

Schistosomula of *Schistosoma mansoni* Use Lysophosphatidylcholine to Lyse Adherent Human Red Blood Cells and Immobilize Red Cell Membrane Components

David E. Golan,*[¶] Carl S. Brown,*[‡] Catherine M. L. Cianci,[†] Stephen T. Furlong,[†] and John P. Caulfield[§]†

Departments of *Medicine, [‡]Pharmacology, and [§]Pathology, Harvard Medical School, Boston, Massachusetts 02115; [¶]Hematology Division and Departments of [¶]Medicine and [†]Rheumatology/Immunology, Brigham and Women's Hospital, Boston, Massachusetts 02115

Abstract. Human red blood cells (RBCs) adhere to and are lysed by schistosomula of *Schistosoma mansoni*. We have investigated the mechanism of RBC lysis by comparing the dynamic properties of transmembrane protein and lipid probes in adherent ghost membranes with those in control RBCs and in RBCs treated with various membrane perturbants. Fluorescence photobleaching recovery was used to measure the lateral mobility of two integral membrane proteins, glycophorin and band 3, and two lipid analogues, fluorescein phosphatidylethanolamine (Fl-PE) and carbocyanine dyes, in RBCs and ghosts adherent to schistosomula. Adherent ghosts manifested 95–100% immobilization of both membrane proteins and 45–55% immobilization of both lipid probes. In separate experiments, diamide-induced cross-linking of RBC cytoskeletal proteins slowed transmembrane protein diffusion by 30–40%, without affecting either

transmembrane protein fractional mobility or lipid probe lateral mobility. Wheat germ agglutinin- and polylysine-induced cross-linking of glycophorin at the extracellular surface caused 80–95% immobilization of the transmembrane proteins, without affecting the fractional mobility of the lipid probe. Egg lysophosphatidylcholine (lysoPC) induced both lysis of RBCs and a concentration-dependent decrease in the lateral mobility of glycophorin, band 3, and Fl-PE in ghost membranes. At a concentration of 8.4 $\mu\text{g/ml}$, lysoPC caused a pattern of protein and lipid immobilization in RBC ghosts identical to that in ghosts adherent to schistosomula. Schistosomula incubated with labeled palmitate released lysoPC into the culture medium at a rate of 1.5 fmol/h per 10^3 organisms. These data suggest that lysoPC is transferred from schistosomula to adherent RBCs, causing their lysis.

SCHISTOSOMA MANSONI lives in intimate contact with human blood cells from the third or fourth day after infection. The surface of this parasite is unusual in that it is bound by a syncytium covered by two membranes (8, 31, 47). The outer membrane appears to be a lipid bilayer almost devoid of protein, since the membrane lacks intramembrane particles by freeze-fracture, whereas the inner membrane resembles the plasma membrane of mammalian cells (8, 31, 47). These membranes do not endocytose multivalent ligands but instead slough the ligands into the culture medium in vitro (42). Labeled surface glycolipids and glycoproteins are also released into the medium, suggesting that the outer membrane, at least, is continually being shed (41).

The tegumental membranes exhibit several unique phenomena in interactions with mammalian blood cells. Human neutrophils fuse with the parasite to form a hybrid membrane that is derived from the cell plasma membrane and the worm outer tegumental membrane (8). This hybrid membrane mixes with the normal outer membrane upon lysis of the neu-

trophil (9). Similar membrane fusions may be responsible for the parasite's acquisition of host membrane components such as murine histocompatibility antigens (23, 24, 43, 44) and membrane glycolipids (14, 15, 29). Further, human neutrophils endocytose multivalent ligands from the parasite surface along with the surface components to which the ligands are bound (10). Paradoxically, the removal of these surface components does not produce structural alteration in the parasite membranes as seen by freeze-fracture or thin-section transmission microscopy. Unlike neutrophils, human red blood cells (RBCs)¹ adhering to the parasite do not fuse with the worm but lyse to form ghosts that remain attached to the parasite surface (6).

1. *Abbreviations used in this paper:* Con A, concanavalin A; diI, carbocyanine dyes; EMA, eosin-5-maleimide; Fl-PE, fluorescein phosphatidylethanolamine; FPR, fluorescence photobleaching recovery; FTSC, fluorescein-5-thiosemicarbazide; lysoPC, lysophosphatidylcholine; PMSF, phenylmethylsulfonyl fluoride; RBC, red blood cell; WGA, wheat germ agglutinin.

The present studies investigate the mechanism of RBC lysis. Because cultures of schistosomula and RBCs contain a few adherent ghosts, many adherent and nonadherent RBCs, and an excess of parasite membrane, the membrane properties of individual cells and ghosts must be measured in situ. Fluorescence photobleaching recovery (FPR) is ideal for this purpose, because it measures the dynamic properties of protein and lipid in a single biological membrane without perturbation of the membrane environment (3). In this technique, a single cell membrane is observed in a fluorescence microscope, using a focused laser beam as the excitation source. A small area of the membrane is exposed to a brief, intense laser pulse, causing irreversible bleaching of the fluorophore in that area. Fluorescence recovery resulting from lateral diffusion of unbleached fluorophore into the bleached area is measured. Analysis of the fluorescence recovery curves yields the fraction of fluorescently labeled protein or lipid that is free to diffuse in the plane of the membrane (the mobile fraction, f), as well as the diffusion coefficient of the mobile fraction (D).

FPR is used to measure the lateral mobility of two integral membrane proteins, glycophorin and band 3, and two lipid probes, fluorescein phosphatidylethanolamine (FI-PE) and carbocyanine dyes (diI), in RBCs and ghosts adherent to schistosomula. These findings are compared with measurements obtained in RBCs subjected to a variety of membrane perturbants. Such experiments suggest that lysophosphatidylcholine (lysoPC) is the agent responsible for RBC lysis on the parasite surface. In independent experiments, worms are labeled with ^3H -palmitate to see whether they metabolize palmitate to lysoPC and, if so, whether the lysoPC is released into the culture medium, as predicted from the shedding of the outer surface membrane.

Materials and Methods

Reagents

Fluorescein-5-thiosemicarbazide (FTSC), eosin-5-maleimide (EMA), and carbocyanine dyes [diI-C₁₂(3), diI-C₁₄(3), diI-C₁₈(3)] were purchased from Molecular Probes, Inc. (Junction City, OR). Diamide and poly-L-lysine were from Sigma Chemical Co. (St. Louis, MO). Wheat germ agglutinin (WGA) and concanavalin A (Con A) were from Vector Laboratories (Burlingame, CA). 9,10- ^3H -Palmitic acid (specific activity, 27.5 Ci/mmol) was from New England Nuclear (Boston, MA). FI-PE and phospholipids (egg phosphatidylcholine, egg lysoPC, egg phosphatidylethanolamine, bovine liver phosphatidylinositol, and bovine brain phosphatidylserine, all >99% pure) were from Avanti Polar-Lipids, Inc. (Birmingham, AL). The fatty acid composition of egg lysoPC is 70.5% palmitic acid, 25.7% stearic acid, 1.8% oleic acid, and 0.7% palmitoleic acid (Avanti Polar-Lipids, Inc., personal communication). RPMI-1640 culture medium was from Gibco (Grand Island, NY). Sodium *m*-periodate and bovine serum albumin (essentially fatty acid and globulin free) (BSA) were from Sigma Chemical Co. EDTA was from Fisher Scientific Co. (Pittsburgh, PA). Phenylmethylsulfonyl fluoride (PMSF) was from Eastman Organic Chemicals (Rochester, NY).

