

# Role of Human Decay-accelerating Factor in the Evasion of *Schistosoma mansoni* from the Complement-mediated Killing In Vitro

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## Summary

Decay-accelerating factor (DAF) is a 70-kD membrane glycoprotein that prevents complement (C)-mediated hemolysis by blocking the assembly or accelerating the decay of C3 convertase. Purified DAF is known to incorporate into the membrane of DAF-deficient cells, inhibiting lysis. Since *Schistosoma mansoni* is a blood-dwelling parasite, we investigated whether DAF can be transferred from human erythrocytes to the worm and protect it against C-mediated killing in vitro. We have found that schistosomula (schla) incubated with normal human erythrocytes (N-HuE), but not with DAF-deficient erythrocytes, become resistant to C damage in vitro. Protected parasites acquire a 70-kD surface protein which can be immunoprecipitated by anti-DAF antibodies. The acquired resistance is abrogated by treatment of N-HuE-incubated parasites with anti-DAF antibody. These results indicate that, in vitro, N-HuE DAF can be transferred to schla, and suggest its participation in preventing their C-mediated killing. This could represent an important strategy of parasites to evade the host's immune response in vivo.

In spite of the immune response that *Schistosoma mansoni*-infected hosts develop against the parasite, adult worms can survive for decades in the blood stream, exposed to immune effectors, without apparent damage (1). One of the mechanisms that have been proposed to account for the successful survival of the adult worm is the masking of parasite target epitopes by molecules from host origin, acquired during worm development (host antigen hypothesis) (2, 3). In vitro studies have shown that schistosomula (schla)<sup>1</sup> in contact with host erythrocytes (E) acquire cell-derived antigens (4–6) and become resistant to immune attack (7). However, despite the presence of a number of host antigens on the parasite surface (4–10) and its correlation with protection against

immune damage (7, 10), this hypothesis is weakened by the findings that resistant parasite stages can still bind antischistosome antibodies (11–13). In this context, an attractive hypothesis to explain the role of host antigens in the parasite's immune evasion is that the molecules incorporated onto the schla surface could provide a specific functional protection against immune attack, as we postulated previously (14). Because C-mediated killing of schla is considered to be one of the mechanisms of immunity in schistosomiasis (15–17) and *S. mansoni* lives in close contact with blood cells, this role could be played by molecules from the E membrane with C inhibitory activity. A potential candidate molecule would be the decay accelerating factor (DAF) from human E, in view of its inhibitory function on the C activation (18–20) and its ability to reincorporate into the membranes of DAF-deficient cells and render these cells resistant to C-mediated lysis (21–22). In fact, our earlier studies provided the first suggestive data that DAF from human E can play such a role (14). In this paper, we further examined the hypothesis that DAF may participate as a functional host antigen by investigating: (a) the ability of normal human erythrocytes (N-HuE) or DAF-deficient E to protect schla against the C-mediated killing in vitro; (b) the presence of human DAF on the surface of N-HuE-protected parasites; and (c) the effect of anti-DAF antibody in the N-HuE-mediated protection of schla in vitro.

<sup>1</sup> Abbreviations used in this paper: DAF, decay-accelerating factor; EBSS, Earle's balanced salt solution; Elac, EBSS with 0.5% lactalbumin hydrolysate and 0.1% glucose; fGPS, fresh guinea pig serum; HRF, homologous restriction factor; NHS, normal human serum; NRbS, normal rabbit serum; N-HuE, normal human erythrocytes; PIP2, phosphatidylinositol-specific phospholipase; PNH, paroxysmal nocturnal hemoglobinuria; PNH-HuE, erythrocytes from PNH patients; schla, schistosomula; a-schla RbS, anti-schistosomula rabbit serum.

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## Materials and Methods

**Parasite.** A Brazilian strain of *S. mansoni* (L.E., Belo Horizonte, Brazil) was used throughout the experiments. Schla were obtained by an in vitro mechanical transformation of cercariae, as previously described (23). Briefly, cercariae were cooled, centrifuged, and resuspended in Earle's balanced salt solution (EBSS) with 0.5% lactalbumin hydrolysate and 0.1% glucose (Elac), containing antibiotics and 10 mM Hepes, pH 7.4. This suspension was vortexed for 45 s and the tails were separated from the cercarial bodies by differential sedimentation at 1 g in 5 ml Elac for 5 min, three times, at room temperature. After a 3-h incubation of the resulting bodies in Elac at 37°C, larvae are considered to be schla (24–26).

