Involvement of Spectrin and ATP in Infection of Resealed Erythrocyte Ghosts by the Human Malarial Parasite, *Plasmodium falciparum*

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ABSTRACT Resealed erythrocyte ghosts were prepared under different experimental conditions and were tested in vitro for susceptibility to infection with the human malarial parasite, *Plasmodium falciparum*. Resealed ghosts, prepared by dialyzing erythrocytes in narrow membrane tubing against low ionic strength buffer that was supplemented with magnesium ATP, were as susceptible to parasite infection as were normal erythrocytes. There was a direct correlation between intraerythrocytic ATP content and susceptibility to parasite infection. Neither $MgCl_2$ nor sodium ATP could be substituted for magnesium ATP in maintaining high intraerythrocytic ATP concentration. When resealed ghosts were loaded with antispectrin IgG, malaria merozoite invasion was inhibited. At an average intracellular antispectrin IgG concentration of 3.5 $\mu g/10^8$ cells, there was a 35% inhibition of parasite invasion. This inhibition was due to spectrin crosslinking within the resealed ghosts, since the monovalent, Fab' fragments of antispectrin IgG had no inhibitory effect on invasion. These results indicate that the cytoskeleton plays a role in the complex process of merozoite entry into the host erythrocyte.

There have been several recent reports on the mechanism of entry of malaria merozoites into erythrocytes (1–6). This process of infection has been shown to involve attachment of the apical end of the merozoite to the host erythrocyte membrane, most likely by specific receptors (4–7). At the point of attachment, an electron-dense junction forms between the merozoite and the red cell membrane and, subsequently, the host membrane invaginates around the entering merozoite (8). As the merozoite invades, the junction moves along the orifice of the invaginating membrane by what has been proposed as a "modified zippering" type of endocytosis (8). Freeze-fracture studies of merozoites in the process of invasion have shown changes in the organization of transmembrane proteins within the invaginating host cell membrane (2, 9); its P face becomes depleted of intramembrane particles (IMPs) and clusters of IMPs appear at the moving junction.

Although the red cell cytoskeleton is known to play a major role in erythrocyte shape, membrane deformability, and receptor distribution (10–12), the role of cytoskeletal components in merozoite invasion has not been examined. Resealed ghosts offer a good experimental system for perturbation of intraerythrocytic components. Recent studies have shown that merozoites of the human malarial parasite, *Plasmodium falciparum*, are capable of infecting resealed ghosts (13, 14), although with a lower efficiency than they do normal erythrocytes. In this study we describe a modified method for the preparation of resealed ghosts which are as susceptible to infection with *P. falciparum* as are intact erythrocytes. This system has enabled us to test whether the major component of the erythrocyte cytoskeleton, spectrin plays a role in infection by *P. falciparum*.

MATERIALS AND METHODS

Preparation of Resealed Ghosts

Using a modification of the gradual hemolysis procedure (15), four types of resealed ghosts were prepared: (*a*) without ATP supplement (RG), (*b*) supplemented with 2.0 mM $MgCl_2$ (Mg-RG), (*c*) supplemented with 2.0 mM sodium ATP (NaATP-RG), and (*d*) supplemented with 2.0 mM magnesium ATP (MgATP-RG). Whole blood in citrate-phosphate-dextrose (CPD) was washed twice in 10 volumes of RPMI-1640-HEPES (RP) (1060 *g*, 5 min) to remove plasma and buffy coat. After a third wash in Dulbecco's phosphate-buffered saline (PBS, Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) with or without magnesium and/or ATP supplement (pH 7.4), an equal

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volume of the same PBS was added to the cell pellet. Cell suspensions were transferred to autoclaved dialysis tubing and dialyzed for 3 h at 4°C on a horizontal rocker (25 rpm) against 100 volumes of lysis buffer (10 mM sodium phosphate with or without magnesium and/or ATP supplement, pH 7.4). Two types of dialysis tubing were used for the cell lysis: Spectra/Por 2 (12,000–14,000 mwco, 10 mm flat width, Spectrum Medical Industries, Inc., Los Angeles, CA) or Spectra/Por semi-micro tubing (12,000–14,000 mwco, 4 mm flat width). Lysates were transferred to test tubes at 4°C and isotonicity was restored by rapid addition of concentrated KCl as the lysates were vortexed. After incubation at 37°C for 30 min, samples were washed twice in 10 volumes of RP (1060 *g*, 5 min). An aliquot of washed erythrocytes in RP was removed prior to lysis for use as normal cells.