Fluorescent Labeling of Human RBCs and RBC Ghosts

Glycophorin. RBC glycophorin was fluorescently labeled by conjugation of FTSC to glycophorin-linked (22) sialic acid moieties (27). Similar methods have been used by Abraham and Low (1) and by Cherry et al. (13) to label glycophorin selectively with other fluorescent probes. Fresh human blood was washed three times by centrifugation in phosphate-buffered saline (PBS) (128 mM sodium chloride, 10 mM sodium phosphate, pH 7.4) with 120 μM PMSF and 1 mM EDTA (PBS/PMSF/EDTA). Washed RBCs were stirred with an equal volume of 2 mM sodium periodate in PBS for 15 min

at 0°C to oxidize selectively sialic acid exposed at the outer membrane surface (22). Unreacted periodate was removed by washing twice in 0.1 M glycerol in PBS (1) and once in PBS. The oxidized RBCs were incubated with an equal volume of FTSC, 0.5 mg/ml in PBS, for 60 min at 0°C, then washed three times in PBS with 1% BSA (PBS/BSA) (1) and once in RPMI. Unless otherwise indicated, all operations were performed at 4°C in the dark. Under these labeling conditions 80% of the membrane-associated fluorescence co-migrated on SDS polyacrylamide gels with the sialoglycoproteins PAS-1,2,3,4. Of this, 75% was coincident with glycophorin A (PAS-1,2) (21). The stoichiometry of labeling as determined by spectrophotometry and RBC membrane protein assay was 1.9 fluorophores per glycophorin monomer, or 1×10^6 fluorophores per RBC.

Band 3. RBC band 3 was fluorescently labeled by the technique of Nigg and Cherry (39). Briefly, fresh human RBCs washed in PBS/PMSF/EDTA were mixed with 0.2 mg/ml (final concentration) EMA in PBS with 10 mM glucose for 45 min at room temperature. Excess fluorophore was removed by washing three times in PBS/BSA and once in RPMI, all at 4°C. Under these conditions >80% of the membrane-associated fluorescence co-migrated on SDS polyacrylamide gels with band 3, as confirmed by selective extraction procedures on EMA-labeled RBC ghosts (39). The stoichiometry of labeling determined as above was 0.8 fluorophores per band 3 monomer, or 1×10^6 fluorophores per RBC.

FI-PE. A modification of the technique of Golan et al. (25) was used to incorporate FI-PE into the membranes of intact RBCs. FI-PE (2 mg) was dried from a stock solution in chloroform, 10 ml of PBS was added, and the mixture was bath sonicated at room temperature until clarified. 75 μl of the FI-PE mixture was added to 1.5 ml of washed, packed RBCs in 3.0 ml PBS and the mixture gently shaken at room temperature for 30 min. Labeled cells were washed three times in PBS/BSA and once in RPMI. The stoichiometry of labeling, determined by comparing the average fluorescence intensities of FTSC- and FI-PE-labeled RBCs, was 8×10^5 fluorophores per RBC or 0.003 mol of FI-PE per mole of endogenous membrane lipid. This concentration of FI-PE does not perturb the dynamic behavior of RBC membrane lipid (unpublished observations). In some cases FI-PE-labeled RBCs were lysed in 40 vol of 5 mM sodium phosphate with 1 mM EDTA and 120 μM PMSF, pH 7.4, for 45 min at 4°C. The resulting ghosts were pelleted by centrifugation and washed three to four times in the lysis buffer and once in RPMI, all at 4°C.

diI. 50 μl of diI, 0.5 mg/ml in ethanol, was added to 0.5 ml of washed, packed human RBCs in 8.0 ml of PBS and the mixture gently shaken at 4°C for 30 min. Excess fluorophore was removed by washing twice in PBS to which 1% BSA had been added (PBS/1% BSA) and once in RPMI/1% BSA. FI-PE-, FTSC-, and EMA-labeled RBCs each comprised a uniform population of fluorescent, intact discocytes, whereas diI labeled only 10% of the total RBCs and lysed ~50% of the RBCs.

Preparation of Schistosomula and Fluorescent Labeling with Carbocyanine Dyes

Schistosomula were prepared as described (40, 42). 2,000 schistosomula were cultured overnight at 37°C and mixed in 1.5 ml PBS with 2 μl of diI, 1.0 mg/ml in ethanol, for 30 min at room temperature. The labeled somules were washed twice in PBS and once in RPMI/0.1% BSA.

Incubation of Schistosomula with RBCs

1,000 cultured schistosomula were mixed with 2 μl of fluorescently labeled RBCs in 100–500 μl of RPMI/0.1% BSA and incubated at 37°C for 24–48 h. Controls were labeled RBCs or RBC ghosts incubated in the absence of somules. Immediately before FPR or photography, samples were treated with 10 mM eserine sulfate to immobilize the worms (42). This concentration of eserine sulfate did not affect the lateral mobility of RBC membrane components in control RBCs or ghosts.

Diamide Treatment of RBCs

RBCs were incubated with either 2 or 20 mM diamide in PBS at 37°C for 30 min (30, 45), washed twice in PBS containing 10% glycerol, and washed once in PBS. Diamide treatment was performed either before or after fluorescent labeling. Both protocols yielded the same FPR results.

Incubation of RBCs on Poly-L-lysine-, WGA-, and Con A-Coated Slides

FTSC-, EMA-, and FI-PE-labeled RBCs were washed twice more in RPMI

and resuspended in 20 vol of this buffer. Poly-L-lysine was prepared as a 1.0 mg/ml solution in H₂O. WGA and Con A were prepared as 0.5 mg/ml solutions in PBS. 100–200 µl of these solutions was dropped on clean glass slides for 10–15 min at room temperature and the slides washed with PBS. 5 µl of fluorescently labeled RBC suspension was then dropped on the slides for 10 min at room temperature. The slides were washed with RPMI and examined immediately.

Incubation of RBCs or Ghosts with LysoPC

25 µl of packed, fluorescently labeled RBCs or RBC ghosts were mixed with 12 ml of PBS and 0–20 µg/ml of egg lysoPC from a freshly prepared stock solution of 10 mg/ml in H₂O. The mixture was vortexed for 1 min at room temperature, washed twice in PBS and once in RPMI with 1% BSA (RPMI/BSA). Samples were diluted in 10 vol of RPMI/BSA and 1-µl aliquots were sealed on RPMI/BSA-treated microscope slides for FPR experiments. Pretreatment with BSA prevented echinocyte formation in control RBC samples.

FPR

A 4-W argon ion laser (164-08; Spectra-Physics Inc., Mountain View, CA) tuned to 488 nm was used as the excitation source for a fluorescence microscope (Leitz Orthoplan) equipped for incident-light (Ploem) illumination. The beam was focused to a waist at the secondary image plane of the microscope by a weak planoconvex lens ($f = 250, 350, \text{ or } 500 \text{ mm}$) and to another waist at the specimen plane by a 100 or 63× phase fluorescence objective (Leitz). An interferometer (Ealing Corp., S. Natick, MA) placed in the excitation path was used to split the beam into measuring and bleaching paths, as described (25). The interferometer mirrors were fitted with remote-control linear actuators (Newport Corp., Fountain Valley, CA) to allow precise alignment of bleaching and measuring beams at the sample plane. The Gaussian beam radius at the sample plane, as determined by a two-dimensional emission scan technique (Brown, C. S., A. H. Stolpen, and D. E. Golan, manuscript submitted for publication), was $0.53 \pm 0.02 \text{ }\mu\text{m}$. Photobleaching power at the sample was $\sim 2 \text{ mW}$. Bleaching times were typically 40 ms for protein diffusion measurements and 5 ms for lipid diffusion measurements. Measuring beam intensities were $\sim 3 \text{ }\mu\text{W}$. The optical apparatus was mounted on a 4' × 6' research quality vibration isolation table (Newport Corp.). Sample temperatures were controlled to $\pm 0.1^\circ\text{C}$ by using a thermal microscope stage (Leitz).

Emitted light was collected by the objective, filtered by the dichroic mirror (Leitz TK510) and suppression filter (Leitz K510), and directed through a series of lenses, beamsplitters, and mirrors (Leitz MPV-3) to an extended S-20 photomultiplier tube (9558QA; Thorn EMI Gencom Inc., Fairfield, NJ) in a thermoelectrically cooled housing (TE-104RF; Products for Research, Inc., Danvers, MA) driven by a stabilized high-voltage power supply (HVS-1; Princeton Applied Research [PAR], Princeton, NJ). An adjustable field diaphragm placed in the image plane was used to discriminate against out-of-plane fluorescence. The photocurrent was fed into an amplifier/discriminator (PAR 1121A), which converted the signal into a series of single-photon pulses. The pulses were counted within specified time intervals (typically 50–200 ms) by a photon counter (PAR 1109), and the number of counts per interval stored in a computer (PDP 11/23; Digital Equipment Corp., Marlboro, MA). The data could be converted into analog form for display on a cathode ray tube (4006-1; Tektronix, Inc., Beaverton, OR) and for hard-copy readout (Tektronix 4631), or plotted directly on an interactive digital plotter (Tektronix 4662/31). The computer was used to direct the sequence of opening and closing shutters for bleaching and measuring pulses, to gate the photon counter in step with the shutters, and to collect and analyze data. Data were fitted by nonlinear least squares analysis (4) to the approximate solution for fluorescence recovery after a "low percent" photobleach introduced by Yguerabide et al. (Eq. 12 of reference 50).