**Sera.** Anti-schla immune sera were produced in rabbits (a-schla RbS) injected intramuscularly with ~30,000 schla in 1 ml PBS emulsified with an equal volume of CFA (Difco Laboratories, Detroit, MI). The rabbits were boosted three times with the same number of parasites in IFA at 2-wk intervals. Sera were assayed for their ability to induce the C-mediated killing of schla using the in vitro cytotoxic assay described below. Normal rabbit sera (NRbS) were obtained from the animals before immunization. Normal human sera (NHS) were obtained from healthy individuals. Fresh guinea-pig serum (fGPS) was used as source of active C. All sera were obtained from clotted blood after 1 h at 4°C and, except those used as C, all samples were inactivated at 56°C for 30 min before storage at -80°C (Bio-Freezer; Forma Scientific, Marietta, OH). FCS was purchased from Cultilab (Campinas, Brazil). Rabbit anti-human DAF serum was kindly donated by Dr. V. Nussen-zweig (New York University Medical School, New York).

**Preparation of E.** N-HuE, kindly provided by Dr. Mário S.A. Neves (HEMOMINAS blood bank, Belo Horizonte), were obtained from healthy donors blood, regardless of the ABO group, with negative tests for hepatitis B, syphilis, Chagas' disease and AIDS. PNH-HuE, with positive Ham's test (27), were obtained from seven individual samples of blood, kindly provided by Dr. Romeu I. Carvalho (Hospital Felício Rocho, Belo Horizonte). SRBC were obtained from animals maintained at the Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte. Blood was conserved for 1–4 wk in equal volume of Alsever's solution at 4°C and were washed in Elac when required. Treatment of N-HuE with trypsin and pronase were carried out as described elsewhere (28, 29). Briefly, N-HuE free of plasma proteins were incubated with pronase (1 mg/ml) or with trypsin (150 µg/ml) in 0.02 M phosphate buffer containing 0.2 M NaCl for 1 h at 37°C and then washed to remove enzymes.

**Preparation of E Ghosts and Membrane Protein Fraction.** N-HuE ghosts and E membrane proteins were prepared as described by Nicholson-Weller et al. (20). Briefly, E were extensively washed with PBS and then lysed with 5 mM phosphate buffer, pH 7.5, containing 1 mM EDTA at 4°C. The resulting E ghosts were washed out of hemoglobin by centrifugation (10,000 g, 30 min), resuspended in the same buffer, and stored at -80°C until required. Alternatively, n-butanol was added to this membrane preparation, with stirring, at 4°C to a final concentration of 20% (vol/vol), and stirring was continued for 30 min. This process was repeated, as described (20), and the butanol-saturated aqueous phase was dialyzed extensively against Earle's solution before incubation with schla. Protein was quantified by the method of Lowry (30).

**Assay of Susceptibility of Schla to C-mediated Killing.** A modified in vitro assay originally described by Clegg and Smithers (7) was used. Our assay consisted of a 24-h incubation of 3-h schla with E or fractions, to allow the acquisition of host antigens by the parasites, followed by a C-mediated cytotoxic assay, to evaluate the de-

gree of protection afforded to the parasites during the previous step. Alternatively, schla incubated with N-HuE were treated with anti-DAF mAb before the cytotoxic assay, as described below.

**Incubation of Schla with E or Fractions.** Approximately 2,000 schla were incubated with 1% packed N-HuE (or equivalent amount of E ghosts or 250 µg/ml membrane protein fraction), PNH-HuE or SRBC in 2 ml Iscove's modified Dulbecco's medium (Sigma Chemical Co., St. Louis, MO), either in the absence or in the presence of 5% FCS or NHS in 24-well plastic culture plates (Tissue Culture Cluster; Costar, Cambridge, MA). After 24 h, parasites were extensively washed by differential sedimentation at 1 g, at room temperature, until free host material was completely removed.

**Treatment of Schla with anti-DAF Antibody.** After 24 h of incubation with N-HuE in the presence of FCS, washed parasites were incubated for 30 min with 10 µl of the IA10 anti-human DAF IgG2a mAb (31) (8.5 mg/ml) (gift of Dr. V. Nussen-zweig, New York University Medical School). The parasites were washed to remove unbound antibodies and then assayed for their susceptibility to C-mediated cytotoxicity.

