Role of phospholipids in the cytotoxic action of high density lipoprotein on trypanosomes

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Abstract Host range among the African trypanosomes, protozoa that cause fatal diseases both in humans and livestock, may be, in part, regulated by toxic properties associated with host high density lipoproteins (HDL). High density lipoproteins from hosts resistant (baboon, human) or susceptible (rabbit, rat) to *Trypanosoma brucei* infection were isolated and their trypanocidal activity was determined in in vitro cell lysis assays. Rabbit and rat HDL were not cytotoxic while baboon and human HDL rapidly lysed trypanosomes within 2 h at 37°C. Analysis of the phospholipid composition of HDL preparations from these species suggested a correlation between trypanocidal activity and low phosphatidylinositol content. Phospholipase digestion of HDL resulted in a loss of trypanocidal activity, indicating the importance of native phospholipids in maintaining this biological activity of HDL. Cell lysis and loss of trypanosome infectivity induced by baboon HDL could be inhibited either by addition of rabbit or rat HDL to the incubation medium or by addition of purified phospholipids, phosphatidylinositol being the most effective inhibitor. Although the mechanism by which HDL lyses trypanosomes remains to be elucidated, these results suggest an important role for phospholipids in determining the specificity of this cytotoxic property of HDL. —**Rifkin, M. R.** Role of phospholipids in the cytotoxic action of high density lipoprotein on trypanosomes. *J. Lipid Res.* 1991. **32:** 639–647.

Supplementary key words *Trypanosoma brucei* • host specificity • cell lysis • phospholipase • liposomes

The ability of a mammalian host to resist infection by certain viruses or protozoa can, in some cases, be correlated with the presence of toxic factors in their serum (or plasma). Thus, mouse serum can neutralize the infectivity of murine xenotropic, but not ecotropic, viruses (1), and serum (or plasma) from baboons and humans, which are resistant to *Trypanosoma brucei* infection, are cytotoxic for this protozoan parasite (2). In both cases, neutralizing or cytotoxic activity has been shown to reside in the high density lipoprotein (HDL) fraction of these sera (3–5). Moreover, in the case of *T. brucei*, sera or HDL from rabbits or rats, animals susceptible to infection by this trypanosome, are not cytotoxic. Thus, the host range of trypanosomes may be partly regulated by or associated with toxic properties of the host's lipoproteins.

The mechanism by which HDL induces cell lysis of trypanosomes is not yet well understood. Lipid exchange, of both cholesterol and phospholipids, between HDL and cells probably plays a role in preserving cellular membrane integrity and function (6, 7). However, in some cases, such lipid exchange may be detrimental to the cell. For example, hemolysis of erythrocytes can be induced by in vitro replacement of the native phosphatidylcholine (PC) with certain synthetic PC (8). It was of interest, therefore, to determine whether the phospholipid composition of cytotoxic and noncytotoxic HDL could be correlated with their trypanocidal activity.

The overall protein and lipid content of baboon, human, rabbit, and rat HDL have been reported (9, 10). While the weight percentage composition of protein, phospholipid, and cholesterol did vary among the HDL, there was no particular composition that distinguished cytotoxic baboon and human HDL from noncytotoxic rat and rabbit HDL. Phospholipid analyses of baboon (10), human (11), and rabbit (12) HDL indicate some striking differences, notably in phosphatidylethanolamine (PE) content, but some of this variation could be due to different methods used for HDL isolation and purification, lipid extraction, and phospholipid analysis. A reinvestigation into the phospholipid composition of HDL from hosts resistant or susceptible to infection by *T. brucei*, prepared and analyzed by identical procedures, was therefore undertaken.

In this report we present evidence that the phospholipid composition of HDL may be important for trypanocidal activity. Cytotoxic HDL were characterized by their relatively higher PC content and lower phosphatidylinositol (PI) content. Trypanocidal activity could be destroyed by phospholipase treatment of cytotoxic HDL. Finally, HDL-induced cell lysis could be inhibited by the

Abbreviations: HDL, high density lipoprotein; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; MEM, minimum essential medium; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SP, sphingomyelin; TLC, thin-layer chromatography.

addition to the incubation medium of liposomes composed of anionic phospholipids.

