL concentration varied from 120 to 740 μg/ml. The presence of a threefold excess of cold HDL in an incubation mix containing 500 μg ^{125}I-HDL reduced the amount of ^{125}I-HDL bound to trypanosomes at 0 time to 23% of control values. Cold rat HDL was almost as effective as cold baboon HDL in decreasing this binding, whereas whole serum reduced this binding only slightly and albumin had no effect. This 4°C binding was not saturable even when ^{125}I-HDL concentrations as high as 1.22 mg/ml were used.

Continued incubation of trypanosomes at 4°C for periods up to 2 hr in the presence of ^{125}I-HDL resulted in a continued increased in binding of HDL which did not appear to reach saturation. The net increase was variable but ranged from 6.5% to 60% at 40 min. Trypsinization of cells, to remove the surface coat, resulted in markedly higher 0 time HDL binding (1,426 cpm for control vs 2,610 cpm for trypsinized cells) but very little increase upon continued incubation, so that at 40 min there was little difference between trypinsized and control cells. Similarly, binding to cultured procyclics, which do not have a surface coat, was three times that of bloodstream forms (10,991 cpm/10^7 procyclis vs 3,124 cpm ^{7} bloodstream forms at 485 μg ^{125}I-HDL/ml). Thus, the presence of a surface coat partially prevents the binding of HDL to trypanosomes.

To study the reversibility of HDL binding to trypanosomes, cells were incubated at 4°C in ^{125}I-HDL for 40 min, washed, and then resuspended in cold (4°C) fresh medium containing either nonradioactive baboon HDL, baboon LDL, rat HDL, albumin, or medium alone and incubated at 4°C or 37°C. The results, shown in Figure 1, indicate that at 4°C in the presence of homologous or heterologous HDL or unrelated proteins a 40–52% decrease was observed within 10 min. The radioactivity continued to decrease over the course of the experiment. In the absence of added protein this decrease was much slower. At 37°C, bound HDL decreased by 90% in 10 min in medium containing cold HDL; again, in the absence of added protein, the

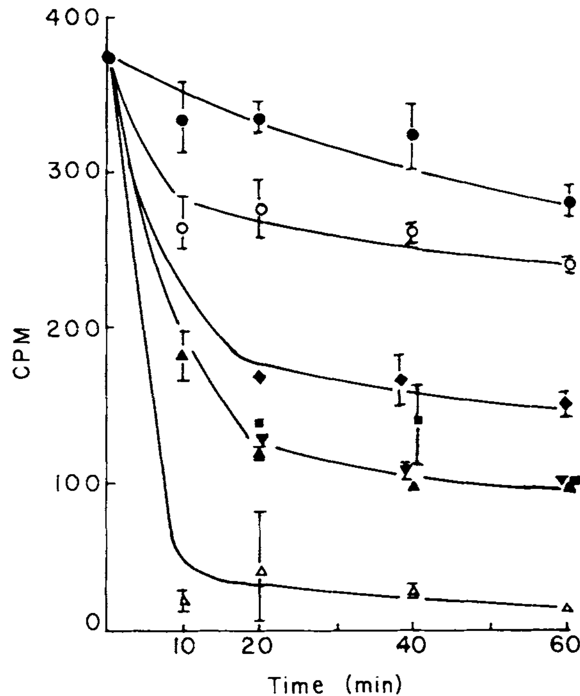

Fig. 1. Dissociation of ^{125}I-HDL bound to trypanosomes at 4°C upon reincubation in fresh medium under different conditions. Trypanosomes were incubated at 4°C in MEM at a concentration of 9.2 × 10^7 cells/ml and 510 μg ^{125}I-baboon HDL/ml. After 40 min, the cells were centrifuged, washed twice with a large volume of MEM, and resuspended in MEM. Subsequent incubation was at a cell concentration of 4.5 × 10^7/ml at 4°C in MEM containing nonradioactive baboon HDL (569 μg/ml) (▲), baboon LDL (360 μg protein/ml) (▼), rat HDL (550 μg/ml) (■), albumin (500 μg/ml) (♦), or medium alone (●), or at 37°C in MEM containing nonradioactive baboon HDL (569 μg/ml) (△) or in MEM alone (○). Aliquots were taken at the indicated time points and processed as described in Methods.

rate of loss of "bound" HDL was significantly slower. Thus the association of HDL with trypanosomes is readily reversible at both 4°C and 37°C.