Incubation of Resealed Ghosts and Normal Erythrocytes

Cells were suspended in RPMI-1640-HEPES, 10% human serum (RPS) at 5 × 10^8 cells/ml and plated in Linbro miniwell plates (Flow General, Inc., Hamden, CT). Cells for ATP and invasion measurements were incubated under identical conditions (16), although parasites were not added to cells used for ATP determination. Culture medium was changed every 4 h.

ATP Measurements

ATP was measured by the firefly extract method (17) using a liquid scintillation counter (18).

Preparation of *P. falciparum*

The FCR3/K+ strain of *P. falciparum* was cultivated in vitro by the method of Jensen and Trager (16) and maintained in synchronous growth within a 6-h window of the 48-h life cycle (19). To initiate infection, schizont-infected cells were enriched from cultures using gelatin separation (20), counted from a hemocytometer and a blood smear, and added to resealed ghosts or normal erythrocytes to give a 1% parasitemia. After 15-h incubation, cells were collected from plates, centrifuged at 600 *g* for 5 min, and adjusted to ~50% cell suspension. Blood smears from the suspension were stained with Giemsa stain, and ring-stage parasite/total number of cells was counted. For each cell type in each experiment, at least 9,000 cells were counted from triplicate cultures.

Preparation of Antispectrin Antibodies

Spectrin was purified from human erythrocytes by the method of Ungewickell and Gratzer (21) and used to immunize New Zealand White rabbits (12). Normal and immune IgG were purified from rabbit serum (22), and antispectrin IgG was separated from total immune IgG by affinity chromatography on spectrin-coupled Sepharose 4B (12). Fab' fragments were prepared (23) and both IgG and Fab' were radioiodinated by the chloramine T method (24). Antibody concentration and specific radioactivity were calculated from measurements of absorbance at 280 nm and CPM/μl in a gamma counter.

Antibody Loading of Resealed Ghosts

Erythrocytes were washed as described above and an equal volume of antibody (0.5–2.0 mg/ml) in PBS was added to the washed cell pellet. Cells were lysed by dialysis in Spectra/Por semi-micro tubing against lysis buffer supplemented with 2.0 mM magnesium ATP. Ghosts were resealed and washed as described above. The average amount of loaded antibody was calculated as follows: CPM per 10^8 cells/CPM per μg antibody. Parasites were added and incubated as described above.

Evaluation of Resealed Ghost Heterogeneity

Erythrocytes were washed as described above and an equal volume of fluorescent antibody (F.I.T.C.-conjugated anti-goat IgG prepared in rabbit, Miles-Yeda Ltd., Rehovot, Israel, code no. 65-176) at 2.0 mg protein/ml in PBS was added to the washed cell pellet. Cells were lysed by dialysis against lysis buffer supplemented with magnesium ATP in (*a*) Spectra/Por 2 dialysis tubing or (*b*) Spectra/Por semi-micro tubing. Ghosts were resealed and washed as described above. Analysis of resealed ghosts was performed using a Becton-Dickinson FAC II fluorescence-activated cell sorter (Becton, Dickinson & Co., Rutherford, NJ) equipped with a 5-W argon-ion laser (laser power—300 mW). Instrument parameters used were: photomultiplier voltage-750 V, light scatter pre-amplifier gain-4/1.0, and fluorescence pre-amplifier gain-4/0.5. The instrument was standardized with 10-μm fluorescent microspheres (Coulter Electronics Inc., Hialeah, FL) and operated at a flow rate of 2,800 particles/s. As an additional test of heterogeneity in antibody loading, acetone-fixed blood smears of normal rabbit IgG-loaded resealed ghosts and control resealed ghosts were stained by indirect immunofluorescence and examined by light and fluorescence microscopy.