MATERIALS AND METHODS

Parasites and sera

Trypanosoma brucei, derived from a clone of strain EATRO 110 (13), was used in all studies. Male NCS mice (Rockefeller University), weighing 20–25 g, were each infected with approximately 10^6 trypanosomes (passaged less than five times since cloning) from a freshly thawed capillary stabilate (14). Three days after infection, trypanosomes were harvested and purified as described previously (13). Trypanosomes were resuspended in Minimum Essential Medium (Eagle) with Earle's salts (MEM, Gibco Laboratories) at 4°C at a cell concentration of 1×10^8/ml and used as soon after isolation as possible.

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 human sera were prepared as described (4).

HDL preparation

HDL was purified essentially as described by Rudel et al. (15). Briefly, serum was adjusted to a density of 1.225 g/ml 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 g/ml. Tubes were centrifuged in a Beckman SW40 rotor for 40 h 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 cm × 90 cm. The column was eluted with 0.15 M NaCl–0.01% EDTA, pH 7.0, containing 0.02% Na azide. 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.

Lipid extraction and phospholipid analyses

Lipoprotein fractions were extracted by the standard chloroform–methanol procedure of Folch, Lees, and Sloane Stanley (16). All solvents were freshly redistilled before use. After drying down the extracts under N_2, they were resuspended in chloroform–methanol 2:1 and stored under N_2 at −20°C. Phospholipids were separated by two-dimensional TLC on Redi-Coat-2D plates (Supelco) with the solvent systems chloroform–methanol–28% ammonium hydroxide 65:25:5 and chloroform–acetone–methanol–acetic acid–water 6:8:2:2:1 (17) and located by exposure of the plates to iodine vapor. Spots were scraped and quantitated directly for phosphorus by the Bartlett method (18). Duplicate plates were run for each phospholipid analysis.

Liposomes

Commercially obtained phospholipids were purified by TLC on preparative 1 mm silica gel 60H (EM Reagents) plates containing 0.1 M Mg acetate with chloroform–methanol–acetone–H_2O 50:30:8:4 as the solvent system before being used for liposome preparation. Multilamellar liposomes were prepared by the method of Bangham, Standish, and Watkins (19) using MEM for resuspension of the lipid.

Assays

Cell lysis assays were performed as described (13). Baboon and human serum (or HDL) were used as the cytotoxic sera (or HDL); control tubes contained noncytotoxic rat or rabbit serum (or HDL). Generally, in vitro incubation was carried out at 37°C in stoppered, 13 × 100 mm silicone-coated glass tubes containing MEM with trypanosomes at a final concentration of 1–2 × 10^7 cells/ml and 25% serum or 1 mg/ml HDL. Final incubation volume was usually 0.2 ml. Cell lysis was monitored either by phase contrast microscopy of fixed cells or by release of [^{3}H[leucine-labeled macromolecules from lysed cells, described in detail elsewhere (13, 20). Briefly, when microscopy was used to assess lysis, a 20- to 25-μl aliquot was removed from the incubation tubes at the indicated times and fixed with an equal volume of a buffered glutaraldehyde solution (13). Fixed cells were kept at 4°C, to prevent clumping of cells, and counted within 1–2 h. Two hundred trypanosomes, in two separate areas of the slide, were scored by morphological criteria to determine the percentage of nonrefractile cell ghosts (= % lysis). Trypanocidal activity of sera or HDL could be expressed quantitatively as 50% trypanolytic units (T_{50} units) per ml or per mg protein (20). Because the extent of lysis is somewhat variable and dependent on the particular trypanosome preparation, data reported are from single experiments. However, all experiments were repeated at least twice.

Trypansome infectivity for mice was estimated by the method of Lumsden et al. (21), which measures the number of infective organisms/inoculum, or by the method of Hawking (22), which is based on determining the length of the prepatent period after intraperitoneal injection of trypanosomes. Protein concentrations were determined using the Bio-Rad assay and bovine plasma albumin as a standard.

Materials

Phospholipase A_2 (Crotalus) was purchased from Worthington Biochemical Corp., phosphatidic acid (Na salt) was obtained from Supelco, and phosphatidylinositol (plant) was obtained from Applied Science Laboratories. The following compounds were all purchased from Sigma: phospholipase A_2 (*Naja naja*), phospholipase C (*B. cereus*), essentially fatty acid-free bovine albumin, cholesterol, cholesteryl linoleate, L-α-phosphatidyl-DL-glycerol, cardiolipin, L-α-phosphatidylcholine (egg yolk, Type V-E), L-α-phosphatidylethanolamine (bovine, Type I, and egg, Type III), L-α-phosphatidylinositol (soy bean, Grade III), and L-α-phosphatidylserine (bovine brain).