The experiments described above suggest that at 4°C an immediate binding of HDL to trypanosomes occurs. This binding is nonsaturable, readily reversible, and specific. There is thus no evidence for a classic high-affinity, saturable receptor for HDL on trypanosomes.

When binding experiments were performed at 37°C rather than at 4°C, a somewhat higher amount of radioactivity (+10% during 40 min incubation) became associated with the trypanosomes. It is possible that this increase is due to uptake of labeled HDL by pinocytosis. In fact, using the published value of 0.5 nl/hr/10^6 trypanosomes for the pinocytic rate of trypanosomes [13], 80% of the increased binding at 37°C could be attributed to pinocytosis. Thus, the amount of ^{125}I-HDL associated with trypanosomes at 37°C is not significantly different from that measured at 4°C. Taken together with the reversibility of HDL-binding (Fig. 1), these data suggest that at 37°C only a transient interaction of HDL with the trypanosome surface occurs.

HDL Binding to VSG

To investigate further whether interaction of VSG and HDL at 37°C could modulate HDL trypanocidal properties, the effect of added purified VSG on HDL-induced trypanolysis was assessed. In addition, the possible formation of an HDL-VSG complex at 37°C was investigated.

Addition of purified VSG to a standard incubation mixture containing trypanosomes (2.5×10^7/ml) and baboon serum (25%) had no effect on either the rate of lysis or the final extent of lysis (data not shown). Control tubes containing either medium alone, or medium + VSG, showed negligible lysis (<5%) after 120 min at 37°C. The amount of VSG added (0.23 mg/ml) was about ten times the total amount of VSG present on the living trypanosomes in the incubation. These results suggest that the presence of excess VSG does not in any detectable manner alter the cytotoxic properties of baboon serum.

The possibility that complex formation between HDL and VSG had nevertheless occurred but that the VSG-HDL complexes were as trypanocidal as HDL could not be ruled out by the experiments described above. However, the results shown in Figure 2 make this possibility unlikely. HDL and VSG were incubated separately or together at 37°C for 1 hr at roughly equimolar concentrations and then fractionated on a Sephacryl S-300 column. The HDL peak (tube 54, MW 300,000) was separated from the VSG peak by two fractions. Interestingly, the bulk of the VSG appeared to elute as a tetramer (tube 56, peak 1, MW 240,000), with trailing peaks 2 (tube 62) and 3 (tube 68) which most likely correspond to VSG dimers (MW 118,000) and monomers (MW 58,000), respectively. However, there was no shift in the elution position of either the protein (mostly HDL) or radioactivity when HDL and VSG were coincubated and cochromatographed, indicating that a significant degree of stable complex formation between HDL and soluble VSG had not occurred.

Effect of Surface Coat Cross Linking on Trypanosome Lysis

The experiments described above are consistent with the hypothesis that there is a transient interaction of HDL with trypanosomes which is probably not mediated by VSG and that the surface coat, by its mere presence, may in fact hinder this interaction to some extent. The idea that the trypanosome surface coat could be artificially made into an impenetrable barrier was tested by asaying the sensitivity of trypanosomes to lysis by HDL after surface coat cross linking (Table I). Cells treated with the higher DMS concentration were less viable (or infective) (see Methods) and showed more nonspecific lysis at 37°C. Thus, at 120 min when untreated cells incubated in noncytotoxic rabbit HDL were still 99% unlysed, 35% lysis had occurred in 200 μg/ml DMS-treated cells. At this time, control cells in baboon HDL exhibited 85% lysis. This nonspecific lysis was less pronounced with 100 μg/ml DMS-treated cells which showed 7% and 16% lysis in rabbit and baboon HDL, respectively. The results in Table I show that cross linking of the surface coat clearly renders the trypanosomes resistant to HDL-mediated lysis.