Unless otherwise indicated, samples were sealed on RPMI/1% BSA-treated microscope slides for FPR experiments. Blood was donated by four normal individuals. Experiments were performed two to five times and the results pooled, since there were no significant differences among results from different donors.

Incubation of Schistosomula with Labeled Palmitate

1 mCi of 9,10-³H-palmitic acid was dried under nitrogen, 200 µl of RPMI was added, and the mixture vortexed vigorously for 1 min. 60,000 schistosomula cultured overnight at 37°C were added to the radiolabel and the volume brought to 1.0 ml with RPMI/0.1% BSA. The incubation was carried out

for 90 min at 37°C under a 5% CO₂ atmosphere. After incubation the schistosomula were harvested by centrifugation at 200 g, washed three times in RPMI/0.1% BSA, and resuspended to 1,200 worms/ml in fresh RPMI/0.1% BSA. Aliquots consisting of 1.0 ml of the worm suspension per 1.5-ml conical centrifuge tube were incubated under a 5% CO₂ atmosphere at 37°C for up to 12 h. Cultures were sampled at 0, 3, 6, and 9 h (two experiments) or 0, 6, and 12 h (one experiment). Either four (two experiments) or five (one experiment) tubes were pooled for each of three separate determinations at each time point. Somules were separated from the culture medium by centrifugation and both pellet and medium were analyzed for phospholipid.

Phospholipid Analysis

Medium and pellet were extracted in chloroform/methanol (17) and the phases separated by centrifugation. The upper phase was discarded and the lower phase immediately dried under nitrogen. Phospholipids from the lower phase were separated immediately using high performance liquid chromatography on a Waters automated gradient control system (Waters Instruments, Inc., Rochester, MN) equipped with a model 510 pump, a "Z" module, a U6K injector, a 10-µm Radial-Pak µPorasil cartridge, and a model 441 fixed wavelength detector. Samples were suspended in hexane/isopropanol/water (3/4/1), injected, and eluted isocratically with acetonitrile/methanol/85% phosphoric acid (130/6/1.5) (12). Radioactivity was quantitated by liquid scintillation counting. Retention times for the various phospholipids were compared with those of standard phospholipids detected at a wavelength of 214 nm.

Results

Adherence of Human RBCs to Schistosomula Leads to Lysis and Immobilization of Membrane Components

Incubation with schistosomula for 24–48 h at 37°C caused many human RBCs to adhere to the worm surface and undergo profound morphological change (Fig. 1). Two general types of adherent RBCs were recognized. First, there were intact RBCs, many of which were deformed and attached by membrane tethers to the parasite surface. A few attached cells were echinocytic. Second, there were lysed RBCs (ghosts) which were visualized best by fluorescence microscopy (cf. Figs. 1 and 2 of reference 6). After a 24-h incubation three to five ghosts and 50–100 intact RBCs were attached to each organism. The ultrastructure of RBCs and ghosts adherent to schistosomula has been described (6).

The lateral mobilities of the major transmembrane proteins, band 3 and glycophorin, and a phospholipid analogue, FI-PE, were measured at 23°C in both adherent RBCs and ghosts. The diffusion coefficients of both transmembrane proteins and FI-PE were two- to threefold less in deformed RBCs adherent to the parasite surface than in control RBCs incubated under the same conditions. The fractional mobilities of deformed and control RBCs were not significantly different (Table I). RBC ghosts on the worm surface exhibited 100% immobilization of both glycophorin and band 3 and $\sim 50\%$ immobilization of FI-PE (Figs. 2 and 3; Table I). Since 100% of the membrane protein was immobilized, both adherent and non-adherent portions of the RBC ghost membranes must have been perturbed by the RBC-parasite interaction.

Hypotonically lysed RBC ghosts incubated in the absence of schistosomula for 24 h at 0°C manifested the slow protein diffusion and rapid lipid diffusion previously observed in intact RBCs and ghosts (5, 25, 28, 33, 34, 46). Unlike the ghosts found on the surface of schistosomula, these controls had fractional mobilities of $\sim 60\%$ for glycophorin, 20% for

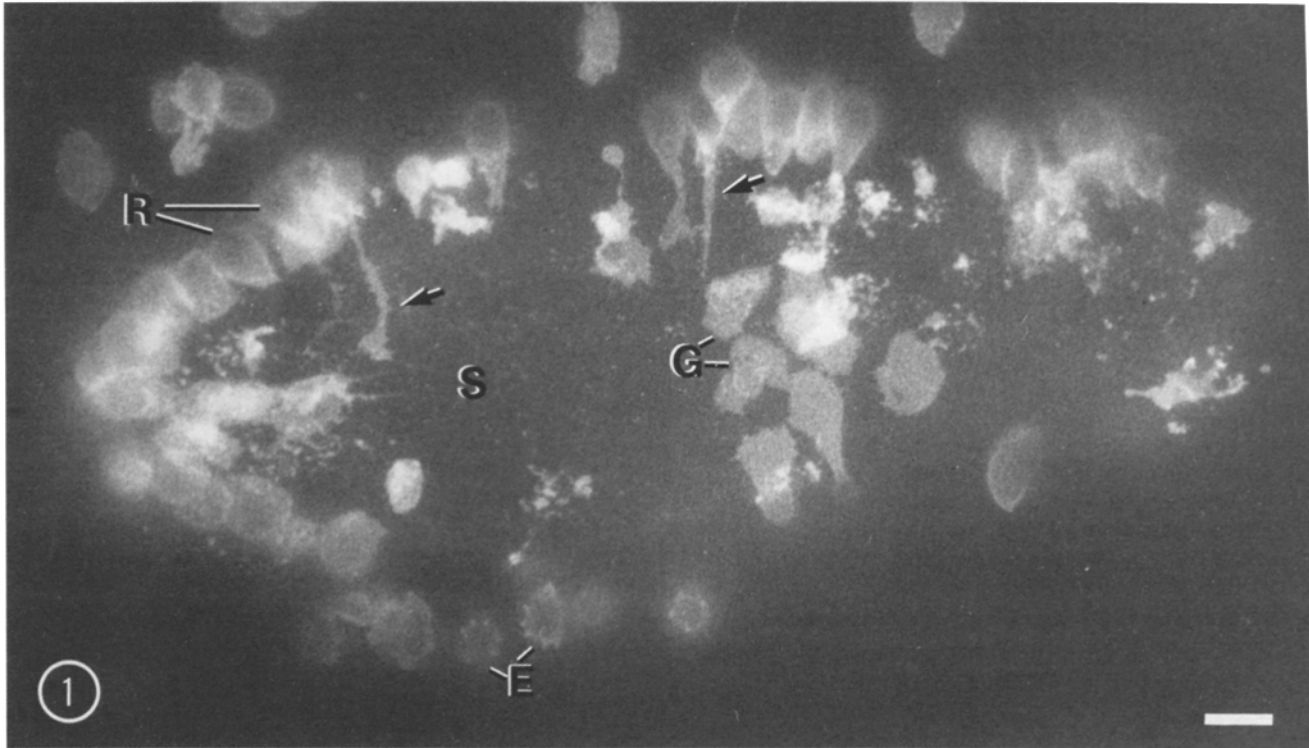


Figure 1. Fluorescence micrograph of a schistosomulum incubated with FTSC-labeled RBCs (*R*). Many of the adherent cells are deformed and attached to the parasite by tethers (*arrows*). Some cells are deformed into echinocytes (*E*) and others are lysed to form ghosts (*G*). Both echinocyte formation and lysis are observed after lysoPC treatment of intact RBCs. Bar, 10 μ m.

band 3, and 100% for FI-PE (Table I). Incubation of ghosts for 24 h at 37°C, conditions which resembled those of the RBC-worm incubation, led to 50-fold increases in the diffusion coefficients of the transmembrane proteins and increases in the fractional mobilities to 100% for glycophorin and 80% for band 3 (Table I). These increases are consistent with a relaxation of the cytoskeletal restrictions on the lateral mobility of transmembrane proteins seen in intact RBCs and ghosts incubated at 0°C (28, 34). In addition, these controls indicate that buffer and incubation conditions did not induce the immobilization of RBC membrane protein and lipid measured in ghosts adherent to schistosomula.