RESULTS

Trypanocidal properties of sera and HDL from different animals

In vitro incubation of purified bloodstream trypanosomes in MEM containing either baboon or human serum resulted in 85–100% lysis within 2 h at 37°C (**Fig. 1**). Rat and rabbit serum, which were not cytotoxic by themselves, when mixed with cytotoxic sera partially inhibited the trypanocidal activity of human serum (Fig. 1) and of baboon serum (data not shown). Because baboon serum had a higher titer of trypanocidal activity than human serum, inhibition of lysis by rat or rabbit serum was less pronounced. Rat serum was more effective than rabbit serum in inhibiting human serum trypanocidal activity.

Trypanocidal activity of human serum is associated with purified HDL (4, 5). To test whether the inhibitory property of rabbit or rat serum might also reside in a specific lipoprotein fraction, rat and rabbit lipoproteins were assayed in mixing experiments. The results in **Table 1** show that purified rat and rabbit HDL were effective in significantly inhibiting cell lysis induced by human serum. Rat HDL was a more potent inhibitor than rabbit HDL. Inhibition by rabbit low density lipoprotein was somewhat variable, ranging from no inhibition to about 50% inhibition, but in no case did it approach the inhibition produced by rabbit HDL.

TABLE 1. Inhibition of human serum-induced trypanosome lysis by rabbit serum, rat serum, and HDL

	T_{50} Units/ml	% of Control
Human serum	14	(100)
Human serum + rabbit serum	5.1	36
Human serum + rat serum	2.1	15
Human serum + rabbit HDL	2.3	17
Human serum + rat HDL	0.6	4
Rabbit or rat serum	0.3	2
Rabbit or rat HDL	0.3	2

Incubation tubes contained 25% (v/v) human and/or rabbit serum, rabbit HDL (3.9 mg/ml final conc.), or rat HDL (4 mg/ml final conc.) either separately or together. Incubation was at 37°C for 2 h at a cell concentration of 2×10^7 trypanosomes/ml. Trypanocidal titers were determined as described (13) and expressed as 50% trypanolytic units ($T_{50}/\mu l$).

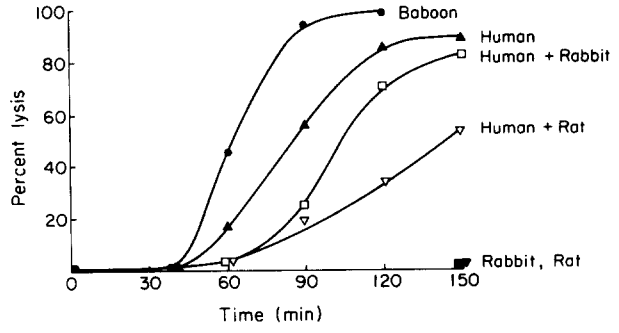

Fig. 1. Lysis of trypanosomes by various animal sera. Trypanosomes, purified from infected mouse blood, were incubated at 1×10^7 trypanosomes/ml at 37°C in MEM containing 25% (v/v) serum from different animal species. At each time point, aliquots were removed and the percent cell lysis was determined by phase contrast microscopy of fixed cells (20). In serum mixing experiments, each serum was present at 25% (v/v).

Infectivity assays (21) of trypanosomes incubated in either baboon or rat HDL separately or together at 37°C for 70 min showed that after exposure to baboon HDL (1.47 mg/ml final concentration) only 1 in 339 trypanosomes was infective. However, after incubation in baboon HDL with added rat HDL (2.1 mg/ml final conc.) 1 in 2 trypanosomes was infective, which was equivalent to the infectivity of control trypanosomes incubated either in MEM or rat HDL alone. These results are consistent with the idea that addition of noncytotoxic HDL prevents formation of the primary lesion, which eventually leads to cell death.

Because rat and rabbit serum (or HDL) are not cytotoxic, an assay for their "activity" relies on their ability to inhibit trypanocidal sera. In such assays, as described above, rat serum and HDL are more inhibitory than rabbit serum and HDL. Thus, for comparative purposes, the animal sera to be discussed in this paper will be arranged in order of decreasing trypanocidal activity as follows: baboon (most cytotoxic), human, rabbit, rat (most inhibitory, or least cytotoxic).