RESULTS

Preparation of Resealed Ghosts

In a series of experiments, ghosts were prepared by lysis of erythrocytes in Spectra/Por 2 dialysis tubing (SP2) suspended in hypotonic buffer, with or without sodium ATP (NaATP). When these ghosts were resealed and used as host cells for *Plasmodium falciparum*, the suspectibility of both NaATP-RG-SP2 and RG-SP2 to parasite infection was less than that of normal erythrocytes and was inconsistent from preparation to preparation, from 30% to 80% of the normal infection.

In these same experiments, the ATP content of RG-SP2 and NaATP-RG-SP2 was measured as a function of incubation time. As in the parasite infection results, there was a great deal of variation in ATP content from one experiment to the next, as demonstrated by the enormous standard deviation when the data of all the experiments were pooled (Table I). Despite the variation, there was a distinct trend; during incubation, the ATP concentration in RG-SP2 gradually increased relative to normal erythrocytes from ~20% initially to 38% at 36–70 h. ATP in NaATP-RG-SP2 decreased over the same period, from a mean of 114% of normal erythrocyte ATP to 60% at 36–70 h. The inconsistencies could not be explained by inadequate resealing of different ghost preparations because no significant amount of hemoglobin was detected in the culture medium of incubated resealed ghosts.

Microscopic examination of Giemsa-stained resealed ghosts showed different intensities of staining, indicating heterogeneity in the amount of hemoglobin within populations of resealed ghosts. This heterogeneity could have been the cause of incon-

TABLE I

ATP Content of SP2 Resealed Ghosts Throughout Incubation

		ATP content* as a function of hours incubation				
Cell type	Hours: 0 (n = 13)‡	4–6 (n = 8)	8–12 (n = 10)	14–18 (n = 6)	20–30 (n = 7)	36–70 (n = 7)
RG-SP2	20 ± 9§	25 ± 10	31 ± 13	26 ± 10	37 ± 16	38 ± 22
NaATP-RG-SP2	114 ± 64	78 ± 53	74 ± 22	64 ± 45	62 ± 22	60 ± 35

Results were pooled from 13 independent experiments in which resealed ghosts were prepared in SP2 tubing, with sodium ATP supplement (NaATP-RG-SP2) or without (RG-SP2). Samples were prepared for ATP measurement before incubation (0 h) and at various points throughout incubation. Each individual experiment had from 3 to 6 time-point, ATP determinations.

* ATP content is expressed as a percentage of the ATP content measured in normal erythrocytes under identical conditions.

‡ n refers to the number of ATP determinations for each cell type that were averaged in a given time interval.

§ Standard deviation of the mean.