Cross-linking Agents Do Not Immobilize an RBC Membrane Lipid Probe

Cross-linking agents were tested on RBCs for their ability to duplicate the pattern of protein and lipid immobilization ob-

served in ghosts adherent to schistosomula. Diamide at a concentration of 2 mM has been shown to cross-link the cytoskeletal protein spectrin into a high molecular weight complex (30) and to cause a slowing of transmembrane protein diffusion in the RBC membrane (45). Both glycophorin and band 3 diffusion were slowed by 30–50% after treatment with 2 mM diamide (Table II), in agreement with earlier findings (45). Diamide had no effect, however, on the rate of FI-PE diffusion or on the fractional mobility of either protein or lipid. Treatment with 20 mM diamide yielded identical FPR results (data not shown), suggesting that the effects of 2 mM diamide were maximal. Intracellular cross-linking of the RBC cytoskeleton is unlikely to cause the immobilization of transmembrane proteins and FI-PE seen in ghosts adherent to schistosomula.

In an attempt to mimic the attachment of RBCs to the surface of schistosomula, RBCs were incubated on glass slides

Table I. Effect of Adherence to Schistosomula on Lateral Mobility of RBC Membrane Components

	Glycophorin			Band 3			FI-PE		
	<i>D</i>	<i>f</i>	<i>n</i>	<i>D</i>	<i>f</i>	<i>n</i>	<i>D</i>	<i>f</i>	<i>n</i>
Intact RBC control, 37°C	3.6 ± 1.7*	56 ± 12	10	2.3 ± 2.0	58 ± 20	17	410 ± 110	99 ± 6	13
Adherent deformed RBC	1.4 ± 0.5	71 ± 28	8	0.8 ± 0.5	50 ± 11	12	220 ± 80	85 ± 14	9
Adherent RBC ghost	—	2 ± 3	16	—	2 ± 3	12	160 ± 100	48 ± 9	15
RBC ghost control, 37°C	47 ± 21	98 ± 6	12	79 ± 57	78 ± 24	11	300 ± 90	95 ± 5	8
RBC ghost control, 0°C	1.1 ± 0.4	63 ± 20	15	1.4 ± 2.3	18 ± 19	8	340 ± 100	101 ± 5	8

Glycophorin was labeled with FTSC, band 3 with EMA, and RBCs with FI-PE. Schistosomula were mixed with fluorescently labeled RBCs and incubated for 24 h at 37°C. As controls, intact RBCs and hypotonically lysed RBC ghosts were incubated in the absence of worms for 24 h at either 37°C or 0°C. Lateral mobility at 23°C was measured by FPR. *D*, diffusion coefficient, $\times 10^{11}$ cm² s⁻¹. *f*, fractional mobility, %. *n*, number of measurements. —, *D* cannot be determined for *f* < 20%.

* Mean ± SD.

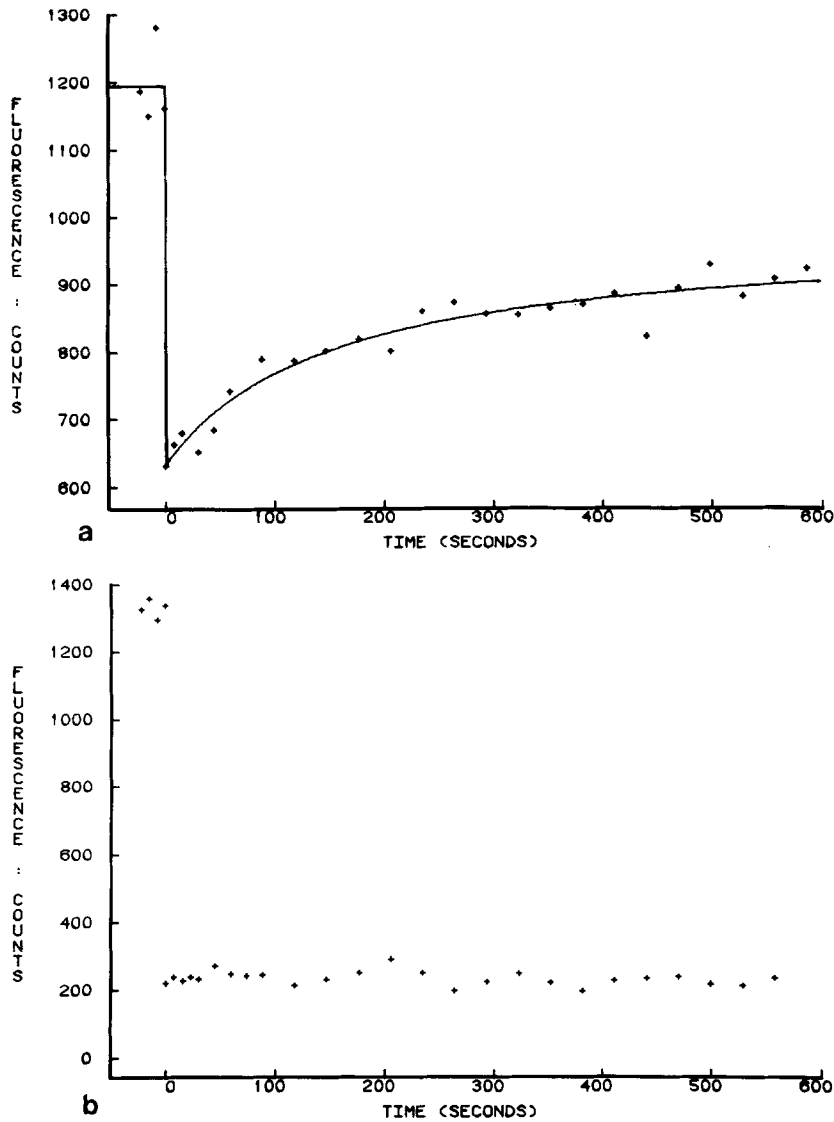


Figure 2. Representative FPR curves of glyophorin lateral mobility in intact control RBCs (a) and in RBC ghosts adherent to schistosomula (b). FTSC-labeled RBCs were incubated for 24 h at 37°C in the absence (a) and in the presence (b) of schistosomula. Crosses represent fluorescence counts at various times before and after the photobleaching pulse, which occurs at time = 0. Recovery curves are fit by nonlinear least squares analysis to theoretical curves for diffusion with a Gaussian laser beam. Adherent RBC ghosts exhibited complete immobilization of glyophorin. (a) Diffusion coefficient $D = 2.95 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$; fractional mobility $f = 59.8\%$. (b) $f = 0\%$.

coated with cross-linking reagents. Both polylysine, which primarily binds to glyophorin-linked sialic acid residues, and WGA, which primarily binds to glyophorin-linked *N*-acetylglucosamine residues (2), should cross-link transmembrane glyophorin molecules on the external surface of the RBC membrane. Treatment with both of these agents

lysed all RBCs adherent to the slide. As shown in Table II, polylysine and WGA partially immobilized glyophorin and band 3, each of which manifested a fractional mobility of ~5–20%. Fl-PE fractional mobility was unaffected. Con A, which primarily binds to band 3-linked mannose residues (16, 20) and should cross-link transmembrane band 3 mole-

Table II. Effect of Cross-linking Agents on Lateral Mobility of RBC Membrane Components