**Cytotoxic Assay.** 250 schla were incubated in 250 µl Elac for 30 min in 96-well microplates (Microtest II, Tissue Culture Plate; Falcon Plastics, Oxnard, CA) with or without 100 µl of a-schla RbS (final dilution 1:64), washed, and incubated with fGPS (final dilution 1:25), as source of C. After 18–24 h, schla were examined at 40× with an inverted microscope (Olympus; model, IMT, Japan) and the percentage of dead or damaged schla was determined. These parasites were easily recognized by their immobility and opaque granular appearance. Incubations were carried out in 5% CO<sub>2</sub>/95% air at 37°C (Water Jacketed CO<sub>2</sub> incubator, model 3157; Forma Scientific, Marietta, OH) and the whole experiment was carried out under sterile conditions.

All experiments throughout this work were repeated two to five times and each figure represents a typical result.

**Schla Surface Immunoenzymatic Assay.** Approximately 1,000 live schla, previously incubated for 24 h either in the absence or in the presence of N-HuE, were treated with 100 µl of a-schla RbS or NRbS in 96-well flat-bottomed ELISA plates pre-coated with 1% casein in PBS. Excess proteins were washed out and parasites were incubated with an affinity purified goat anti-rabbit IgG (H+L) horseradish peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA). Each incubation was carried out at room temperature for 40 min and parasites were washed with EBSS containing 0.5% NRbS, to minimize nonspecific binding of conjugate to schla. After last washing (EBSS without serum), parasites were incubated with 100 µl of a solution of 0.02% (wt/vol) orthophenylenediamine and 0.02% (vol/vol) H<sub>2</sub>O<sub>2</sub> in a phosphate-citrate buffer pH 5.0 (0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.05 M citric acid). After 15 min of enzymatic reaction, supernatants were removed and added to clean wells containing 20 µl of 20% H<sub>2</sub>SO<sub>4</sub> to be read at 492 nm in an ELISA reader (Uniskan; Eflab, Eflab Oy, Helsinki, Finland).

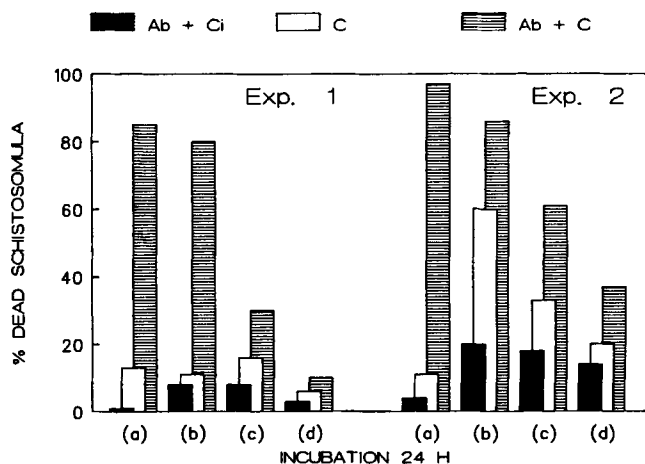
**Immunoprecipitation.** About 300,000 3-h or 24-h schla were incubated with N-HuE ghosts, in the absence of serum, and washed extensively until all E membrane traces were removed. The parasites were then surface iodinated with 500 µCi <sup>125</sup>I by the method of Iodogen (Pierce Chemical Co., Rockford, IL). Labeled molecules were extracted with 1% NP-40 in 50 mM Tris and 150 mM NaCl, containing the following protease inhibitors: PMSF (10 mM), EDTA (10 mM), iodoacetamide (10 mM), antipain (0.1 mg/ml), and leupeptin (0.1 mg/ml). Aliquots of the extracts, containing 3 × 10<sup>6</sup> cpm, were treated with staphylococcal protein A-Sepharose beads previously incubated with either the monoclonal mouse anti-human DAF IgG2a (IA10) (31) or a rabbit anti-human DAF serum (32). The protein A-Sepharose antibody-antigen complexes

were subjected to a 5–13% gradient SDS-PAGE under reducing conditions (33, 34) followed by autoradiography of dried gels.