The nonspecific lysis seen with some DMS-treated cells suggested that DMS treatment had not "fixed" the cells and thus rendered them incapable of rounding up and lysing. This conclusion was confirmed by observing the rate of swelling and lysis in hypotonic media. Cells were resuspended in either low-ionic-strength triethanolamine buffer (21.2 mM triethanolamine, 26 mM glycine, pH 7.2) or a 1 PSG:9 10 mM Tris, pH 7.4, solution at room temperature and monitored by phase-contrast

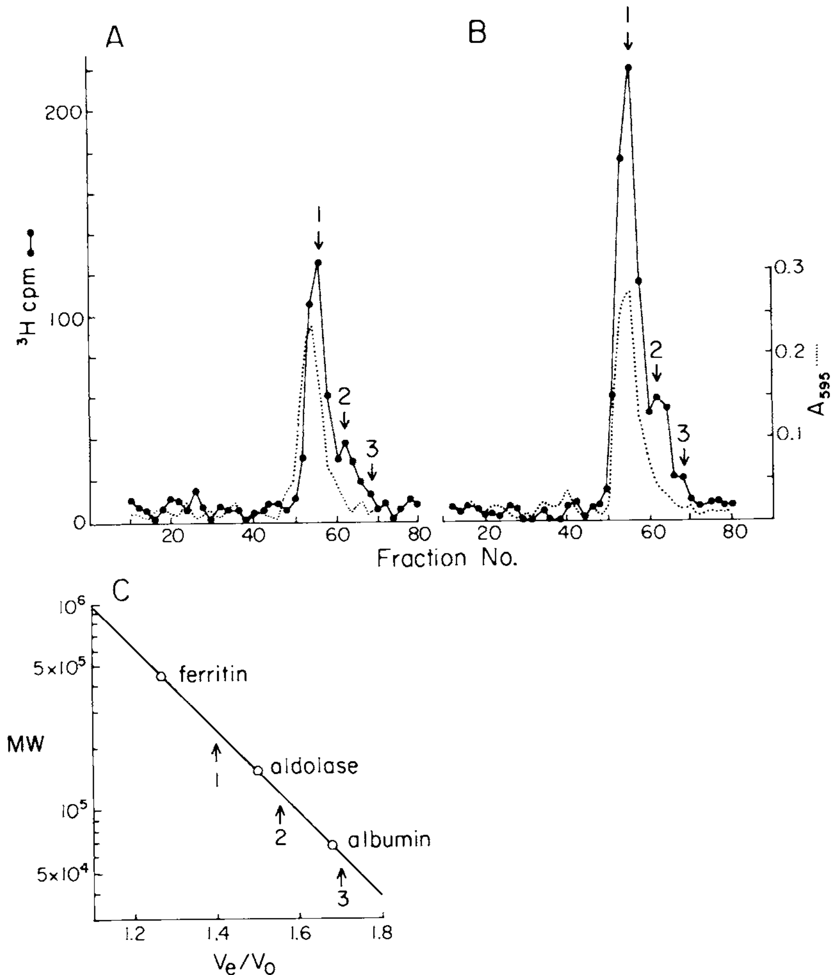

Fig. 2. Sephacryl S-300 elution profiles of HDL and ^{3}H-leucine-labeled VSG incubated and fractionated separately (panel A: elution profiles of 2 separate columns superimposed) or incubated and chromatographed together (panel B). One milliliter MEM containing 0.95 mg baboon HDL (3.1 μM) or 0.31 mg ^{3}H-leucine-labeled VSG (5.2 μM) (see Methods) alone or together was incubated at 37°C for 1 hr and then fractionated on a 1.5 × 52.5 cm Sephacryl S-300 column. The column was eluted with 0.15 M NaCl–0.01% EDTA, pH 7, at a flow rate of 5 ml/hr. Ten-minute fractions were collcted; 0.1 ml of each fraction was taken for protein determination (OD_{595}), and 0.5 ml was mixed with 10 ml Ultrafluor (National Diagnostics) for radioactivity determination by scintillation counting (cpm). The column was calibrated (panel C) with Blue Dextran (V_0), ferritin (MW 440,000), aldolase (MW 158,000), and albumin (MW 68,000).

microscopy for 30 min. No difference was seen between control and 100 μg/ml DMS-treated cells.