RBC Treatment	Glyophorin			Band 3			Fl-PE		
	<i>D</i>	<i>f</i>	<i>n</i>	<i>D</i>	<i>f</i>	<i>n</i>	<i>D</i>	<i>f</i>	<i>n</i>
Intact control	$2.3 \pm 1.3^*$	82 ± 15	29	2.1 ± 1.4	45 ± 17	31	300 ± 90	99 ± 5	16
Hypotonic lysis	1.8 ± 0.6	62 ± 15	8	1.1 ± 1.0	47 ± 17	7	300 ± 90	95 ± 5	8
Diamide, 2 mM	1.8 ± 0.7	92 ± 5	12	0.8 ± 0.4	52 ± 10	8	290 ± 80	97 ± 2	8
Polylysine slide	—	6 ± 10	7	—	8 ± 13	9	80 ± 30	87 ± 13	7
WGA slide	12 ± 7	21 ± 7	7	7 ± 6	16 ± 15	8	290 ± 80	94 ± 5	8
Con A slide	1.9 ± 0.4	65 ± 9	7	ND	ND		ND	ND	

Glyophorin was labeled with FTSC, band 3 with EMA, and RBCs with Fl-PE. Labeled RBCs were incubated under various conditions, as described in Materials and Methods. Lateral mobility was measured by FPR. All FPR measurements were performed at 23°C except those for glyophorin and band 3 mobility in the intact control and diamide-treated samples, which were performed at 37°C. *D*, diffusion coefficient, $\times 10^{11} \text{ cm}^2 \text{ s}^{-1}$. *f*, fractional mobility, %. *n*, number of measurements. ND, not done. —, *D* cannot be determined for $f < 20\%$.
* Mean \pm SD.

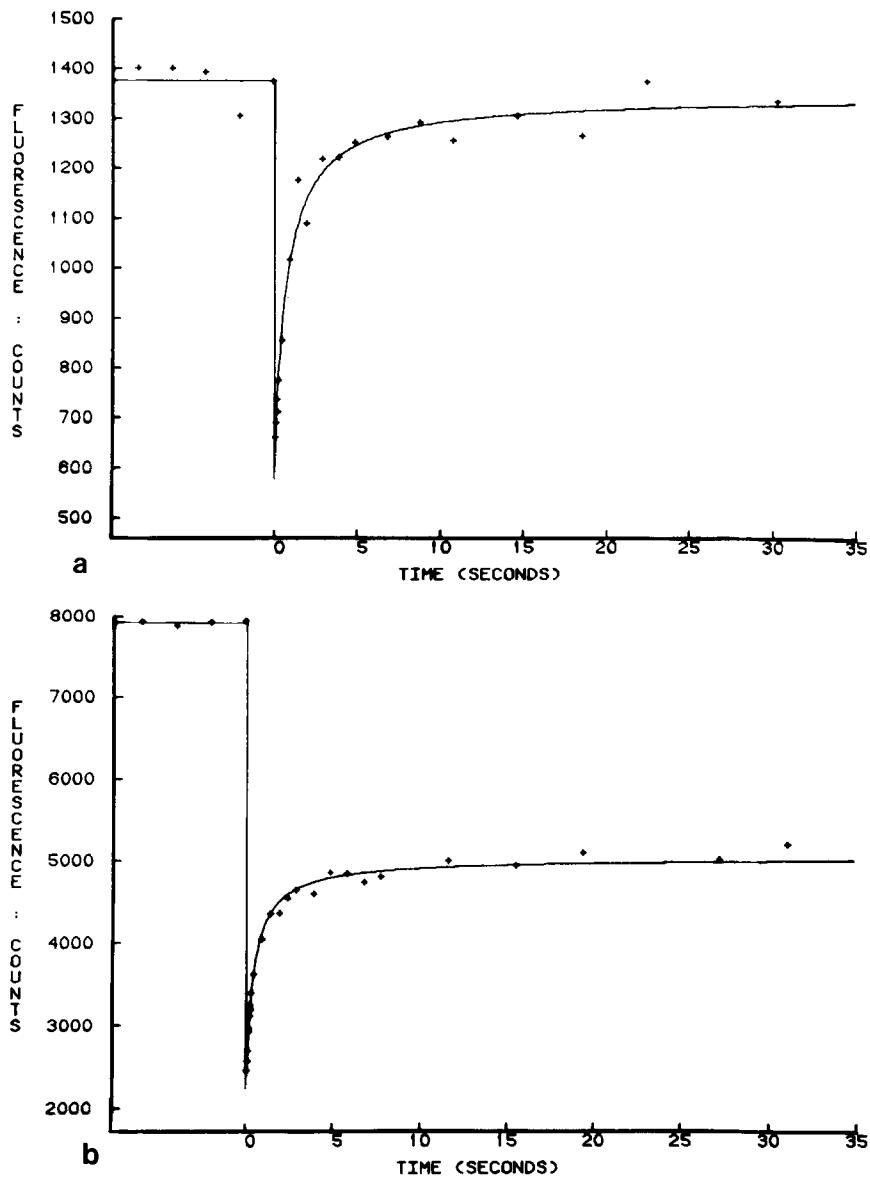


Figure 3. Representative FPR curves of FI-PE lateral mobility in intact control RBCs (a) and in RBC ghosts adherent to schistosomula (b). FI-PE-labeled RBCs were incubated for 24 h at 37°C in the absence (a) and in the presence (b) of schistosomula. Experimental data are presented and analyzed as described in the legend to Fig. 2. Adherent RBC ghosts exhibited significant immobilization of FI-PE. (a) $D = 4.21 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$; $f = 96.1\%$ (b) $D = 2.03 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$; $f = 49.0\%$.

cules on the extracellular membrane surface, lysed 10–50% of RBCs. It had no effect on glycoprotein diffusion (Table II). Extracellular cross-linking of RBC transmembrane proteins is unlikely to be the mechanism by which schistosomula induce protein and lipid immobilization.

Three Carbocyanine Dyes Are Immobilized in Ghosts Adherent to Schistosomula

Carbocyanine dyes have been used in FPR experiments on both the schistosome membrane (32) and the RBC membrane (5, 33, 46), and they have been shown to transfer from adherent RBCs to the parasite membrane (6). RBCs were labeled with diI_s having acyl chain lengths of 12, 14, and 18 carbons, and then incubated with schistosomula. All three dyes manifested fractional mobilities of 80–90% in deformed RBCs and 40–50% in ghosts adherent to the parasite (Table III). The fractional mobility of diI in control RBCs or RBC ghosts incubated in the absence of worms was 90–100% (Table III). The fractional mobility of diI in both adherent and control RBCs and RBC ghosts was similar to

that of FI-PE (Table I). The diffusion coefficient of diI in RBC controls (Table III) was between two- and fivefold greater than that of FI-PE in control cells (Table I). This difference may have been caused either by the different incubation times at 37°C of the two preparations (48 h and 24 h, respectively) or by the diI(3) probes themselves, which may disrupt membrane architecture (5, 33). The lateral mobility of diI was also measured in schistosome membranes in which dye had been acquired either through incubation with labeled RBCs or through direct labeling. The fractional mobility of all three diI species was 65–80%, and was independent of the method by which the parasite acquired the dye (Table III). Similarly, the diffusion coefficients of diI-C14 and diI-C18 were not significantly different in the two schistosome preparations (Table III).

LysoPC Induces Lysis of RBCs and Causes a Concentration-dependent Immobilization of RBC Membrane Components

Since intracellular and extracellular RBC membrane protein

Table III. Effect of Adherence to Schistosomula on Lateral Mobility of Carbocyanine Dyes in RBCs

	dil-C12(3)			dil-C14(3)			dil-C18(3)		
	<i>D</i>	<i>f</i>	<i>n</i>	<i>D</i>	<i>f</i>	<i>n</i>	<i>D</i>	<i>f</i>	<i>n</i>
Intact RBC control	20.1 ± 10.5*	95 ± 7	3	ND	ND		19.6 ± 5.4	94 ± 2	3
Adherent deformed RBC	ND	ND		7.4 ± 4.4	81 ± 11	4	2.1 ± 1.6	89 ± 7	5
Adherent RBC ghost	5.2 ± 3.6	48 ± 13	6	2.8 ± 3.7	54 ± 21	7	2.4 ± 0.8	44 ± 12	6
RBC ghost control	7.2 ± 1.1	102 ± 4	3	9.3 ± 3.2	95 ± 5	9	8.3 ± 3.1	94 ± 5	3
Schistosomula membrane (acq.)	12.9 ± 7.5	66 ± 1	3	3.5 ± 1.2	82 ± 7	3	6.4 ± 4.9	81 ± 11	3
Schistosomula membrane (dir.)	ND	ND		2.0 ± 0.9	69 ± 15	42	1.1 ± 0.8	67 ± 18	16

Schistosomula were mixed with dil-labeled RBCs and incubated for 48 h at 37°C. Lateral mobility at 23°C was measured by FPR. The lateral mobility of dil acquired by the worm membrane during the incubation with labeled RBCs is shown (acq.), as well as that of dil incorporated directly into the worm membrane (dir.). *D*, diffusion coefficient, $\times 10^9 \text{ cm}^2 \text{ s}^{-1}$. *f*, fractional mobility, %. *n*, number of measurements. ND, not done.