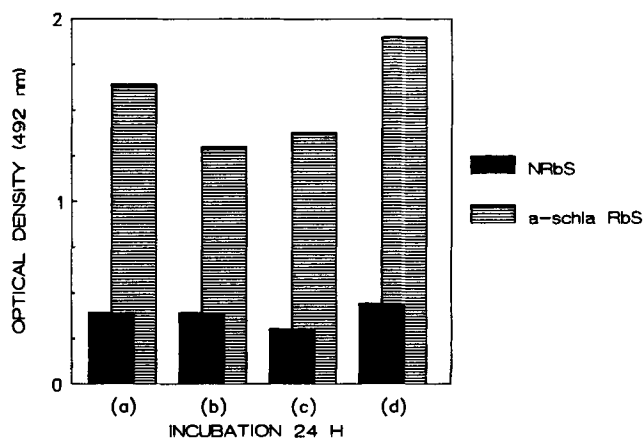
## Results

**Effect of N-HuE in the Susceptibility of Schla to the C-mediated Killing.** Two representative experiments show the susceptibility of schla to C-mediated killing in vitro before and after incubation with N-HuE (Fig. 1). In experiment 1 it is observed that, whereas 85% of recently transformed larvae (3-h schla) (a) and 80% of the parasites incubated in chemically defined medium (24-h schla) (b) are killed by C when coated with anti-schla antibodies, only 30% of the schla previously cultured in the presence of N-HuE (24-h N-HuE schla) (c) are susceptible to immune damage. These results show that N-HuE have the ability to confer resistance to schla against the antibody-dependent C-mediated killing in vitro. The N-HuE-mediated protection is even greater when 24-h N-HuE schla are incubated in the presence of FCS (d) or NHS (not shown). In these cases, almost 100% of the parasites become resistant to the lethal effect of antibody and C. The protective effect of N-HuE is also observed when schla is exposed only to the alternative pathway of C activation. This is particularly evident when mortality of 24-h schla exposed to C alone (b) happens to be high, as occurs in the analogous experiment 2. This finding indicates that N-HuE-mediated mechanism of schla protection acts at the level of C activation.

**Binding of IgG Anti-schla to the Surface of N-HuE-protected Parasites.** To verify whether the resistance of schla to antibody and C-mediated killing is due to the masking of target epitopes by N-HuE-derived molecules, we investigated the ability of anti-schla antibody to bind to the surface of N-HuE-protected parasites. The antibody binding was determined

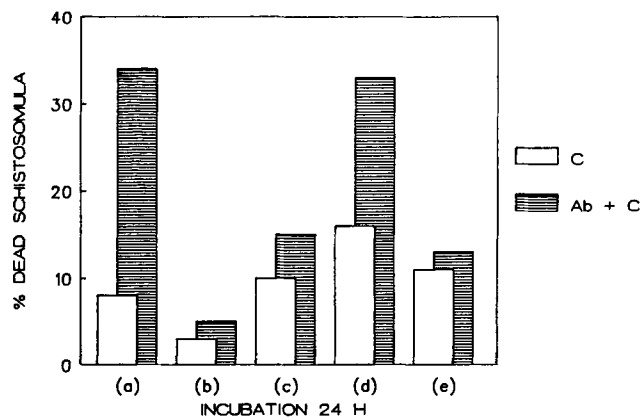


**Figure 1.** Effect of N-HuE in the susceptibility of schla to C-mediated killing. Values shown in analogous Exps. 1 and 2 are the mean percent of parasite killing mediated by C, Ab + C or Ab + Ci from duplicate samples of 3-h schla (a) or schla previously cultured for 24 h in medium chemically defined (b) or supplemented with N-HuE (c) or N-HuE + FCS (d). All experiments throughout this work were repeated two to five times with similar results.



**Figure 2.** Binding of IgG anti-schla to the surface of N-HuE-protected parasites. 3-h schla (a) or schla previously cultured for 24 h in medium chemically defined (b) or supplemented with N-HuE (c) or N-HuE + FCS (d) were incubated with a-schla RbS or NRbS and then with goat anti-rabbit IgG. Each point represents the mean OD from duplicate supernatant samples after the addition of substrate to Ab-coated schla. Parasites were taken from the same preparation used in the experiment represented in Exp. 1, Fig. 1.

by an immunoenzymatic reaction on the surface of schla as described in Materials and Methods. Fig. 2 shows that protected parasites previously incubated with N-HuE either in the absence (c) or in the presence of FCS (d) are still able to bind anti-schla IgG, as compared to the controls with NRbS and parasites which did not have any contact with N-HuE (a and b). These results, confirmed by immunofluorescence (data not shown), suggest that the resistance conferred by N-HuE to schla is not due to a failure of anti-schla antibodies



**Figure 3.** Effect of N-HuE ghosts, hemoglobin or membrane proteins in the susceptibility of schla to C-mediated killing. Values shown are the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of schla previously cultured for 24 h in medium supplemented with FCS (a), FCS + N-HuE (b), FCS + N-HuE ghosts (c), FCS + N-HuE hemoglobin-rich fraction (d), or FCS + N-HuE membrane proteins (e). Background mortality in the presence of Ab + Ci (<17%) was subtracted from mortality mediated by C or Ab + C.

to bind to the surface of the parasite. These findings support the previous suggestion that the protective effect seems to be due to an inhibition of C activation on the parasite surface.