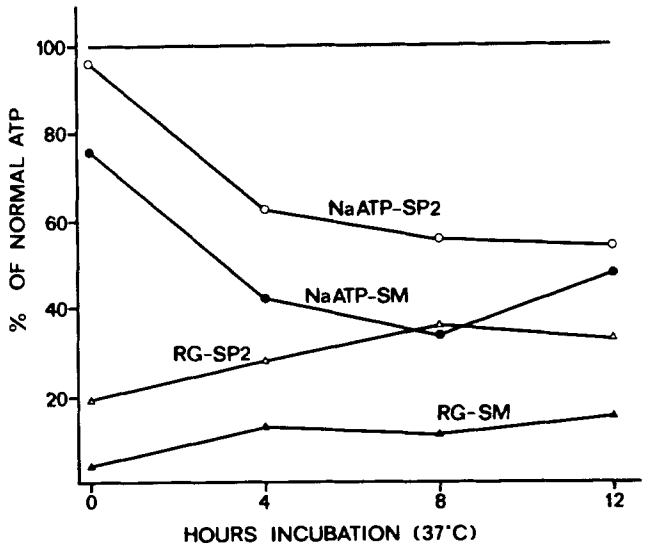

FIGURE 1 Cells were incubated under the standard culture conditions used to maintain *P. falciparum* growth in vitro (see Materials and Methods). ATP measurements were made from samples collected throughout incubation at 37°C. The cells included resealed ghosts prepared in SM tubing without ATP supplement (RG-SM, ▲) or with sodium ATP supplement (NaATP-SM, ●) and resealed ghosts prepared in SP2 tubing without ATP supplement (RG-SP2, △) or with sodium ATP supplement (NaATP-SP2, ○). Values for resealed ghosts are expressed as a percentage of the ATP measured in normal erythrocytes (mean 1.45 mM ± 0.12 SD).

sistencies in the SP2 preparations. Also, if there was a significant proportion of inadequately lysed cells in the preparations, these cells could mask any possible correlation between intraerythrocytic ATP content and susceptibility to parasite infection. Measurement of the average ATP content of the total population of cells would not reveal the presence of cells with deviant ATP contents.

To reduce the degree of heterogeneity in the population, narrow, rapidly dialyzing Spectra/Por semi-micro tubing (SM) was used, making possible a more "simultaneous" erythrocyte lysis. Fig. 1 shows a comparison of the ATP content during incubation of resealed ghosts when SP2 and SM preparations were tested in the same experiment. Cells prepared in SP2 (NaATP-RG-SP2 and RG-SP2) maintained higher average ATP concentrations than their counterparts prepared in SM (NaATP-RG-SM and RG-SM). When infection of these resealed ghosts by *P. falciparum* was measured (Table II), in this particular experiment, the cells prepared in SP2 showed a higher level of parasitemia than those prepared in SM. SM resealed ghosts that had been supplemented with ATP showed higher infection than the nonsupplemented ghosts (Table II, RG-SM – 30%, NaATP-RG-SM – 55%), whereas this difference was not apparent in the two types of resealed ghosts prepared in SP2 (RG-SP2 – 81%, NaATP-RG-SP2 – 80%). The most likely explanation for these results is that the SP2 resealed ghosts contain a larger fraction of inadequately lysed cells than SM resealed ghosts, i.e., cells that are susceptible to infection; therefore, the effect of ATP content on parasite infection was masked.

ATP and Malaria Infection

Although it was possible to improve parasite infection of SM resealed ghosts by supplementing with sodium ATP, the susceptibility to infection remained lower than that of normal erythrocytes. Because it is known that magnesium ATP (MgATP) is a critical substrate in glycolytic metabolism (25), attempts were made to improve ATP maintenance and susceptibility to parasite infection by preparing resealed ghosts in the presence of MgATP. In a series of experiments, MgATP–, NaATP–, $MgCl_2$–, and non-supplemented resealed ghosts were prepared in SM and tested for ATP content and parasite infection.

ATP measurements on these cells are shown in Fig. 2. Resealed ghosts that had been prepared by lysis in the absence of ATP (RG) maintained low levels of ATP throughout incubation. Resealed ghosts supplemented with NaATP or with MgATP during lysis contained near normal concentrations of ATP before incubation at 37°C; however, NaATP-RG showed a very precipitous drop in ATP during incubation, to a concentration ~30% of normal. An additional experiment revealed

TABLE II

Effect of Preparation Method of Resealed Ghosts on Invasion by P. falciparum

Cell type	Invasion
	%
Normal erythrocytes	100*
RG-SP2	81
NaATP-RG-SP2	80
RG-SM	30
NaATP-RG-SM	55

Results represent the same experiment described in Fig. 1.
* Values for resealed ghosts are expressed as a percentage of invasion of normal erythrocytes (6.03 ring-stage parasites per 100 cells).

