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MECHANISMS OF EVASION OF *SCHISTOSOMA MANSONI* SCHISTOSOMULA TO THE LETHAL ACTIVITY OF COMPLEMENT

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Schistosomula of Schistosoma mansoni *became resistant to antibody-dependent complement damage* in vitro *after pre-incubation with normal human erythrocytes (NHuE) whatever the ABO or Rh blood group. Resistant parasites were shown to acquire host decay accelerating factor (DAF), a 70 kDa glycoprotein attached to the membrane of NHuE by a GPI anchor. IgG2a mAb anti-human DAF (IA10) immunoprecipitated a 70 kDa molecule from ^{125}I-labeled schistosomula pre-incubated with NHuE and inhibited their resistance to complement-dependent killing* in vitro. *Incubation of schistosomula with erythrocytes from patients with paroxismal nocturnal hemoglobinuria (PNHE) or SRBC, which are DAF-deficient, did not protect the parasites from complement lesion. Supernatant of 100,000 x g collected from NHuE incubated for 24 h in defined medium was shown to contain a soluble form of DAF and to protect schistosomula from complement killing. Schistosomula treated with trypsin before incubation with NHuE ghosts did not become resistant to complement damage. On the other hand, pre-treatment with chymotrypsin did not interfere with the acquisition of resistance by the schistosomula. These results indicate that,* in vitro, *NHuE DAF can be transferred to schistosomula in a soluble form and that the binding of this molecule to the parasite surface is dependent upon trypsin-sensitive chymotrypsin-insensitive polypeptide(s) present on the surface of the worm.*

Key words: *Schistosoma mansoni* schistosomula – complement – human decay accelerating factor

Parasites use a variety of strategies to endure immune attack, persisting and reproducing in their hosts. *Schistosoma mansoni* developed a particular ability to influence the vertebrate host by escaping immune damage and at same time, directing this attack to the newly incoming schistosomula, thus preventing superinfection and death of the host (Smithers & Terry, 1969). This phenomenon designated concomitant immunity (Smithers & Terry, 1969), was suggested to be dependent on the acquisition of host antigens by the developing parasite (Smithers et al., 1969; McLaren & Terry, 1982). The host antigen hypothesis proposes that molecules from the host incorporate into the surface of the parasite disguising the worm as a self structure. This hypothesis was strongly supported by the detection of a variety of host molecules on the surface of adult schistosomes recovered from experimental animals (Clegg et al., 1971; Dean & Sell, 1972; Dean, 1974; Sher et al., 1978; McLaren, 1980). On the other hand, the suggestion that the immune system would be tolerant to the parasite surface bearing a self coat was negated by further work demonstrating that antibodies, although unable to mediate killing the worm, bind to the surface of adult *S. mansoni* (McLaren et al., 1978; Simpson & Cioli, 1982).

To explain the paradox of escape of the adult worm despite the antibody binding, we recently proposed a novel hypothesis on the role of host antigens. In our view, the acquisition of host antigens would not prevent the antibody production and binding. Instead, it would block the later stages of surface damage to the parasite. Because we had substantial evidence that, protective immunity is dependent on the presence of an intact complement system and IgG (Horta & Ramalho-Pinto, 1984, 1987) but not on eosinophils (Goes & Ramalho-

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Pinto, 1981, 1991), we proposed that schistosomula would incorporate host antigens with the biological activity of interfering with complement activation (Ramalho-Pinto, 1987). Two lines of evidence influenced our views. The first one was earlier work by the Mill Hill group in the late sixties and early seventies, demonstrating that host antigens and escape from complement mediated killing could be acquired by schistosomula incubated with monkey red cells (Clegg & Smithers, 1972). The second one was the demonstration of the importance of decay accelerating factor (DAF) present in human erythrocytes in controlling complement activation on those surfaces (Hoffmann, 1969a, b). DAF is a 70-kDa glycoprotein, attached to the cell membrane by an anchor of phosphatidylinositol (GPI) that prevents the assembly of C3 convertases of complement on those surfaces (Nicholson-Weller et al., 1982). As the activity of C3 convertases on its substrate is the main amplification step in the complement cascade its impairment is reflected upon the lytic effect of complement activation by either the classical or the alternative pathways.