**The Protection of Schla Is Mediated by N-HuE Membrane Proteins.** To identify the cellular component involved in the schla protection, E were lysed, and schla were incubated with either the membrane- or the hemoglobin-rich fraction, in the presence of FCS, to maximize the degree of schla protection. As seen in Fig. 3, while the membrane fraction (c) is able to protect the larvae from immune damage, the mortality of schla incubated with the hemoglobin-rich fraction (d) is equivalent to that observed for the larvae incubated in cell-free FCS-containing medium (a). Schla incubated with the delipidated protein fraction of N-HuE membrane (e) become totally resistant to the antibody-dependent C-mediated damage. These results show that the protection of schla is mediated by N-HuE membrane proteins.

**Effect of Trypsin- and Pronase-treated N-HuE in the Susceptibility of Schla to the C-mediated Killing.** DAF and the CR1 C3b receptor are two N-HuE membrane proteins that down regulate C activation (20, 35). Trypsin is known to remove CR1 and glycophorin from N-HuE surface, without affecting DAF activity (28, 29, 36). On the other hand, the treatment of N-HuE with pronase abolishes the DAF activity rendering the cells C-sensitive (29). To investigate whether trypsin or pronase treatment affects the ability of N-HuE to protect the parasite, intact cells were treated with these enzymes and then incubated with schla in the presence of FCS (Fig. 4).

Trypsin-treated N-HuE fully retain their ability to confer protection to parasites against C-mediated damage in vitro (Exp. 1): ghosts from trypsinized (b) as well as from non-trypsinized N-HuE (a) confer equivalent levels of protection

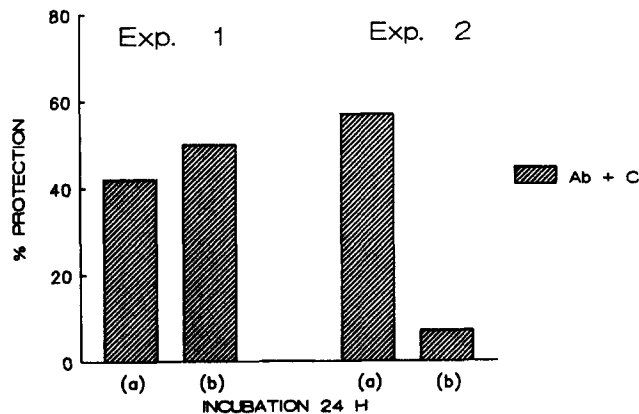
to schla, in relation to larvae incubated with FCS alone. This does not occur when pronase is used (Exp. 2). On the contrary, treatment of N-HuE with pronase (b) completely abolishes the protective effect of N-HuE (a).

Pronase- but not trypsin-treated N-HuE protein fractions lost the 70-kD band recognized by anti-DAF antibodies by immunoblotting after SDS-PAGE, showing the efficient removal of DAF by pronase. The efficiency of trypsin was verified by the fact that E treated with the enzyme was totally depleted of glycophorin, the major E surface protein, as indicated by the periodic acid Schiff staining after SDS-PAGE (data not shown).

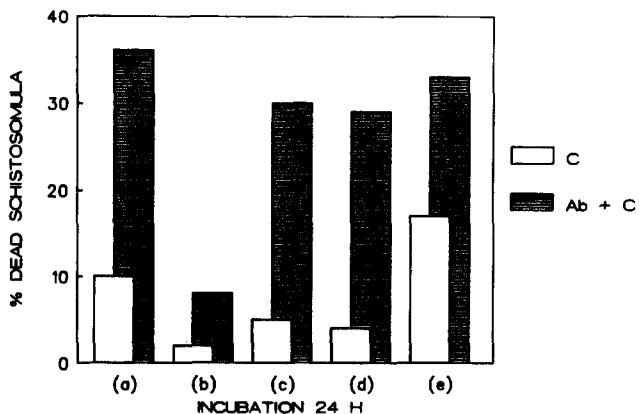
These results rule out the participation of glycophorin and probably of CR1 in the N-HuE-mediated protection of schla and indicate the possible involvement of DAF.

**Effect of DAF-deficient E in the Susceptibility of Schla to C-mediated Killing.** PNH-HuE (29, 37) and SRBC (21), both extremely sensitive to C-mediated lysis, are known to be naturally deficient in DAF. We, therefore, investigated whether these DAF-deficient cells are able to protect schla from the C-mediated killing. In Fig. 5, we can observe that the mortality of schla incubated with either PNH-HuE (c and d) or SRBC (e) in the presence of FCS is equivalent to that observed for the larvae incubated in cell-free FCS-containing medium (a), demonstrating that neither cell type is able to protect schla as N-HuE (b). All PNH-HuE donors were in the crisis period of the disease at the time of blood collection. DAF deficiency was confirmed by a dot blot screening of the PNH-HuE membrane proteins which did not react with anti-DAF antibodies as opposed to N-HuE (not shown).