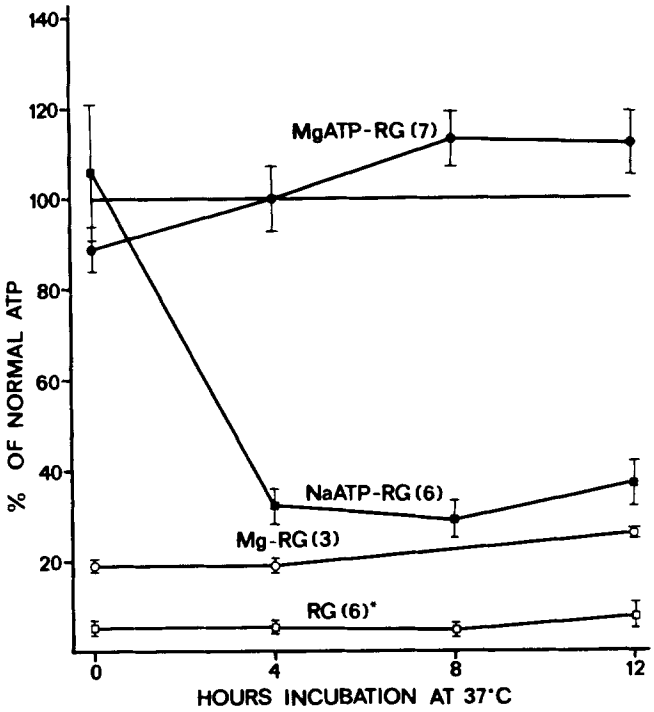

FIGURE 2 ATP content of resealed ghosts prepared in semi-micro tubing. Samples included resealed ghosts prepared by lysis in the presence of 2 mM magnesium ATP (MgATP, ●), 2 mM sodium ATP (NaATP, ■), 2 mM $MgCl_2$ (Mg-RG, ○), and no ATP or magnesium (RG, □). Values for resealed ghosts are expressed as a percentage of the ATP measured in normal erythrocytes (1.72 mM ± 0.22 SD). Vertical bars indicate standard error of the mean. * Numbers in parentheses indicate the number of independent preparations tested.

that ~75% of the decline occurred during the first hour of incubation. The decrease in the ATP content of NaATP-RG could not be accounted for by leakage from the cells, because the intracellular ATP lost during the first 4 h of incubation was not detectable in the culture medium. MgATP-RG were able to maintain a high ATP concentration which gradually increased, exceeding normal cell values. To determine whether the maintenance of high ATP levels was due to the supplement of magnesium or ATP, resealed ghosts were lysed in the presence of 2 mM $MgCl_2$. These cells showed ATP levels only slightly elevated from those of RG (Fig. 2, Mg-RG). Therefore, for resealed ghosts to maintain normal ATP concentration, both magnesium and ATP were required because neither magnesium alone (Mg-RG) nor ATP alone (NaATP-RG) was sufficient.

The susceptibility of resealed ghosts to infection by *P. falciparum* was also measured in the same experiments described above. The results (Table III) showed a qualitative correlation between susceptibility to parasite infection and intraerythrocytic ATP concentration. Resealed ghosts with the lowest levels of ATP (RG) were the least susceptible to infection (39% of normal). Resealed ghosts supplemented with 2 mM $MgCl_2$ or 2 mM NaATP during lysis were intermediate in susceptibility to infection (53% and 65%, respectively) and resealed ghosts with ATP levels most like normal erythrocytes (MgATP-RG) were as susceptible to infection as were normal cells ($p < 0.005$). Magnesium ATP supplement and semi-micro tubing were used in the preparation of resealed ghosts for the antispectrin experiments described below.

Invasion of Antispectrin-loaded Resealed Ghosts

To examine the effect of cross-linking of spectrin on merozoite invasion, resealed ghosts were prepared in the presence of normal IgG (NRIgG) or antispectrin IgG (ASIgG) and invasion of these cells by the parasite was assessed. The results are expressed relative to resealed ghosts prepared without IgG. Although NRIgG is the true control for ASIgG, in a given experiment, it was difficult to prepare two preparations with the same average amount of loaded NRIgG and ASIgG, respectively. To achieve nearly equivalent loading, it was necessary to add higher concentrations of NRIgG than ASIgG to the erythrocytes before lysis. Presumably, ASIgG loaded more efficiently due to its binding to spectrin within the ghosts.