Phospholipase treatment destroys HDL trypanocidal activity

Trypanocidal activity of HDL is destroyed by protein denaturing agents (dithiothreitol, urea) or trypsin (20). To assess the role of phospholipids in HDL trypanocidal activity, baboon and rabbit HDL were treated with a variety of phospholipases. Phospholipase A_2 treatment (25 U/ml, 37°C, 1 h) did not alter the elution pattern of HDL on a Sephacryl S-300 column; phospholipase C treatment (same conditions) rendered the peak slightly broader but

did not alter the elution position of the peak (data not shown). The extent of phospholipase action was determined by phospholipid analysis of phospholipase A_2-treated HDL. A reduction in PC and PE content with a concomitant increase in their respective lysophospholipids was found (**Table 2**).

Although phospholipase-treated HDL was repurified before testing of trypanocidal activity, the possibility that residual phospholipase adhering to the HDL might influence the cytotoxicity assays needed to be ruled out. Repurified HDL was tested for residual phospholipase A_2 activity. The assay for residual phospholipase A_2 activity involved the titration with base of free fatty acid liberated from lecithin (23); the liberation of acid-soluble phosphorus was used as the assay for residual phospholipase C activity (24). Both untreated and phospholipase-treated HDL exhibited background activity in these assays which could detect less than 0.05 units of enzyme.

When trypanocidal activity of phospholipase-treated HDL was tested, both baboon and rabbit HDL preparations were found to extremely cytotoxic, leading to complete lysis of the cells within 60 min. Addition of fatty acid-free albumin, which had no effect on the trypanocidal activity of native HDL, was able to block this nonspecific, phospholipase-induced cytotoxicity (**Table 3**). Higher concentrations of albumin (20 mg/ml) completely inhibited the activity associated with phospholipase A_2-treated baboon HDL and further decreased the activity of phospholipase C-treated baboon HDL fourfold, while only inhibiting the activity of untreated baboon HDL by 25%. It seems likely, therefore, that toxic phospholipid degradation products (e.g., free fatty acids) were responsible for the trypanocidal activity of phospholipase-treated HDL. These results are consistent with the idea that native phospholipid, as well as protein, components are necessary for conferring specificity to the trypanocidal activity of HDL.

TABLE 2. Phospholipid composition of phospholipase-treated baboon HDL

Phospholipid	Untreated	*Crotalus* Phospholipase A_2	*Naja naja* Phospholipase A_2
	% of total phospholipid phosphorus		
PC	80.4	58.3	42.9
PE	4.2	3.7	–
SP	8.8	9.2	9.3
PI (+ PS)	2.5	6.8	6.2
LPC	3.9	21.9	39.3
LPE	–	–	2.2

Baboon HDL (final conc. 5.7 mg/ml) was incubated in MEM at 37°C for 1 h without and with phospholipase (25 U/ml). HDL was repurified on a Sephacryl column, concentrated by ultrafiltration, and extracted, and phospholipids were quantitated after separation by two-dimenstional TLC, as described in Materials and Methods. Data are the average of two determintions.

TABLE 3. Trypanocidal activity of phospholipase-treated HDL

HDL	No Addition	+ Albumin (5 mg/ml)
	% lysis	
Baboon, untreated	100	100
Baboon, phospholipase A_2-treated	100	24
Baboon, phospholipase C-treated	100	82
Rabbit, untreated	3	1
Rabbit, phospholipase A_2-treated	97	8
Rabbit, phospholipase C-treated	99	7

Phospholipase treatment of HDL was as in Table 2. *Naja naja* phospholipase A_2 and *Crotalus* phospholipase C were used. HDL was repurified on a Sephacryl column before trypanocidal activity was tested. Trypanocidal assays were performed as described in Fig. 1 except that the final HDL concentration was 0.5 mg/ml.

Phospholipid composition of cytotoxic and noncytotoxic HDL

HDL was purified from serum of nonpermissive (baboon, human) and permissive (rabbit, rat) hosts by ultracentrifugal flotation and Sepharose chromatography (see Materials and Methods). Phospholipids were extracted and separated by two-dimensional TLC and quantitated to identify a possible correlation between phospholipid composition and trypanocidal activity. The results (**Table 4**) show that the most cytotoxic HDL (baboon HDL) had relatively the lowest PI and LPC and highest PC content, while the inverse (high PI, low PC values) was true for the least cytotoxic HDL (rat HDL). Because PI and phosphatidylserine (PS) were not always well separated on these plates, the combined PI + PS region was analyzed. Further chromatography of material from this region with different solvent systems indicated that PI was the major phospholipid. The positive correlation between trypanocidal activity and PC content and the negative correlation between this activity and PI content suggested the possibility of attempting to modulate trypanocidal activity by altering the phospholipid composition of HDL.