So far, our results demonstrate that resistance of schla upon contact with N-HuE is conferred by a pronase-sensitive,



**Figure 4.** Effect of trypsin- and pronase-treated N-HuE in the susceptibility of schla to C-mediated killing. N-HuE were treated with trypsin (Exp. 1) or pronase (Exp. 2) before incubation with parasites. Data represent the mean percent protection against killing mediated by Ab + C of duplicate samples of schla were previously cultured for 24 h in medium supplemented with FCS + N-HuE (a) and FCS + enzyme-treated N-HuE (b) in relation to parasites incubated with FCS alone. Background mortality in the presence of Ab + Ci (<14%) was subtracted from mortality mediated by Ab + C, before calculations were done.

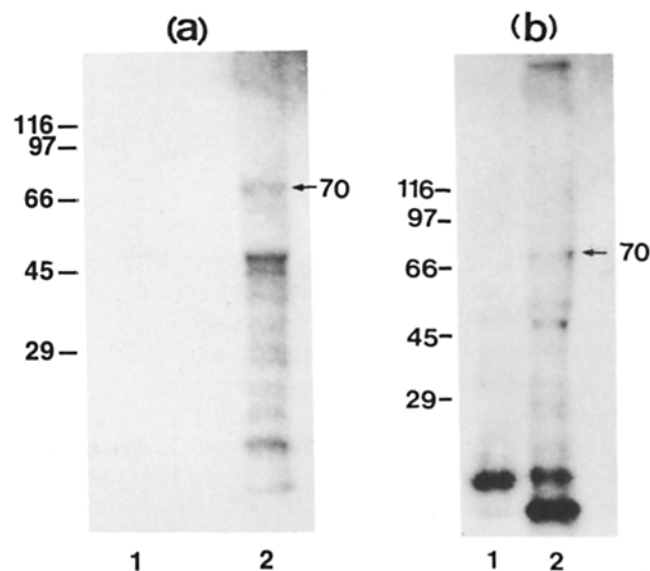


**Figure 5.** Effect of DAF-deficient E in the susceptibility of schla to C-mediated killing. Each point represents the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of schla previously cultured for 24 h in medium supplemented with FCS (a), FCS + N-HuE (b), FCS + PNH-HuE (c, patient 1, d, patient 2, representative of seven that presented identical results), and SRBC (e). Background mortality in the presence of Ab + Ci (<10%) was subtracted from mortality mediated by C or Ab + C.

trypsin-resistant C inhibitor protein present on the surface of N-HuE and absent from SRBC and PNH-HuE. Thus this inhibition could conceivably be due to DAF.

**Presence of DAF on the Surface of N-HuE-incubated Schla.** To investigate for the presence of human DAF on the surface of schla, parasites protected by N-HuE ghosts in the absence of serum as well as 3-h schla were surface labelled with  $^{125}\text{I}$ , and NP40-soluble proteins were immunoprecipitated with anti-human DAF antibodies, known to react with the 70-kD form of human E (31, 32). As shown in Fig. 6, both the monoclonal (mouse IgG2a) (a) and the polyclonal (rabbit serum) (b) anti-human DAF antibodies are able to immunoprecipitate from extracts of protected schla a surface protein of Mr 70 kD (lane 2), absent from 3-h susceptible parasites (lane 1). Other bands are visible in immunoprecipitates from 24-h N-HuE schla but not 3-h schla, namely 110, 140, and >200 kD (b), a doublet between 66 and 45 kD, two bands between 45 and 29 kD, and four other bands below 29 kD (a and b). The low molecular mass band seen in the control of the polyclonal antibody (b) seems to be due to nonspecific binding, as usually occurs when total serum is used. The presence of anti-DAF-reactive proteins in 24-h N-HuE schla and their absence in 3-h worms clearly demonstrate that schla can acquire DAF from the human red cells.