To facilitate interpretation, the data from individual experiments were arbitrarily grouped by IgG content and invasion scores averaged. Fig. 3 shows the histogram for the average inhibition scores. Assuming that there are 2×10^5 spectrin dimers/cell, 5 μg IgG/10^8 cells represents an equimolar ratio of spectrin to ASIgG. In the range between 2.0–2.9 and 3.0–3.9 μg IgG/10^8 cells, the average inhibition of infection was 23% and 35%, respectively. These values are significantly different from those for NRIgG ($p < 0.002$, Mann-Whitney U test). In the ranges between 1.0–1.9 and 4.0–4.9 μg IgG/10^8 cells, there are not sufficient data to evaluate statistical significance. However, if all the inhibition scores from 1.0 to 4.0 μg IgG/10^8 cells are pooled, ASIgG inhibition scores are significantly different from those for NRIgG ($p < 0.002$, Mann-Whitney U test). Thus, although the inhibitory effect of antispectrin was not complete (i.e., was not 100%), the effect was highly significant. To demonstrate that the inhibitory effect of ASIgG was due to spectrin cross-linking, the monovalent Fab' fragments of ASIgG (ASFab') were tested in resealed ghosts. Table IV shows that when ASFab' were included in resealed ghosts at concentrations equivalent in spectrin binding capacity to the 3.0–3.9 μg ASIgG/10^8 cells range, no inhibition of invasion was observed relative to NRFab'.

TABLE III

Effect of ATP on Invasion of Resealed Ghosts by P. falciparum

Cell type	Invasion
	%
Normal erythrocytes (6)*	100‡
Resealed ghosts (6)	39 ± 0.8§
$MgCl_2$ resealed ghosts (3)	53 ± 2.4
NaATP resealed ghosts (6)	65 ± 4.1
MgATP resealed ghosts (7)	96 ± 3.1

Results are from the same experiments described in Fig. 2 legend.
* Number of separate preparations tested.
‡ Values for resealed ghosts are expressed as a percentage of invasion of normal erythrocytes (mean % parasitemia = 6.5 ± 0.5 SEM).
§ SEM

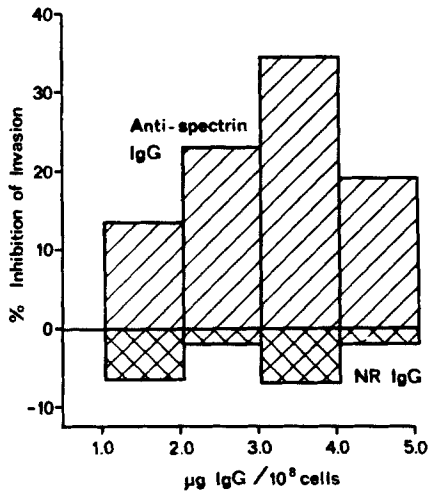

FIGURE 3 Effect of antispectrin IgG on invasion of resealed ghosts by *P. falciparum*. Results represent data from four experiments pooled by IgG content. For each group (1.0–1.9, 2.0–2.9, 3.0–3.9, 4.0–4.9 μg IgG/10^8 cells), % inhibition values were averaged and plotted. Single hatching represents antispectrin IgG; cross hatching represents normal rabbit IgG.

Heterogeneity of Resealed Ghost Preparations

Although the inhibitory effect of spectrin cross-linking on parasite invasion was highly significant, it was not complete. Because selective infection of cells with less than maximal spectrin cross-linking could contribute to the incomplete inhibition, the heterogeneity in antibody loading of SM resealed ghosts was analyzed. SP2 resealed ghosts were also analyzed and compared to SM resealed ghost in order to objectively confirm the microscopic observation that the use of SM had reduced the degree of heterogeneity relative to SP2.