Reconstitution experiments with baboon and rabbit HDL in which the apolipoproteins were recombined with both homologous and heterologous lipids by the method of Ritter and Scanu (25) were performed. While the reassembled lipid-protein complexes appeared similar to native HDL in hydrodynamic properties, the reconstituted particles had lost all specificity when assayed for biological activity. That is, HDL reconstituted from homologous apolipoprotein and lipids, whether derived from baboon or rabbit HDL as starting material, were equally very cytotoxic. Thus, this approach was not pursued further.

In an attempt to alter the phospholipid composition of rat HDL, two groups of rats were placed on synthetic diets with or without myo-inositol (26) for 4 weeks. Inositol is an essential nutrient for mammalian cells and

TABLE 4. Phospholipid composition of HDL from different animal species

Species	PC	PE	PI (+PS)	SP	LPC
			% of total phospholipid phosphorus		
Baboon (10)	81.9 ± 0.9	5.9 ± 0.4	3.6 ± 0.2	5.6 ± 0.2	2.4 ± 0.2
Human (8)	78.8 ± 0.9	3.3 ± 0.2	3.6 ± 0.3	10.6 ± 1.7	2.6 ± 0.4
Rabbit (10)	72.5 ± 1.2	4.8 ± 0.6	5.2 ± 0.4	9.2 ± 0.4	6.7 ± 0.8
Rat (6)	68.2 ± 4.3	0.9 ± 0.3	7.8 ± 0.7	11.0 ± 1.8	11.3 ± 1.5

Values are means ± SEM. Phospholipids were extracted from purified HDL preparations and separated by two-dimensional TLC as described in Materials and Methods. In each case two to four separate HDL preparations were extracted. The number in parentheses indicates the number of TLC plates analyzed.

inositol-deficient diets lead to alterations in lipid metabolism in rats (26). Since low HDL-PI content was correlated with increased trypanocidal activity, it was hoped that HDL isolated from rats on inositol-deficient diets might be more cytotoxic than normal rat HDL. When the trypanocidal activity of HDL isolated from the two groups of rats was compared, both in direct trypanosome lysis assays and in their ability to inhibit the trypanocidal activity of human serum or HDL, no differences were found. This finding is consistent with the subsequent phospholipid analysis of these rat HDLs which showed no significant quantitative differences in the percentage phospholipid composition of HDL derived from rats maintained on diets with or without myo-inositol. Additional experiments showed that both groups of rats were equally susceptible to trypanosome infection and trypanosomes grown in these two groups of rats did not differ in their susceptibility to lysis by baboon HDL (data not shown).

Exogenous phospholipids modulate HDL trypanocidal activity

Because the addition of rabbit or rat HDL to incubation media containing cytotoxic HDL could inhibit trypanosome lysis (Table 1), addition of liposomes, made from purified phospholipids, to the trypanocidal assay incubation mix was studied. **Fig 2A** shows that addition of PC liposomes had no effect on the trypanocidal activity of baboon serum while the presence of PI liposomes almost completely inhibited both baboon serum and baboon HDL-induced trypanosome lysis (Fig. 2B).

The inhibition of trypanosome lysis by PI liposomes was dose-dependent (**Table 5**). Maximum inhibition of lysis was reached at a final PI concentration of about 1 mg/ml.

The effect of added PI on the loss of cell infectivity was assessed by incubating trypanosomes for 60 min (other conditions as in Fig. 2B) in the presence and absence of