Inhibition of Lysis By Various Drugs

Cytochalasins. Initial experiments indicated that adddition of cytochalasins C and E could inhibit the lysis of trypanosomes by baboon serum (Table II). Cytochalasin B at 25 μg/ml was not effective. Cytochalasin A, at concentrations as low as 1

TABLE I. Effect of Surface Coat Cross Linking on Lysis

Treatment	Percent lysis Baboon HDL	Rabbit HDL
None	72	1
DMS 100 μg/ml	5	3
DMS 200 μg/ml	12	8

Trypanosomes were treated with DMS as described in Methods and then incubated at 37°C for 90 min in MEM with baboon or rabbit HDL. Final concentrations were 2×10^7 trypanosomes/ml and 0.72 mg baboon HDL/ml or 1.39 mg rabbit HDL/ml. Cells were fixed and assayed for lysis by phase-contrast microscopy.

TABLE II. Inhibition of Lysis by Cytochalasins

Cytochalasin	Concentration	Percent lysis Baboon serum	Rabbit serum
B	25 μg/ml	68	10
C	100 μg/ml	29	13
E	100 μg/ml	34	10
—(DMSO)	1%	68	5

Stock solutions of cytochalasins were made up in DMSO so that the final DMSO concentration in the incubation medium would not exceed 1%. Trypanocidal assays were performed at 37°C for 120 min in MEM containing 25% serum and 2×10^7 trypanosomes/ml. Lysis was assessed by phase-contrast microscopy.

μg/ml, was toxic to the cells (data not shown). Preincubations of cells in 100 μg/ml cytochalasin C or E for 1 hr at 37°C followed by subsequent incubation at 37°C for 90 min also resulted in partial protection of lysis in unfractionated baboon serum. Equivalent protection by cytochalasin C and E pretreatment was observed (data not shown). These results suggest that cytochalasin inhibits lysis by interacting with the cell rather than with the trypanocidal factor.

Cytochalasin preincubation could also effectively protect trypanosomes from lysis by purified baboon HDL (Table III). Concentrations of cytochalasins lower than those used in the experiments desribed in Table III were less effective. For example, preincubation of cells in 10 μg/ml cytochalasin E resulted in only a 50% inhibition of lysis in baboon HDL. The results in Table III indicate that the effect of cytochalasin E was essentially irreversible under the experimental conditions, whereas the protective effects of cytochalasins D and C were more reversible. Lysis of cytochalasin-pretreated cells in hypotonic media (see similar experiments for surface coat cross-linked cells above) was equivalent to that of control, untreated cells.

Metabolic activity and infectivity of trypansosomes pretreated with cytochalasin E at 25 μg/ml for 1 hr at 37°C was asessed. In separate experiments ^{3}H-leucine and ^{3}H-uracil incorporation into TCA-precipitable material during 60 min at 37°C in MEM was 84–124% of control values (incorporation by untreated trypanosomes), indicating that the biosynthetic capability of the cells had not been impaired by

TABLE III. Effect of Cytochalasin Pretreatment on Lysis

Pretreatment		Percent lysis	
		Baboon HDL	Rabbit HDL
Cytochalasin C	100 μg/ml	63	3
Cytochalasin D	25 μg/ml	44	6
Cytochalasin E	25 μg/ml	5	5
DMSO	1%	86	2

Cells were pretreated with cytochalasins or DMSO at the indicated concentrations in MEM at 37°C for 1 hr, washed twice with MEM, and incubated in MEM + HDL (1 mg/ml) at 37°C for another 3 hr. Lysis was assessed by phase-contrast microscopy.

cytochalasin pretreatment. Infectivity of cytochalasin-treated trypanosomes, however, was about 10% that of control trypanosomes.

Local anesthetics. Local anesthetics, like cytochalasins, affect the mobility of surface macromolecules [14,15] and were therefore tested for their effect on trypanolysis. Addition of dibucaine, tetracaine, and procaine, at the concentrations indicated, all inhibited, to varying degrees, lysis of trypanosomes by baboon serum (Table IV). Addition of fourfold to tenfold lower concentrations of the drugs had no effect whereas addition of higher concentrations led to rapid nonspecific lysis. Even at the concentrations that partially inhibited baboon serum-induced lysis there was significant release of radioactivity in normally noncytotoxic rabbit serum (Table IV), representing some nonspecific lysis. The data obtained by the radioactive assay were confirmed by light-microscopic observation of the trypanosomes at the end of the incubation.