Fig. 4 shows the cell sorter analysis of SP2 (*A*) and SM (*B*) resealed ghosts. By microscopic examination, the cells did not differ dramatically from normal erythrocytes in cell size. However, the histogram for light scattering (Fig. 4*a*), very roughly an indication of cell size distribution, showed that SP2 resealed ghosts (*A*) were more heterogeneous than SM resealed ghosts (*B*). Specifically, there were more "small" cells.

The fluorescence intensity histogram (Fig. 4*b*) showed that SP2 resealed ghosts were more heterogeneous with respect to antibody than were SM resealed ghosts. SM resealed ghosts showed a single peak of antibody loading; however, within the

TABLE IV

Invasion of Fab'-loaded Resealed Ghosts by P. falciparum

Antibody loaded	Parasitemia	Inhibition*
	%	%
Control	7.57 ± 0.98‡	0
NR Fab'§	7.23 ± 0.46	4
Antispectrin Fab'‖	7.25 ± 0.33	4

All resealed ghosts were prepared using magnesium ATP-supplemented lysis buffer and SM dialysis tubing. Controls contained no added antibody. The results are averages from three separate experiments, two preparations from one blood donor and one from a second.

* % Inhibition = 100 − 100 (parasitemia of sample/parasitemia of control).

‡ Standard deviation.

§ 2.85 ± 0.12 SD μg Fab'/10^8 cells.

‖ 3.12 ± 0.32 SD μg Fab'/10^8 cells.

population, some cells had less and some more antibody than the average amount trapped within the cells. Thus, although the average IgG content could be determined in the antispectrin experiments, in a given preparation there was a range of antispectrin concentrations.

DISCUSSION

Resealed Ghost Preparation and the Effect of ATP

During the course of studies aimed at improving and standardizing the malaria/resealed ghost system, two important factors that had not been addressed in previous work (13, 14) became apparent. First, heterogeneity in populations of resealed ghosts was recognized as a serious problem when experimental reagents were tested for their effect on merozoite infection (Table II). Because the holes in the erythrocyte membrane produced by hypotonic lysis are only transient (15), heterogeneity would be expected if all the cells do not lyse simultaneously; cells that lyse first, lyse into a buffer environment. Those that lyse later, open to a solution rich in hemoglobin and cellular enzymes. Although the use of narrow, rapidly dialyzing membrane tubing (SM) did not abolish heterogeneity in resealed ghost populations (Fig. 4), the reduction in heterogeneity was sufficient to discern the effects of ATP on infection (Table III).

The second factor in the preparation of resealed ghosts that appeared to be critical was magnesium ATP. The presence of magnesium ATP during erythrocyte lysis resulted in preparations of resealed ghosts that were consistently indistinguishable from normal erythrocytes with respect to parasite infection (Table III). Neither ATP nor magnesium alone was effective. In a recent study, Dluzewski et al. (14) reported infection of resealed ghosts with *P. falciparum*. The low efficiency of infection that they observed may possibly be due to the absence of magnesium ATP during red cell lysis. They do not specify the type of dialysis tubing used in their preparation. The rapid decrease in ATP concentration that we observed during incubation of NaATP-RG (Fig. 2) was not due to leakage of ATP from these cells. Therefore, a catabolic use of intracellular ATP must be considered. The failure of these resealed ghosts to maintain normal ATP during incubation may be a consequence of magnesium deficiency, caused by dilution during cell lysis. Conversely, the fact that Mg-RG were not able to restore ATP to normal levels may be a consequence of the initial ATP deficiency in these cells. Magnesium ATP requirements for glycolytic energy regeneration (25) and ion pumps (26) are well documented.