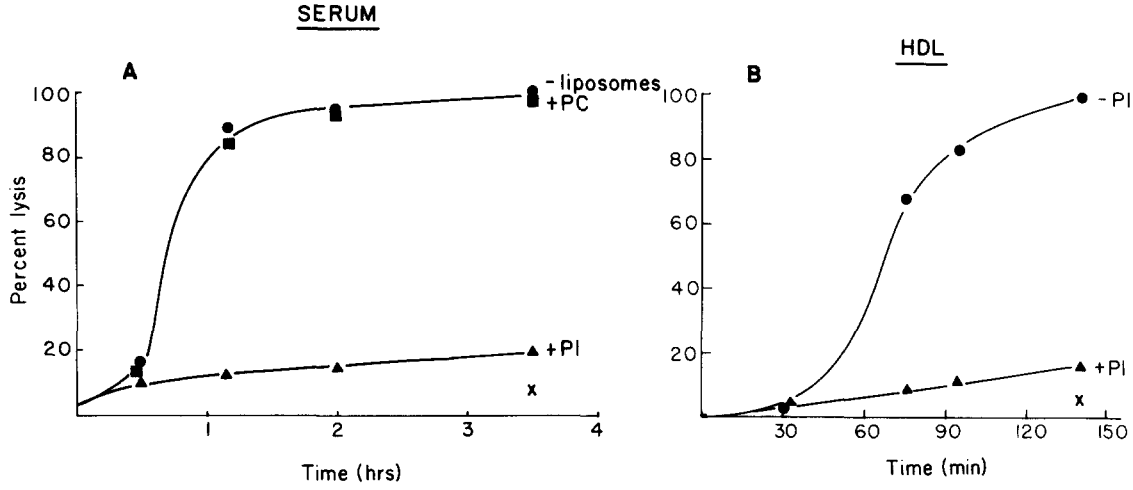

Fig. 2. Inhibition by PI of trypanosome lysis induced by baboon serum (A) or HDL (B). A) Cell lysis assay conditions were the same as in Fig. 1. PC and PI liposomes were present at 1 mg/ml final concentration. Liposomes and the incubation medium were preincubated at 37°C for 30 min before addition of trypanosomes; X, percent lysis observed in control tubes containing rabbit serum with PC or PI liposomes. B) PI liposomes (final concentration 1.25 mg/ml) were added to incubation tubes containing baboon HDL (final concentration 3 mg/ml) or MEM and preincubated at 37°C for 30 min before addition of trypanosomes; X, percent lysis observed in control tubes containing MEM with or without PI liposomes.

TABLE 5. Inhibition of trypanosome lysis by phosphatidylinositol

PI Final Concentration	Percent Lysis
mg/ml	%
0	98
0.025	96
0.075	92
0.25	84
1.25	5

Trypanosome lysis was assayed under standard conditions: 25% baboon serum, 10^7 trypanosomes/ml, 37°C, 2 h.

PI liposomes. The trypanosomes were washed and assayed for infectivity by injecting mice (groups of five mice/incubation tube) intraperitoneally with 5×10^4 trypanosomes and evaluating the length of the prepatent period (22). Trypanosomes incubated in noncytotoxic media (rabbit HDL) with or without PI liposomes gave patent infections in 1.30 ± 0.27 days (mean ± SD) and 1.35 ± 0.38 days, respectively. Incubation in baboon HDL resulted in a much longer prepatent period, 4.23 ± 0.84 days, indicating that the trypanosomes had been rendered 400-fold less infective. Trypanosomes incubated in baboon HDL plus PI liposomes were almost as infective as control trypanosomes and produced a patent infection in 1.65 ± 0.14 days.

Thus, the addition of PI liposomes not only inhibited trypanosome lysis, as judged by phase microscopy, but also inhibited loss of trypanosome infectivity. These results are consistent with the hypothesis that added exogenous PI prevents formation of the initial lesion that leads to cell death. Whether this occurs by blocking the interaction of cytotoxic HDL with the trypanosome surface or by some other mechanism remains to be elucidated.

Inhibition of cell lysis by exogenous PI required the simultaneous incubation of cytotoxic HDL, PI liposomes, and trypanosomes (**Table 6**). Preincubation of trypanosomes in PI, followed by washing of the trypanosomes and subsequent incubation in baboon HDL, did not affect the extent of cell lysis observed (Expt. 1). Preincubation of baboon serum with either PC or PI liposomes, followed by HDL purification, did not alter the trypanocidal properties of the purified baboon HDL (Expt. 2). These results do not rule out the possibility that PI may be binding to the cell, thereby preventing interaction of HDL with the trypanosome surface, but do suggest that if such binding occurs the PI is readily washed off the cells.