Trypanosomes pretreated with 0.1 mM procaine in MEM for 1 hr at 37°C and subsequently washed and incubated in baboon or rabbit serum showed some protective effect of procaine pretreatment. Control cells exhibited 94% lysis in baboon serum compared to 54% lysis for procaine-pretreated cells. Incubation in rabbit serum resulted in 3% and 2% lysis for control and preincubated cells, respectively. These results suggest that in the case of procaine, as with the cytochalasins, inhibition of lysis is probably due to an effect of the drug on some property of the cell surface which affects its response to the trypanocidal effect of HDL.

Trypanosomes preincubated at 37°C for 1 hr in either 0.1 mM tetracaine or 1 mM procaine and then resuspended in hypotonic media at room temperature were found to be indistinguishable from control, non-drug-treated cells, suggesting that drug treatment did not prevent the cells from swelling and lysing under hypotonic conditions, but had a specific effect on HDL-induced lysis.

Zinc. Zn^{++} has been reported to have a membrane-stabilizing effect [16,17] and to inhibit the release of VSG from trypanosomes [18]. Addition of Zn acetate had a marked effect on the extent of trypanosome lysis induced by baboon HDL (Fig. 3). At the higher Zn^{++} concentrations some nonspecific lysis was observed, presumably due to toxic effects of Zn^{++} on the cell.

The above experiments on inhibition of lysis by a variety of compounds which all have in common a membrane-stabilizing effect, taken together with the finding that the presence of the surface coat decreases HDL binding at low temperature,

TABLE IV. Inhibition of Lysis by Local Anesthetics

Addition		cpm released	
		Baboon serum	Rabbit serum
—		1,742	32
Dibucaine	0.025 mM	543	268
Tetracaine	0.1 mM	546	117
Procaine	1 mM	956	684

Local anesthetics were dissolved in DMSO and kept as 0.5 M stock solutions at −20°C. Trypanosomes were preincubated in 40 μc/ml ^{3}H-leucine in MEM at 37°C for 30 min at a cell concentration of 2.5 × 10^7/ml. Cells were washed twice and resuspended in MEM. Subsequent incubation in MEM was at 37°C for 80 min at 2.5 × 10^7 cells/ml with 25% serum and drugs added at the indicated final concentrations. Lysis was assayed by measuring release of TCA-precipitable radioactivity [7]. 0 time cpm values have been subtracted. Control cells in baboon serum exhibited over 90% lysis as estimated by phase-contrast microscopy.

suggest that VSG when restricted in its mobility in the plane of the plasma membrane might act as a barrier preventing the interaction of HDL with the trypanosome by steric hindrance. However, the possibility that inhibition of HDL-induced lysis is due to some other effect of these drugs on the plasma membrane cannot be ruled out.

DISCUSSION

Although the uptake of LDL by cultured fibroblasts has been extensively studied and shown to be due to a high-affinity, specific, saturable, temperature-independent plasma membrane receptor [19,20], the nature of the interaction of HDL with different cell types is variable. Some investigators have reported that in vitro uptake of HDL by fibroblasts [21] and endothelial cells [22] can be acounted for by fluid pinocytosis, while others have described a saturable, low-afffinity binding to cells or plasma membrane fractions from rat liver [23–25] or rat ovary [26]. However, HDL binding was reversible [24], pronase-insensitive [25], and temperature-dependent [26], and could be inhibited by LDL [22]. This HDL binding, as well as the binding of HDL to trypanosomes described in this paper, displays more similarities to LDL binding to erythrocytes, which is probably due to nonionic adsorption [27], than to the LDL-fibroblast receptor model.

Our inability to define a high-affinity receptor on the trypanosome surface agrees with other biological observations of the interaction of cytotoxic serum (or HDL) with trypanosomes. Thus, trypanosomes incubated in cytotoxic serum (or HDL) at 4°C for up to 2 hr show no loss of infectivity to mice (Rifkin, submitted for publication), suggesting that HDL, if bound to trypanosomes at 4°C, is lost sufficiently rapidly at 37°C that no permanent cell damage occurs. This is consistent with our finding that rapid loss of bound HDL occurs at 37°C (Fig. 1). In addition, we have been unable to adsorb out the trypanocidal activity in serum at 4°C or 37°C using large numbers of either living or fixed trypanosomes (Rifkin, unpublished observations).