**Effect of Anti-DAF Antibody in the Acquired Resistance of Schla to the C-mediated Killing.** 24-h N-HuE schla incubated in the presence of FCS and washed were treated with anti-DAF mAb before the cytotoxic assay, and the results are presented



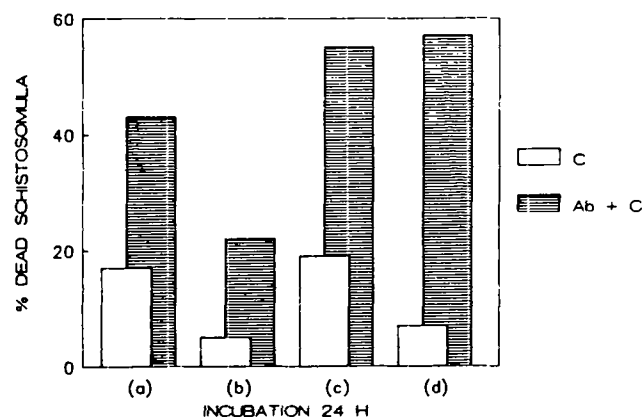
**Figure 6.** Detection of anti-DAF reactive molecules on the surface of N-HuE-protected schla. Susceptible 3-h schla (lane 1) or schla protected from C-mediated killing after incubation for 24 h in culture medium containing N-HuE ghosts (lane 2) were extensively washed and parasites surface was radioiodinated. Extracts were immunoprecipitated with monoclonal murine IgG2a (a) or polyclonal rabbit serum (b) anti-human DAF followed by resolution of proteins by SDS-PAGE on a 5–13% gradient gel. Size of standards are shown to the left in kiloDaltons.

in Fig. 7. Parasites treated with the mAb (c) become as susceptible to C-mediated killing as those incubated with FCS only (a) or with PNH-HUE plus FCS (d). This clearly shows that the anti-DAF antibody completely abrogates the protection afforded by N-HuE, indicating that the human DAF molecules present on the surface of schla are involved in the protection of the parasite against the C-mediated killing in vitro.

## Discussion

DAF is a 70-kD glycosylphosphatidylinositol-anchored membrane glycoprotein that prevents the assembly and disassembles the membrane-bound (20, 21) and fluid phase (38) classical and alternative pathway C3 convertases. In the present study, we described for the first time that DAF from human E can be transferred to the *S. mansoni* schla surface, and we present evidence that strongly suggest that it plays a substantial role in the parasite evasion from C-mediated killing in vitro.

Our initial observation that schla incubated with N-HuE become more resistant to antibody plus C-mediated killing in vitro (Fig. 1) has already been reported by Clegg and Smithers (7) using rhesus monkey E in the presence of homologous serum. This protection was attributed to the failure of antibody binding due to the masking of target epitopes by host molecules (2–6). However, like others (10–13), we have shown that anti-schla IgG can still bind to the surface of N-HuE-protected schla (Fig. 2 and our unpublished indirect immunofluorescence results). The suggestion that the protective effect occurs mainly at the level of C activation, rather than antibody binding, is supported by our observation that N-HuE treatment also inhibits the parasite killing due to the activation of the alternative pathway of C, (more evident in Fig. 1, exp. 2 [c and d]), but also in Fig. 5 [b] and Fig. 7 [b]).



**Figure 7.** Effect of anti-DAF antibody in the susceptibility of schla to C-mediated killing. Data represent the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of schla previously cultured for 24 h in medium supplemented with FCS (a), FCS + N-HuE (b), FCS + N-HuE, anti-DAF-treated (c), or FCS + PNH-HuE (d, patient 3). Background mortality in the presence of Ab + C (<20%) was subtracted from mortality mediated by C or Ab + C.

The protective activity we describe here resides in the protein fraction of N-HuE membrane (Fig. 3). The nature of this molecule was further investigated by taking advantage of two proteolytic enzymes, known to have different behaviors towards DAF. Pronase can remove DAF from the N-HuE surface, causing the cells to become susceptible to lysis by C (29). Trypsin does not affect either the integrity of DAF or the resistance of cells to C (29, 36), but removes the CR1 C3b receptor (29), another H-like protein, and glycophorin (28, 36). The observation that pronase, but not trypsin, abolishes the protective effect of N-HuE (Fig. 4) is consistent with the notion that this protection is DAF-mediated. Moreover, the fact that trypsin-treated N-HuE, depleted of glycophorin, fully retain the ability to confer protection to schla against C-mediated damage in vitro rules out the participation of glycophorin and suggests that CR1 is not involved in this protection.

The first approach to validate our hypothesis that N-HuE DAF is involved in the protection phenomenon was based on the argument that if DAF were responsible for schla escape from immune attack, then DAF-deficient cells would be unable to protect parasites from C-mediated killing in vitro. In fact, this was the case. The finding that PNH-HuE and SRBC, which lack DAF (21, 29, 37), have no ability to protect the parasite from C damage in vitro (Fig. 5) strongly indicates that this molecule may be involved in the protection conferred by N-HuE to schla.