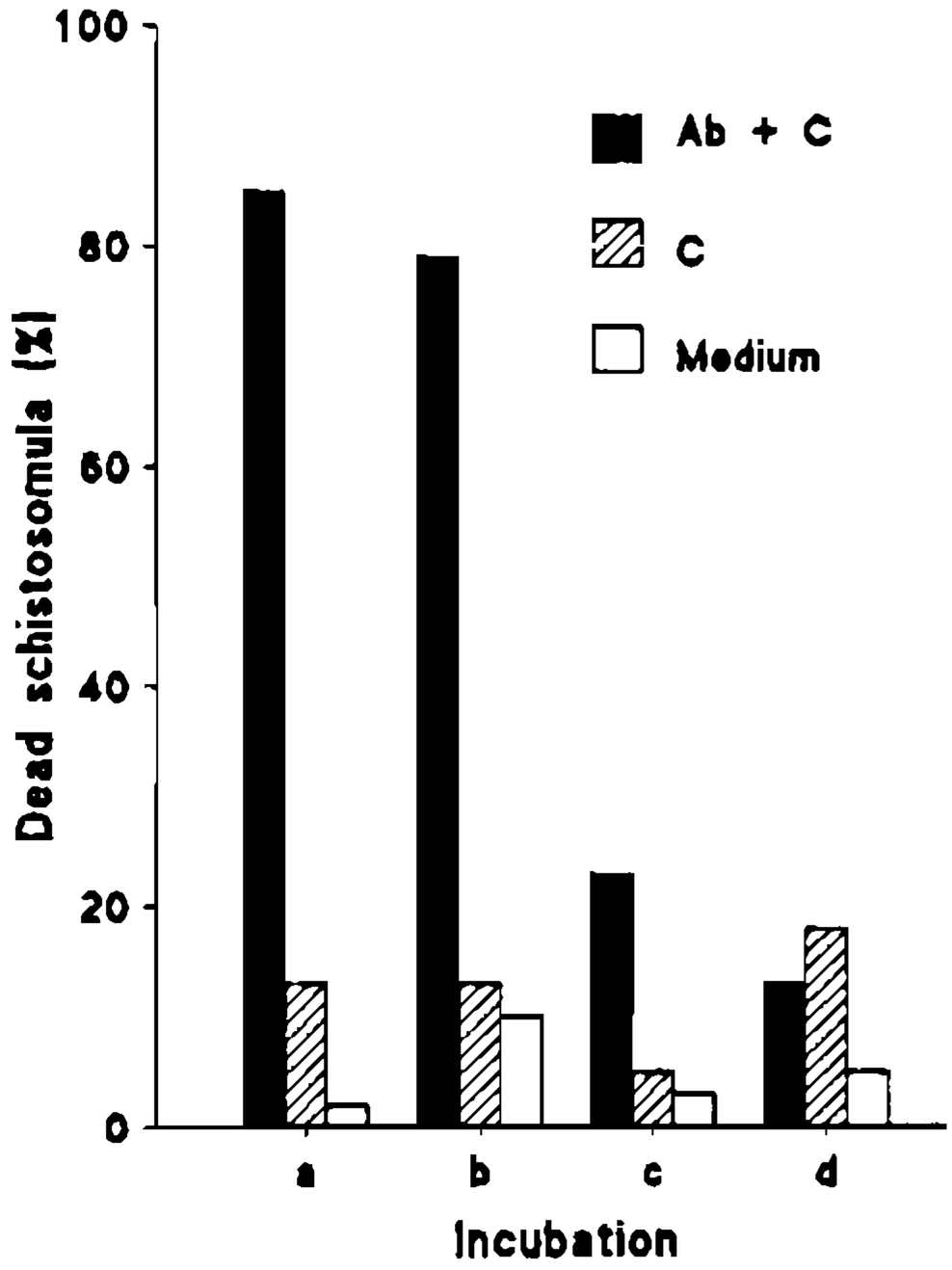

Fig. 1: effect of NHE and FCS in the susceptibility of schla to C-mediated killing. Values shown are the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of 3-h shla (a) or schla previously cultured for 24 h in medium chemically defined (b) or suplemented with FCS (c) or NHE + FCS (d).

In a series of experiments we demonstrated that when a population of schistosomula recently transformed from cercariae is incubated with immune serum from patients with schistosomiasis in the presence of normal guinea-pig serum as a source of complement almost 100% of the larvae are killed. This sensitivity to complement damage by this so called lethal antibody is maintained in schistosomula incubated for 24 h in indefined medium. On the other hand, when parasites are incubated in medium containing 10% fetal calf serum (FCS), the percentage of parasites capable of escaping complement damage is markedly increased. This number is even greater when normal human erythrocytes (NHE) is added to this medium (Fig. 1). The ability to enhance survival of larvae in the presence of antibody plus complement is the same among different ABO or Rh blood groups. (Fig. 2). This result indicated that the protective factor is not contained in the blood group determining molecules. In further experiments RPMI 1640 was replaced by Iscove's medium and the need for FCS was eliminated. Under these conditions the incubation of schistosomula for 24 h in 10% NHE usually promoted the survival of more than 65% of the schistosomula when assayed for lethal antibody (Fig. 3).