The mechanism by which HDL cholesterol and phospholipids readily exchange with the corresponding lipids of cellular plasma membranes remains a controversial question. According to the "collision theory" [28], surface components are thought

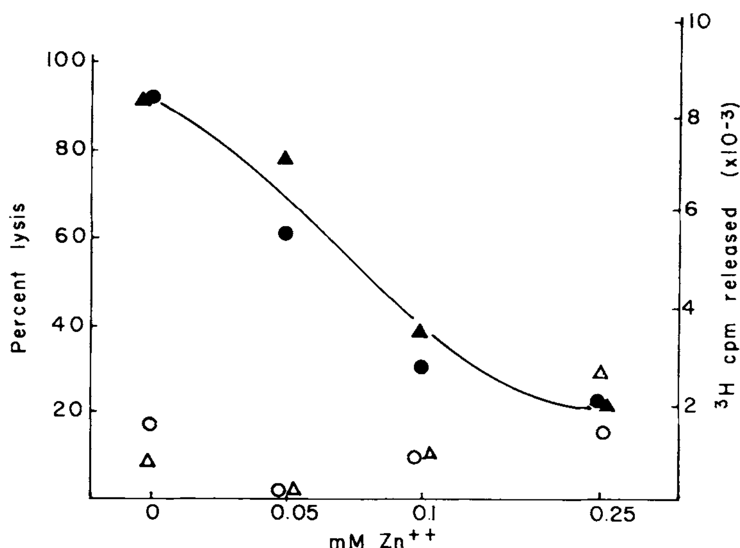

Fig. 3. Inhibition of trypanosome lysis by Zn^{++}. Trypanosomes were preincubated in ^{3}H-leucine, washed, and then incubated at 37°C in MEM at 2.5 × 10^7 cells/ml in the presence of 1.17 mg baboon HDL/ml (●, ▲) or 1.22 mg rabbit HDL/ml (○, △) and Zn acetate at the indicated final concentrations. Cell lysis was monitored by both phase-contrast microscopy (▲, △) and TCA-precipitable radioactivity released (●, ○).

to exchange during transient collision of lipoproteins with cell membranes. Alternatively, in the "phase partition theory" [29], exchange may occur by diffusion of these molecules from donor to acceptor particles (or membranes) through a water space of small but finite distance (a few hundred Å). The rate of exchange would be determined by the rate of desorption out of the donor membrane into the aqueous phase and the distance of diffusion to the aceptor molecule. Specific interactions between the cell and lipoprotein molecule, influenced by the nature of the cellular glycocalyx as well as other factors, would modulate the rate of transfer. This theory allows for more selectivity in lipid or protein transfer than would be observed in a collision complex, where a proportional transfer of all membrane components might be expected.

Lysis of trypanosomes by serum or HDL from nonpermissive hosts is the end result of HDL-mediated damage to the permeability properties of the trypanosome plasma membrane (Rifkin, submitted for publication). How close to the plasma membrane must HDL get in order to have an effect on the viability of the cell? The data reported here suggest that normally, at 37°C, VSG does not prevent HDL from interacting with the cell but that steric hindrance by VSG can be induced by rendering VSG relatively more immobile (or more firmly anchored in the lipid bilayer) by low temperature, by chemical cross linking, or by the action of various membrane-active drugs. Using the cross linker dithiobis(succinimidylpropionate) (DSP), Strickler and Patton [11] observed extensive intermolecular cross linking of VSG with the rapid appearance of oligomers, equal to or greater than octomers. We have no data on the extent of surface coat cross linking indued by DMS treatment, but expect it to be analogous to that described using DSP since the reactive groups on the protein would

be the same (ie, lysine) and the lengths of the cross linkers are similar (11.9 Å for DSP, 11 Å for DMS). The finding that DMS-treated cells were indeed resistant to lysis (Table I) is consistent with the hypothesis that VSG can, when appropriately modified, be made into a barrier preventing HDL-cell interaction.