The effect of ATP depletion (27) and magnesium ATP (10, 11) on erythrocyte membrane shape and deformability could explain the observed correlation between intraerythrocytic ATP and susceptibility to *P. falciparum* infection (Table III). However, it is not yet possible to state definitively that the inhibition of infection of low ATP resealed ghosts is due to membrane alterations that prevent merozoite entry. A count of ring-stage parasites as an assay of invasion, though offering a large sample size, measures not only merozoite penetration but also morphological transformation and survival up to 15 h. Although there was no evidence of parasite degeneration within infected resealed ghosts prepared without ATP, the possibility of parasite starvation in the early ring stage, due to dependence on host energy metabolism (28–30), cannot be rigorously excluded. For the purposes of this study, direct assays of invasion, by video image enhanced light microscopy (31) or electron microscopy (8), pose technical limitations and do not offer sufficient sample size for quantification. In blood smears, examination of invading, free merozoites soon after infection does not differentiate between true, successful invasion and mere adhesion or adventitious superposition of merozoites and erythrocytes.

Spectrin and Merozoite Entry

Spectrin cross-linking causes a decrease in membrane deformability (11) and alterations in membrane receptor distribution and mobility (12). The invading merozoite causes a remarkable deformation of the erythrocyte membrane and brings about near depletion of intramembrane particles in the forming vacuolar membrane (2, 9). Thus, the inhibition of invasion of antispectrin IgG-loaded resealed ghosts (Fig. 3) could be due to restricted membrane deformability, inhibition of transmembrane protein mobility, or possibly an unfavorable arrangement of erythrocyte surface receptors for the merozoite. The latter possibility is of interest in view of the fact that glycophorin, a protein recently implicated in merozoite invasion of erythrocytes (4, 5), is markedly effected by antibody-induced spectrin crosslinking (12).

The inherent heterogeneity in antibody loading of resealed ghosts, even in SM preparations, is likely to be the major cause of failure to observe complete blockage of merozoite invasion of ASIgG-loaded resealed ghosts. Because the parasites invade only ~5% of the resealed ghosts, the presence of cells with less than maximal spectrin cross-linking (due to either spectrin excess or antispectrin excess) could lead to a reduction in the

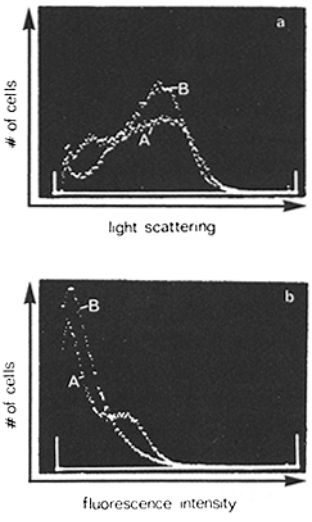

FIGURE 4 FAC II fluorescence-activated cell sorter analysis of F.I.T.C.-conjugated IgG-loaded resealed ghosts (see Materials and Methods). (*a*) light scattering distribution and (*b*) fluorescence intensity distribution of (*A*) resealed ghosts prepared by lysis in Spectra/Por 2 tubing and (*B*) resealed ghosts prepared by lysis in Spectra/Por semi-micro tubing. Histograms represent 100,000 particles analyzed for each sample.

amount of inhibition attainable. The notion that the malaria merozoite can selectively infect particular cells within a heterogeneous population is well-documented (32, 33, 34). Despite the problem of heterogeneity in the system described here, the inhibitory effect of antispectrin IgG on merozoite invasion was highly significant.

The molecular arrangement of the cytoskeleton during merozoite penetration is not known. The organization of the erythrocyte cytoskeleton has been shown to undergo alterations during nuclear extrusion (35) and during concanavalin A-induced endocytosis in neonatal erythrocytes (36). The present study has indicated the involvement of spectrin in malaria invasion, suggesting a change in the cytoskeletal configuration during merozoite penetration. The establishment of a method to prepare resealed ghosts that are highly susceptible to parasite infection now makes it possible to examine other internal membrane constituents involved in invasion. Further studies will be needed to define these components and, ultimately, the mechanism by which the merozoite interacting with the external surface of the erythrocyte induces changes across the host plasma membrane.

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