* Mean ± SD.

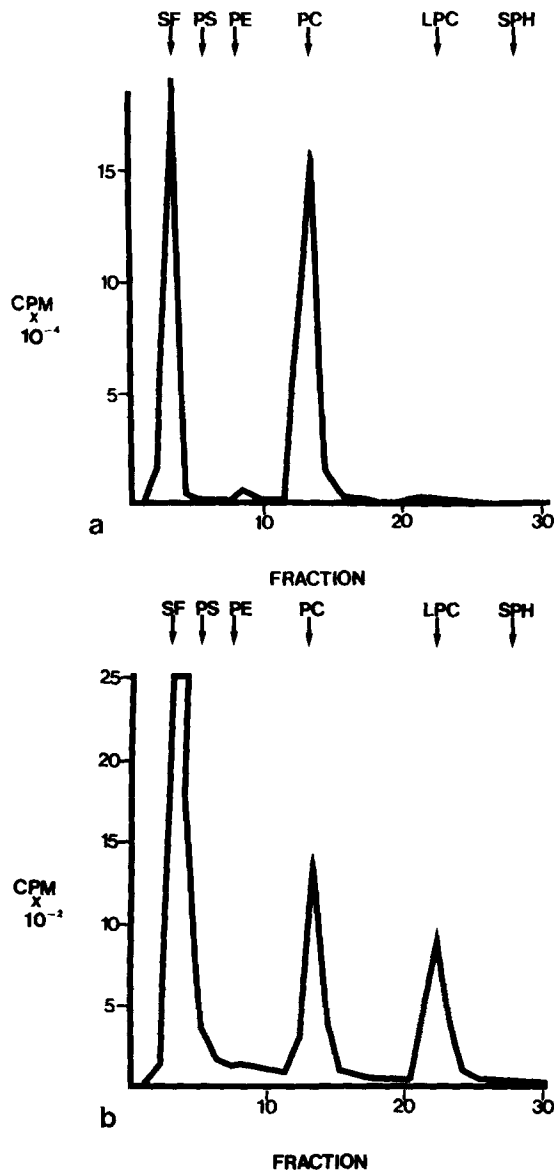


Figure 4. High performance liquid chromatography chromatograms of ^3H -palmitate-labeled lipid from intact schistosomula (a) and culture medium (b). Labeled lipid was eluted from a 10- μm silica cartridge ($\mu\text{Porasil}$) with acetonitrile/methanol/85% phosphoric acid (130/6/1.5) at a flow rate of 4.0 ml/min. Fractions were collected at 30-s intervals and radioactivity quantitated by liquid scintillation counting. Lipid standards were detected by ultraviolet absorption at 214 nm. Schistosomula released significant quantities of lysoPC into the incubation medium.

cross-linking techniques could not duplicate the immobilization of lipid probes in RBC ghosts adherent to schistosomula, we examined the effects of lysoPC on RBCs. This lysoPC agent directly perturbs the RBC lipid bilayer and therefore might immobilize membrane lipid as well as transmembrane protein. Low concentrations of egg lysoPC (1.0–1.5 $\mu\text{g}/\text{ml}$) caused lysis of 50% of intact RBCs after 1-min incubation at room temperature. Higher concentrations of egg lysoPC ($\geq 4 \mu\text{g}/\text{ml}$) caused lysis of 100% of intact RBCs. In intact discocytes incubated in the absence of lysoPC, glycophorin and band 3 had identical diffusion coefficients, $D = 1\text{--}2 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$, and fractional mobilities (*f*) of 68 and 44%, respectively. Control values for Fl-PE mobility were $D = 5 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ and $f = 96\%$ (Table IV). As the concentration of egg lysoPC was increased from 0 to 8.4 $\mu\text{g}/\text{ml}$, there was a monotonic decrease in the fractional mobility of glycophorin, without change in the diffusion coefficient of the mobile fraction. Complete immobilization of both glycophorin and band 3 ($f < 10\%$) was observed at a lysoPC concentration of 8.4 $\mu\text{g}/\text{ml}$ (Table IV). Both the diffusion coefficient and the fractional mobility of Fl-PE decreased monotonically over the same concentration range, although $\sim 40\%$ of the lipid probe remained mobile at a lysoPC concentration, 8.4 $\mu\text{g}/\text{ml}$, which had totally immobilized the transmembrane proteins. A higher concentration of egg lysoPC, 16.8 $\mu\text{g}/\text{ml}$, induced complete immobilization of the lipid probe as well (Table IV). These effects were not a direct consequence of lysoPC-induced RBC lysis, since identical results were obtained upon lysoPC incubation of RBC ghosts which had been produced by osmotic lysis (data not shown).

Schistosomula Synthesize LysoPC and Release It into the Culture Medium

Independent experiments were performed to determine whether schistosomula metabolize labeled palmitate to lysoPC. Fig. 4, a and b show typical chromatograms of ^3H -phospholipids in cultured schistosomula and in the culture medium, respectively, after incubation for 90 min with ^3H -palmitate and culture for 6 h in cold medium. In the worms one major phospholipid peak was observed and this peak co-eluted with a PC standard. In the culture medium there were two major phospholipid peaks and these co-eluted with PC and lysoPC standards. At this time point lysoPC comprised $< 2\%$ of the total labeled phospholipid in intact schistosomula, while it was 34% of the total labeled phospholipid in the culture medium. The rate of appearance of labeled

Table IV. Effect of Egg LysoPC on Lateral Mobility of RBC Membrane Components

LysoPC μg/ml	Glycophorin			Band 3			Fl-PE		
	D	f	n	D	f	n	D	f	n
0	1.2 ± 0.3*	68 ± 5	5	1.6 ± 0.5	44 ± 16	6	510 ± 190	96 ± 4	10
2.1	1.2 ± 0.7	35 ± 13	9	ND	ND		310 ± 100	87 ± 9	8
4.2	1.2 ± 0.6	22 ± 6	6	ND	ND		230 ± 170	79 ± 14	8
8.4	—	9 ± 7	9	—	3 ± 6	18	140 ± 120	37 ± 27	10
16.8	ND	ND		ND	ND		—	3 ± 5	8

Egg lysoPC was incubated with fluorescently labeled RBCs for 1 min at room temperature in RPMI, and lateral mobility at 23°C was measured by FPR. D, diffusion coefficient, $\times 10^{11}$ cm² s⁻¹. f, fractional mobility, %. n, number of measurements. ND, not done. —, D cannot be determined for $f < 20\%$.

* Mean ± SD.

lysoPC in the culture medium was linear up to 12 h. In three separate experiments an average of 1.5 ± 0.3 fmol lysoPC/h per 10^3 organisms (mean ± SD) was released into the culture medium.

Discussion

The present studies demonstrate that RBC ghosts adherent to schistosomula exhibit complete immobilization of two integral membrane proteins, glycophorin and band 3. Two lipid probes, fluorescein-phosphatidylethanolamine and diI, are partially immobilized. These findings are not duplicated by membrane protein cross-linking techniques. An identical pattern of protein and Fl-PE immobilization is found in RBCs lysed with egg lysoPC at a concentration of 8.4 μg/ml. Finally, parasites incubated with labeled palmitate release lysoPC into the culture medium at a rate of 1.5 fmol/h per 10^3 organisms. Taken together, these data suggest that lysoPC is transferred from schistosomula to adherent RBCs, causing their lysis.