The idea that lateral mobility of VSG, or membrane fluidity, might be important in allowing HDL to approach sufficiently close to the plasma membrane to induce lysis is supported by the finding that membrane stabilizers were found to inhibit the lytic effect of HDL. Cytochalasins and local anesthetics are amphipathic molecules which bind to plasma memranes and inhibit many membrane-fluidity-dependent phenomena, such as capping [15,30], cell movement [14,31], and cell fusion [14,32]. The ability of cytochalasins to render cells resistant to lysis by HDL, as described in this paper, is not due to an inhibition of glucose transport since cytochalasins C,D, and E have no effect on this uptake process. In fact, the extreme sensitivity of trypanosomes to cytochalasin A may be due to an inhibition of glucose uptake as trypanosome metabolism requires a high rate of glucose consumption [13]. The relative effectiveness of the cytochalasins in inhibiting HDL-induced cell lysis—namely, cytochalasin E > cytochalasin D > cytochalasin C—is comparable to that found for inhibition of capping [15]. It is interesting to note that cytochalasin pretreatment of two other parasitic protozoa, Leishmania tropica amastigotes [34] and Plasmodium knowlesi merozoites [35], prevented their uptake into host cells by phagocytosis, a process which probably requires the redistribution of cell surface proteins.

The relative order of potency of the local anesthetics tested for inhibition of trypanosome lysis was dibucaine > tetracaine > procaine (Table IV). This sequence is similar to that found by Singer [36] when these drugs were assayed for their effect on enzymatic activity of an integral membrane protein (mitochondrial cytochrome oxidase), and is also identical to their relative anesthetic potencies and the relative strength of their interaction with phospholipids. Although our data are consistent with the hypothesis that VSG sterically hinders HDL-cell surface interaction when membrane fluidity is altered, we cannot rule out the possibility that resistance to lysis is due to the effect of these drugs on some other membrane property.

The mode of attachment of VSG to the trypanosome plasma membrane is as yet undefined. Until recently, VSG was believed to be a peripheral membrane protein readily solubilized from cells following disruption by mechanical or osmotic means [37]. Because the properties of peripheral membrane proteins would not be expected to be modified by membrane-active drugs, the data reported in this paper suggest either that VSG is tightly associated with an integral membrane protein or that VSG is itself an integral membrane protein. After this work was completed, Cardoso de Almeida and Taylor reported that VSG could be isolated as a membrane form, soluble only in the presence of detergents but readily converted upon disruption of the cell to a soluble form [38]. Thus, our data support the conclusion that VSG is an integral membrane protein whose anchoring in the membrane might be affected by membrane-stabilizing agents such as local anesthetics, cytochalasins, and zinc.

It is hoped that ultimately a more complete understanding of the detailed mode of action of cytotoxic HDL will yield some insight into the cellular basis for sensitivity or resistance to lysis. A resistant trypanosome might be one in which VSG acts as a barrier preventing HDL-plasma membrane interaction. This could be the result of

either altered VSG conformation (due either to a diffferent primary sequence or to a different degree of glycosylation resulting in a bulkier molecule) or denser packing of VSG per unit area of cell surface. Altered properties of the hydrophobic C-terminal extension which anchors the VSG in the plasma membrane might also affect the relative sensitivity of a trypanosome to HDL-induced lysis. Alternatively, resistance to lysis might reside in a metabolic difference, resistant cells being able to repair a plasma membrane lesion more efficiently than sensitive cells. Schlager et al [39–41] have reported that resistance of tumor cells to lysis by cytotoxic T lymphocytes can be correlated with the capacity of the target cell to synthesize complex lipids and thereby repair the membrane lesion. A similar explanation has been proposed for the ability of cells to resist lysis in the presence of sublethal doses of complement even though early membrane damage (ion-permeability changes) can be demonstrated [42]. As we know very little about lipid synthesis in trypanosomes, we cannot at this time rule out such a mechanism. The nature of the primary lesion resulting from cytotoxic HDL-cell interaction and the biochemical/cellular basis for the difference between human serum-sensitive (T brucei) and resistant (T rhodesiense) cells, therefore, remains to be elucidated.

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