Fig. 3 shows that other weakly anionic phospholipids are also capable of preventing HDL-induced cell lysis, although not as effectively as PI. Phospholipids from various sources were tested. Thus, PI from both bovine brain and soybean gave similar results; PE from bovine brain was equivalent to PE from egg yolk; only PE from soybean was cytotoxic to the cells in the absence of HDL. Phosphatidic acid was as effective as PI in inhibiting baboon HDL-induced cell lysis, while phosphatidylglycerol at 2.5 mg/ml caused a 50% reduction in cell lysis (data not shown). Other lipids that were found to have no effect on HDL-induced cell lysis were cardiolipin (2.5 mg/ml), cholesterol (2.5 mg/ml), and cholesteryl linoleate (2.5 mg/ml); the latter two compounds were added as albumin complexes. Addition of these phospholipids to incubations containing noncytotoxic (rat, rabbit) serum or HDL never resulted in a significant stimulation of cell lysis.

TABLE 6. Effect of preincubation with phospholipids on trypanosome lysis by baboon serum

Preincubation Conditions	% Lysis[a]
Expt. 1[b]	
Trypanosomes	88
Trypanosomes + PC (0.25 mg/ml)	98
Trypanosomes + PI (0.25 mg/ml)	92
Expt. 2[c]	
Baboon serum + PC (2.5 mg/ml)	99
Baboon serum + PI (2.5 mg/ml)	98

[a]Cell lysis was assayed by phase contrast microscopy (20).
[b]Purified trypanosomes were incubated in MEM at 37°C for 30 min at 2×10^7 trypanosomes/ml as indicated, then washed and incubated at 1×10^7 trypanosomes/ml in 25% (v/v) baboon serum in MEM at 37°C for 2 h. Control tubes contained trypanosomes in rabbit serum and exhibited negligible (0–5%) lysis.
[c]Baboon serum was incubated with phospholipids for 2 h at 37°C before HDL purification. Repurified HDL was then used at 2 mg/ml final concentration in cell lysis assays (conditions as in Expt. 1). Control tubes containing trypanosomes in MEM showed no lysis (<1%).

DISCUSSION

African trypanosomes are protozoan parasites that depend on nutrients in the bloodstream of the host for their

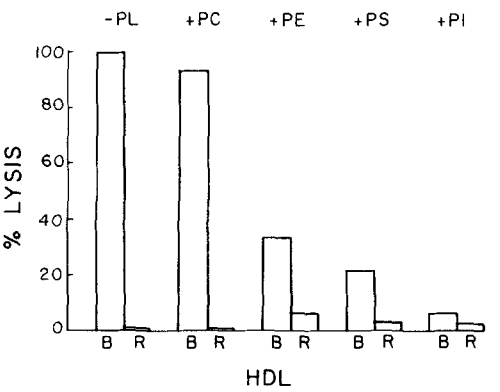

Fig. 3. Effect of added phospholipids on baboon HDL (B-HDL)-induced trypanosome lysis. Assay tubes contained either baboon HDL (final concentration 1 mg/ml) or rabbit HDL (final concentration 1.25 mg/ml), 2×10^7 trypanosomes/ml, and liposomes (final concentration 1 mg/ml) in MEM and were incubated at 37°C for 2 h. Cell lysis was estimated by phase contrast microscopy (20); PL, phospholipid; B, baboon; R, rabbit.

survival. In a mammalian host susceptible to infection by these trypanosomes, this environment allows growth and multiplication of the parasite. The result is either a fulminating parasitemia often leading to death of the host, as in cattle, sheep, or laboratory rodents, or a more chronic infection with no apparent distress to the host, as in game animals, which thus act as reservoir hosts for these parasites. *Trypanosoma brucei* causes the fatal disease Nagana in cattle, but cannot infect humans or baboons.

The curative property of human serum when injected into mice infected with *T. brucei* has been known since 1902 (27). In more recent studies (4, 5), this trypanocidal property was found to be associated with purified serum HDL preparations. The trypanocidal properties of HDL from different species correlated with the susceptibility of these animals to *T. brucei* infection; that is, HDL from permissive hosts was not cytotoxic while HDL from nonpermissive hosts readily destroyed trypanosomes in vitro. It has been suggested, therefore, that the resistance of humans and baboons to *T. brucei* may be due in part to the trypanocidal properties of their HDL (2, 4).