Lysed RBCs adhere to schistosomula via 10–20-nm thick electron-dense plaques, but most of the ghost membrane is not directly attached to the parasite outer membrane (6). The finding that glycophorin and band 3 are completely immobilized in the adherent ghosts suggests that the entire RBC membrane is affected, in both attached and unattached regions. Further, the slowed diffusion of transmembrane proteins and Fl-PE in deformed, adherent RBCs implies that the more extreme lateral mobility changes seen in adherent ghosts are the result of a progressive process initiated by contact between parasite and RBC. Possible mechanisms for such a process include transmembrane protein and lipid interactions with either cytoskeletal proteins or extracellular ligands, transmembrane protein aggregation, and lateral phase separation of membrane lipid. Here, diamide-mediated cytoskeletal cross-linking does not immobilize RBC membrane protein or Fl-PE, although it slows the lateral diffusion of both band 3 and glycophorin. Extracellular cross-linking ligands such as polylysine and WGA also do not decrease the fractional mobility of Fl-PE, although they immobilize transmembrane proteins and slow the lateral diffusion of the lipid probe. In contrast, egg lysoPC induces a concentration-dependent immobilization of both transmembrane proteins and Fl-PE in RBC ghosts. LysoPC at a concentration of 8.4 μg/ml leads to ~50% immobilization of Fl-PE, duplicating the changes observed in ghosts adherent to schistosomula. Thus, lysoPC is most likely the agent responsible for RBC

membrane component immobilization and RBC lysis in the present study.

LysoPC is probably a component of the outer tegumental membrane of schistosomula which transfers directly into the plasma membrane of attached RBCs to produce lysis. Exchange of lysoPC between membranes of different cell types has been observed in other systems (48). When RBC adherence to schistosomula was promoted with lectins, lysis occurred within 1 h (6). Close apposition of parasite and RBC membranes is apparently critical for lysis. In the present studies both lysis of RBCs and immobilization of RBC membrane components are seen only in attached RBCs, again implying that the critical interaction occurs at the parasite surface. Furthermore, while the rate of release of lysoPC by schistosomula is too low to attain lytic concentrations in the medium, it is adequate to achieve a lytic amount of lysoPC at the worm surface. In a 24-h incubation each somule releases $\sim 2 \times 10^7$ labeled lysoPC molecules into the medium. In comparison, $\sim 2 \times 10^7$ lysoPC molecules per RBC are required for lysis (49), and $1-10 \times 10^6$ lysoPC molecules per RBC are needed to produce echinocytes (18, 35, 38). Since the labeled palmitate experiments used a pulse rather than continuous labeling and are not corrected for pool size, it is likely that each somule produces enough lysoPC in a 24-h incubation to lyse three to five RBCs on its surface.

Labeled lysoPC is found in large amounts relative to PC in the culture medium, but in only trace quantities in total worm lipid. Labeled phospholipids in the culture medium may reflect the phospholipid composition of the outer (and, possibly, the inner) tegumental membrane, since surface labeled glycoproteins and glycolipids are shed by schistosomula into the medium (41). Parasite glands may also discharge lipid into the medium, although the effects of such lipids would presumably be manifested in non-adherent as well as adherent RBCs. The metabolic labeling is therefore consistent with the notion that lysoPC is present in the outer membrane, from which it can be transferred into a juxtaposed membrane or shed into the medium.

The presence of lysoPC on the parasite surface may explain interactions previously observed between schistosomes and mammalian cells. First, lysis is seen in other cells besides RBCs. Human eosinophils, which can kill the parasite by degranulation, often lyse on the parasite surface before they exocytose (11). Second, the membrane fusion that occurs between human neutrophils and the outer tegumental membrane (8–10) may be induced by lysoPC, which is a known

fusogen. Third, rat peritoneal mast cells adherent to schistosomula by means of antibody or complement receptors do not degranulate (7). It has recently been shown that lysoPC linearly inhibits compound 48/80-induced degranulation of rat mast cells (37), although other parasite molecules may also inhibit mast cell secretion (36). Fourth, the detergent properties of lysoPC may explain how both the shedding and the endocytosis by neutrophils of parasite surface proteins occur without damage to the outer membrane (10, 42).

There are three generalizations that can be made concerning the role of lysoPC in immunological reactions directed against the parasite. First, lysoPC can cause lysis and can also alter the cell plasma membrane, thereby neutralizing attacking host cells. Second, lysoPC may promote membrane fusion with host cells resulting in the acquisition of host membrane components by the parasite. Finally, the presence of lysoPC may alter antigen presentation and immune recognition of parasite antigens. These three properties may represent a central mechanism by which the parasite escapes host immune responses.

We thank Adam Abrams for expert technical assistance, Ann Hein and Drs. Donald Harn and Alan Kleinfeld for critical review of the manuscript, and Joanne Miccile for expert typing of the manuscript.

This work was supported by National Institutes of Health grants AI-15311, HL-32854, AI-19581, and AI-23083. D. E. Golan is a Fellow of The Medical Foundation, Inc., Boston, Massachusetts. Portions of this work were presented at the 26th Annual Meeting of the American Society of Hematology (26) and the 70th Annual Meeting of the Federation of the American Societies for Experimental Biology (19).

This work is dedicated to the memory of Dr. William R. Veatch.

Received for publication 11 March 1986, and in revised form 19 May 1986.