As also reported by others (4–6), previous results from this laboratory, using anti-N-HuE antibody in the immunoenzymatic assay described here, have indicated the presence of N-HuE molecules on the surface of protected schla that had been incubated for 24 h with N-HuE (our unpublished results). Here, we demonstrate that DAF is among the N-HuE molecules transferred to the parasites by showing in their surface a 70-kD protein recognized by both monoclonal and polyclonal anti-human DAF antibodies (Fig. 6). Preliminary unpublished results have shown that the presence of puromycin during the incubation of schla with N-HuE does not prevent the acquisition of resistance by the parasites. The nonrequirement of protein synthesis in this process suggests that DAF is from host origin.

Recently, additional forms of DAF with  $M_r$  other than 70 kD have been identified. Kinoshita et al. (39) described a HuE membrane molecule of 140 kD, also absent from PNH-HuE, reactive with mAbs anti-human DAF and able to accelerate the decay of C3 convertase of the classical pathway. Seya et al. (40) identified 55-kD monomeric forms of DAF and 450-kD aggregates of 63-kD subunits. In our experiment, the bands of  $M_r$  110, 140, and >200 kD, reactive with the polyclonal anti-DAF (Fig. 6 a) as well as those of  $M_r$  <70 kD found in 24-h N-HuE schla (Fig. 6, a and b), may represent these other forms of DAF. Another possible explanation for the bands of  $M_r$  <70 kD is the possible occurrence of proteolytic degradation of DAF during the manipulation process, since they are not seen in 3-h schla.

The functional aspect of the protective effect was approached based on the assumption that if DAF were transferred to schla surface, anti-DAF antibodies would impair the protection of

the parasites by blocking its activity. Our results that anti-DAF mAb completely prevented the acquisition of protection by the parasite (Fig. 7) corroborated this assumption. It is unlikely that the lack of protection is due to an increase in C3b deposition caused by anti-DAF antibodies since we already had an excess of a-schla antibody and since no significant increase in the killing mediated by C alone was observed. Therefore, we have functional evidence that human DAF molecules in the surface of schla may play a role in the evasion of the parasite in vitro.

Other molecules, such as the homologous restriction factor (HRF), present in N-HuE but absent in PNH-HuE (41), are known to interfere with C activity (42). The participation of HRF in the phenomenon described here is unlikely, since the protective effect is completely blocked by mAbs specific to DAF and since the inhibitory effect was upon heterologous C. It has been shown that DAF does not function in a species restricted manner, at least for the heterologous system involving human DAF-guinea-pig C (18, 19), rabbit DAF-guinea-pig C (43) and human DAF-rabbit C (44).

The above results led us to conclude that, in vitro, DAF from N-HuE can be transferred to the schla surface and participate in the protection of the parasite against C-mediated killing either in the presence or absence of anti-schla antibodies. Other investigators have also suggested the involvement of this molecule in the evasion of the parasite of the alternative C pathway, providing preliminary evidence for the detection of host DAF on the membrane of 14-d-old schla recovered from guinea pig (45).

We are currently investigating the mechanisms whereby DAF is transferred to the surface of schla. Preliminary unpublished data lead us to presume that DAF sheds from the N-HuE membrane, as shown in human polymorphonuclear cells (46), goes into solution and then binds to the surface of the parasite, possibly to a specific protein acceptor molecule.

Certainly, other mechanisms account for *S. mansoni* evasion from the host immune system and it is probable that they act in conjunction. Our findings that, in vitro, N-HuE-mediated resistance of schla is improved in the presence of serum support this view. It is possible that soluble DAF present in serum (47) also binds to the parasite surface contributing to an increase in the parasite resistance. It is also possible that serum phosphatidylinositol-specific phospholipases (PIPL), e.g., PIPLD (48, 49), amplify the release of membrane N-HuE DAF, thus enhancing its uptake by the parasite.

The acquisition of a particular E molecule with C regulatory function, such as DAF, could explain, for instance, why anti-E antibodies can damage adult parasites bearing E antigens in vivo (2, 4), whereas anti-alloantigens do not affect the survival in the host of worms bearing MHC determinants (50). Although several questions still need to be answered, our findings may explain the paradox of the ability of *S. mansoni* to escape immune damage by the acquisition of host antigens despite the presence of specific antibodies on their surface. DAF, as an acquired functional host antigen, might represent an important mechanism used by schistosomes, as well as by other parasites, to evade immune killing mediated by antibody and C in vivo.

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