While neutralization of xenotropic murine viruses by mouse serum was found in the lipid-free apolipoprotein preparation (28), trypanocidal activity could not be attributed to either the apolipoproteins or lipid components of HDL. Apolipoproteins purified from human or rabbit HDL, when tested at 0.4–0.6 mg/ml final concentration, had no trypanocidal activity; HDL lipid extracts derived from cytotoxic human HDL or noncytotoxic rabbit HDL did not differ in their cytotoxic properties (29). Degradation of the HDL protein moieties by trypsin (20) or of the phospholipids by phospholipase digestion, as described in this report, results in inactivation of the trypanocidal activity. Moreover, protein denaturing agents, such as urea or dithiothreitol (20), or lysine modification procedures, such as methylation or acetoacetylation (Rifkin, M. R., unpublished observations), also abolished HDL trypanocidal activity. It is likely, therefore, that both unmodified protein and lipid HDL components are necessary for and contribute to the specificity of the trypanocidal properties of HDL.

Exposure of trypanosomes to cytotoxic HDL results in rapid loss of infectivity, probably due to acute, irreversible damage to the ion permeability properties of the plasma membrane, which then leads to cell lysis by a colloid osmotic mechanism (30). Trypanosomes do not appear to have a saturable receptor for HDL (31) and there is currently no evidence to suggest that HDL must be taken up by the cell to exert its cytotoxic effect. The exact mechanism of HDL action, however, remains unclear.

HDL and its apolipoproteins have been reported to be able to affect net loss of cholesterol from tissue culture cells (32, 33) and cholesterol removal leads to increased permeability and osmotic fragility in red blood cells (34). Cholesterol flux from the cell's plasma membrane to HDL is believed to occur by passive diffusion, driven by the lower cholesterol to phospholipid ratio of HDL as compared to that of the plasma membrane. However, the cholesterol to phospholipid ratio of HDL from nonpermissive hosts (baboons, humans) is not significantly different from that of HDL from permissive hosts (rabbits, rats) (9, 10) and thus this property alone could not account for the selective cytotoxicity of baboon and human HDL for *T. brucei*. In addition, HDL apolipoproteins are not cytotoxic by themselves and we have been unable to detect differences in cholesterol efflux from cells prelabeled with radioactive cholesterol and incubated in baboon and rabbit HDL (29). It is unlikely, therefore, that trypanosome lysis is primarily due to cholesterol removal from the plasma membrane by HDL.

Trypanosome killing may be due to the insertion of a particular HDL-lipid species into the trypanosome plasma membrane. Because trypanosomes are limited in their capacity to synthesize lipids, it is believed that the organisms derive many of their required lipids from their environment (35). It is likely that serum lipoproteins serve as donors of preformed lipids since in vitro growth of trypanosomes in lipoprotein-free medium requires the addition of either high density or low density lipoproteins; lipid-depleted lipoproteins could not substitute for the native lipoproteins (36). This lipid uptake does not require binding and uptake of lipoproteins and may be mediated by desorption and diffusion of the lipids from the lipoproteins to the trypanosomes (37). It is possible, then, that certain lipid species may have an adverse effect on the trypanosomes and lead to cell death. Similarly, red blood cells can tolerate only limited changes in their phospholipid composition and lyse when native phospholipids are replaced with different phospholipid species.

HDL has been reported to stimulate 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (38) and glycolipid synthesis (39). It is possible that cytotoxic HDL might specifically stimulate some trypanosome enzyme activity that could indirectly influence membrane lipid composition in an adverse manner and thereby lead to cell lysis. An alteration in membrane lipid composition can affect the normal function of membrane proteins whose properties are dependent on the degree of fluidity or the composition of the membrane (40). We are currently investigating this possibility by comparing the lipid composition of isolated trypanosome plasma membranes before and after interaction with HDL.

The ability of exogenous phospholipid, in particular phosphatidylinositol, to inhibit the cytotoxic action of baboon HDL, as described in this report, is not well understood. Preincubation of cells in PI did not protect the cells from subsequent lysis. The required presence of added phospholipid during the incubation of trypanosomes in cytotoxic HDL suggests two possibilities. The exogenous phospholipid might inhibit either some deleterious en-

zyme activity present as an intrinsic component of HDL or a cellular enzyme induced by interaction of the cell with HDL. On the other hand, the exogenous phospholipid may be utilized by the cell to repair the membrane lesions caused by HDL. Schlager, Ohanian, and Borsos (41) have reported that certain tumor cell lines are resistant to complement-mediated lysis due to their ability to synthesize complex lipids, which restore normal membrane function before permanent cellular damage occurs. Trypanosomes are very restricted in their lipid synthetic capabilities (36), and the added phospholipid in the medium may be used as a lipid substrate for membrane repair. There is at the present time, however, no experimental data to favor either of these hypotheses.

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