References

1. Abraham, G., and P. S. Low. 1980. Covalent labeling of specific membrane carbohydrate residues with fluorescent probes. *Biochim. Biophys. Acta.* 597:285-291.
2. Adair, W. L., and S. Kornfeld. 1974. Isolation of receptors for wheat germ agglutinin and the Ricinus communis lectins from human erythrocytes using affinity chromatography. *J. Biol. Chem.* 249:4676-4704.
3. Axelrod, D., D. E. Koppel, J. Schlessinger, E. Elson, and W. W. Webb. 1976. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophys. J.* 16:1055-1069.
4. Bevington, P. R. 1969. *Data Reduction and Error Analysis for the Physical Sciences.* McGraw-Hill, Inc., New York. 336 pp.
5. Bloom, J. A., and W. W. Webb. 1983. Lipid diffusibility in the intact erythrocyte membrane. *Biophys. J.* 42:295-305.
6. Caulfield, J. P., and C. M. L. Cianci. 1985. Human erythrocytes adhering to schistosomula of *Schistosoma mansoni* lyse and fail to transfer membrane components to the parasite. *J. Cell Biol.* 101:158-166.
7. Caulfield, J. P., A. Hein, G. Moser, and A. Sher. 1981. Light and electron microscopic appearance of rat peritoneal mast cells adhering to schistosomula of *Schistosoma mansoni* by means of complement or antibody. *J. Parasitol.* 67:776-783.
8. Caulfield, J. P., G. Korman, A. E. Butterworth, M. Hogan, and J. R. David. 1980. The adherence of human neutrophils and eosinophils to schistosomula: evidence for membrane fusion between cells and parasites. *J. Cell Biol.* 86:46-63.
9. Caulfield, J. P., G. Korman, A. E. Butterworth, M. Hogan, and J. R. David. 1980. Partial and complete detachment of neutrophils and eosinophils from schistosomula: evidence for the establishment of continuity between fused and normal parasite membrane. *J. Cell Biol.* 86:64-76.
10. Caulfield, J. P., G. Korman, and J. C. Samuelson. 1982. Human neutrophils endocytose multivalent ligands from the surface of schistosomula of *S. mansoni* before membrane fusion. *J. Cell Biol.* 94:370-378.
11. Caulfield, J. P., H. L. Lenzi, P. Elsas, and A. J. Dessein. 1985. Ultrastructure of the attack of eosinophils stimulated by blood mononuclear cell products on schistosomula of *Schistosoma mansoni*. *Am. J. Pathol.* 120:380-390.
12. Chen, S. S., and A. Y. Kou. 1982. Improved procedure for the separation of phospholipids by high performance liquid chromatography. *J. Chromatog.* 227:25-31.
13. Cherry, R. J., E. A. Nigg, and G. S. Beddard. 1980. Oligosaccharide motion in erythrocyte membranes investigated by picosecond fluorescence polarization and microsecond dichroism of an optical probe. *Proc. Natl. Acad. Sci. USA.* 77:5899-5903.
14. Dean, D. A. 1974. *Schistosoma mansoni*: adsorption of human blood group A and B antigens by schistosomula. *J. Parasitol.* 60:260-263.
15. Dean, D. A., and K. W. Sell. 1972. Surface antigens on *Schistosoma mansoni*. II. Adsorption of a Forssman-like host antigen by schistosomula. *Clin. Exp. Immunol.* 12:525-540.
16. Findlay, A. 1974. The receptor proteins for concanavalin A and *Lens culinaris* phytohemagglutinin in the membrane of the human erythrocyte. *J. Biol. Chem.* 249:4378-4403.
17. Folch, J., M. Lees, and G. H. Sloan-Stanley. 1957. A simple method for isolation and purification of total lipid from animal tissues. *J. Biol. Chem.* 226:497-509.
18. Fujii, T., and A. Tamura. 1983. Dynamic behavior of amphiphilic lipids to penetrate into membrane of intact human erythrocytes and to induce change in the cell shape. *Biomed. Biochim. Acta.* 11/12:S81-S85.
19. Furlong, S. T., and J. P. Caulfield. 1986. Characterization of lipid synthesized by cultured schistosomula of *Schistosoma mansoni*. *Fed. Proc.* 45:623. (Abstr.)
20. Furthmayr, H., I. Kahane, and V. T. Marchesi. 1976. Isolation of the major intrinsic transmembrane protein of the human erythrocyte. *J. Membr. Biol.* 26:173-187.
21. Furthmayr, H., M. Tomita, and V. T. Marchesi. 1975. Fractionation of the major sialoglycopeptides of the human red blood cell membrane. *Biochem. Biophys. Res. Commun.* 65:113-121.
22. Gahmberg, C. G., and L. C. Andersson. 1977. Selective radioactive labeling of cell surface sialoglycoproteins by periodate-tritiated borohydride. *J. Biol. Chem.* 252:5888-5894.
23. Gitter, B. D., and R. T. Damian. 1982. Murine alloantigen acquisition by schistosomula of *Schistosoma mansoni*: further evidence for the presence of K, D, and I region gene products on the tegumental surface. *Parasite Immunol. (Oxf.)* 4:383-393.
24. Gitter, B. D., S. L. McCormick, and R. T. Damian. 1982. Murine alloantigen acquisition by *Schistosoma mansoni*: presence of H-2K determinants on adult worms and failure of allogeneic lymphocytes to recognize acquired MHC gene products on schistosomula. *J. Parasitol.* 68:513-518.
25. Golan, D. E., M. R. Alecio, W. R. Veatch, and R. R. Rando. 1984. Lateral mobility of phospholipid and cholesterol in the human erythrocyte membrane: effects of protein-lipid interactions. *Biochemistry.* 23:332-339.
26. Golan, D. E., C. S. Brown, C. M. Cianci, and J. P. Caulfield. 1984. *Schistosoma mansoni* induces lysis of human erythrocytes and immobilization of glycophorin. *Blood.* 64:26a. (Abstr.)
27. Golan, D. E., P. A. Singer, and W. R. Veatch. 1984. Calcium-induced immobilization of glycophorin in the human erythrocyte membrane. *Biophys. J.* 45:202a. (Abstr.)
28. Golan, D. E., and W. Veatch. 1980. Lateral mobility of band 3 in the human erythrocyte membrane studied by fluorescence photobleaching recovery: evidence for control by cytoskeletal interactions. *Proc. Natl. Acad. Sci. USA.* 77:2537-2541.
29. Goldring, O. L., J. A. Clegg, S. R. Smithers, and R. J. Terry. 1976. Acquisition of human blood group antigens by *Schistosoma mansoni*. *Clin. Exp. Immunol.* 26:181-187.
30. Haest, C. W. M., D. Kamp, G. Plasa, and B. Deuticke. 1977. Intra- and intermolecular cross-linking of membrane proteins in intact erythrocytes and ghosts by SH-oxidizing agents. *Biochim. Biophys. Acta.* 469:226-230.
31. Hockley, D. J., and D. J. McLaren. 1979. *Schistosoma mansoni*: changes in the outer membrane of the tegument during development from cercaria to adult worm. *Int. J. Parasitol.* 3:13-25.
32. Johnson, P., P. B. Garland, P. Campbell, and J. R. Kusel. 1982. Changes in the properties of the surface membrane of *Schistosoma mansoni* during growth as measured by fluorescence recovery after photobleaching. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 141:132-135.
33. Kapitza, H.-G., and E. Sackmann. 1980. Local measurement of lateral motion in erythrocyte membranes by photobleaching technique. *Biochim. Biophys. Acta.* 595:56-64.
34. Koppel, D. E., M. P. Sheetz, and M. Schindler. 1981. Matrix control of protein diffusion in biological membranes. *Proc. Natl. Acad. Sci. USA.* 78:3576-3580.
35. Lange, Y., and J. M. Slayton. 1982. Interaction of cholesterol and lysophosphatidylcholine in determining red cell shape. *J. Lipid Res.* 23:1121-1127.
36. Mazingue, C., D. Camus, J. P. Dessaint, M. Capron, and A. Capron. 1980. In vitro and in vivo inhibition of mast cell degranulation by a factor from *Schistosoma mansoni*. *Int. Arch. Allergy Appl. Immunol.* 63:178-189.
37. Mio, M., A. Ikeda, M. Akagi, and K. Tasaka. 1985. Inhibitory effect of lysophosphatidylcholine on the histamine release from rat peritoneal mast cells. *Agents Actions.* 16:113-117.
38. Mohandas, N., A. C. Greenquist, and S. B. Shohet. 1978. Bilayer balance and regulation of red cell shape changes. *J. Supramol. Struct.* 9:453-458.
39. Nigg, E. A., and R. J. Cherry. 1979. Influence of temperature and cholesterol on the rotational diffusion of band 3 in the human erythrocyte membrane. *Biochemistry.* 18:357-365.
40. Ramalho-Pinto, F. J., G. Gazzinelli, R. E. Howells, T. A. Mota-Santos, E. A. Figueiredo, and J. Pelligrino. 1974. *Schistosoma mansoni*: defined system for a step-wise transformation of cercariae to schistosomula in vitro. *Exp.*

Parasitol. 36:360-372.

41. Samuelson, J. C., and J. P. Caulfield. 1982. Loss of covalently labeled glycoproteins and glycolipids from the surface of newly transformed schistosomula of *S. mansoni*. *J. Cell Biol.* 94:363-369.

42. Samuelson, J. C., J. P. Caulfield, and J. R. David. 1982. Schistosomula of *Schistosoma mansoni* clear concanavalin A from their surface by sloughing. *J. Cell Biol.* 94:355-362.

43. Sher, A., B. F. Hall, and M. A. Vadas. 1978. Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. *J. Exp. Med.* 148:46-57.

44. Simpson, A. J. G., D. Singer, T. F. McCutchan, D. L. Sacks, and A. Sher. 1983. Evidence that schistosome MHC antigens are not synthesized by the parasite but are acquired from the host as intact glycoproteins. *J. Immunol.* 131:3315-3330.

45. Smith, D. K., and J. Palek. 1982. Modulation of lateral mobility of band 3 in the red cell membrane by oxidative cross-linking of spectrin. *Nature*

(*Lond.*). 297:424-425.

46. Thompson, N. L., and D. Axelrod. 1980. Reduced lateral mobility of a fluorescent lipid probe in cholesterol-depleted erythrocyte membranes. *Biochim. Biophys. Acta.* 597:155-165.

47. Torpier, G., M. Capron, and A. Capron. 1977. Structural changes of the tegumental membrane complex in relation to developmental stages of *Schistosoma mansoni* (Platyhelminthes: Trematoda). *J. Ultrastruct. Res.* 61:309-324.

48. Weltzien, H. U. 1979. Cytolytic and membrane-perturbing properties of lysophosphatidylcholine. *Biochim. Biophys. Acta.* 559:259-287.

49. Weltzien, H. U., B. Arnold, and R. Reuther. 1977. Quantitative studies on lysolecithin-mediated hemolysis: use of ether-deoxy lysolecithin analogs with varying aliphatic chain-lengths. *Biochim. Biophys. Acta.* 466:411-421.

50. Yguerabide, J., J. A. Schmidt, and E. E. Yguerabide. 1982. Lateral mobility in membranes as detected by fluorescence recovery after photobleaching. *Biophys. J.* 39:69-75.