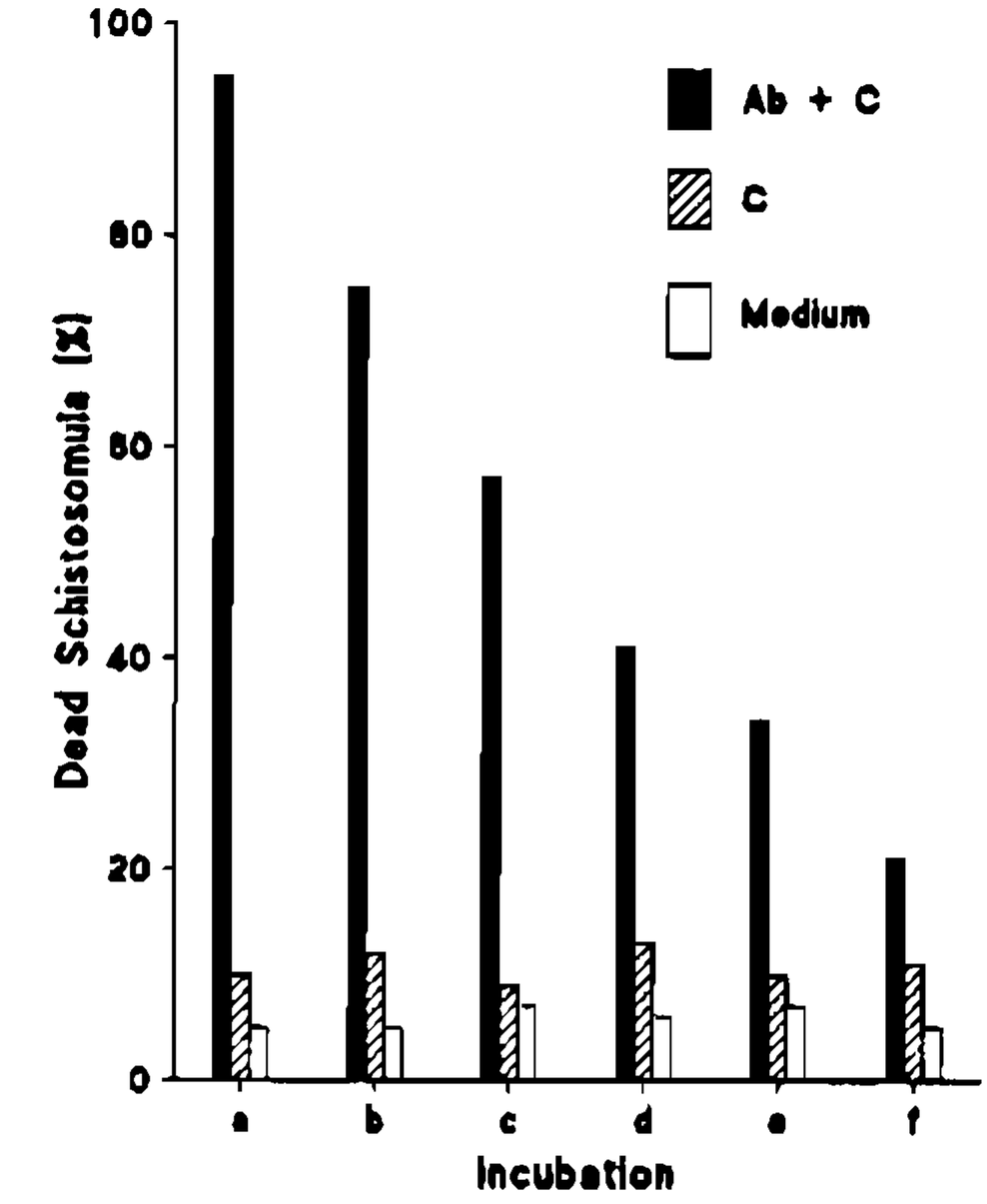

Fig. 2: effect of ABO and Rh blood groups in the susceptibility of schla to C-mediated killing. Data represent the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of schla previously cultured for 24 h in medium chemically defined (M) or suplemented with NHE of blood group O+, A+, B–, AB+, B+ or O–.

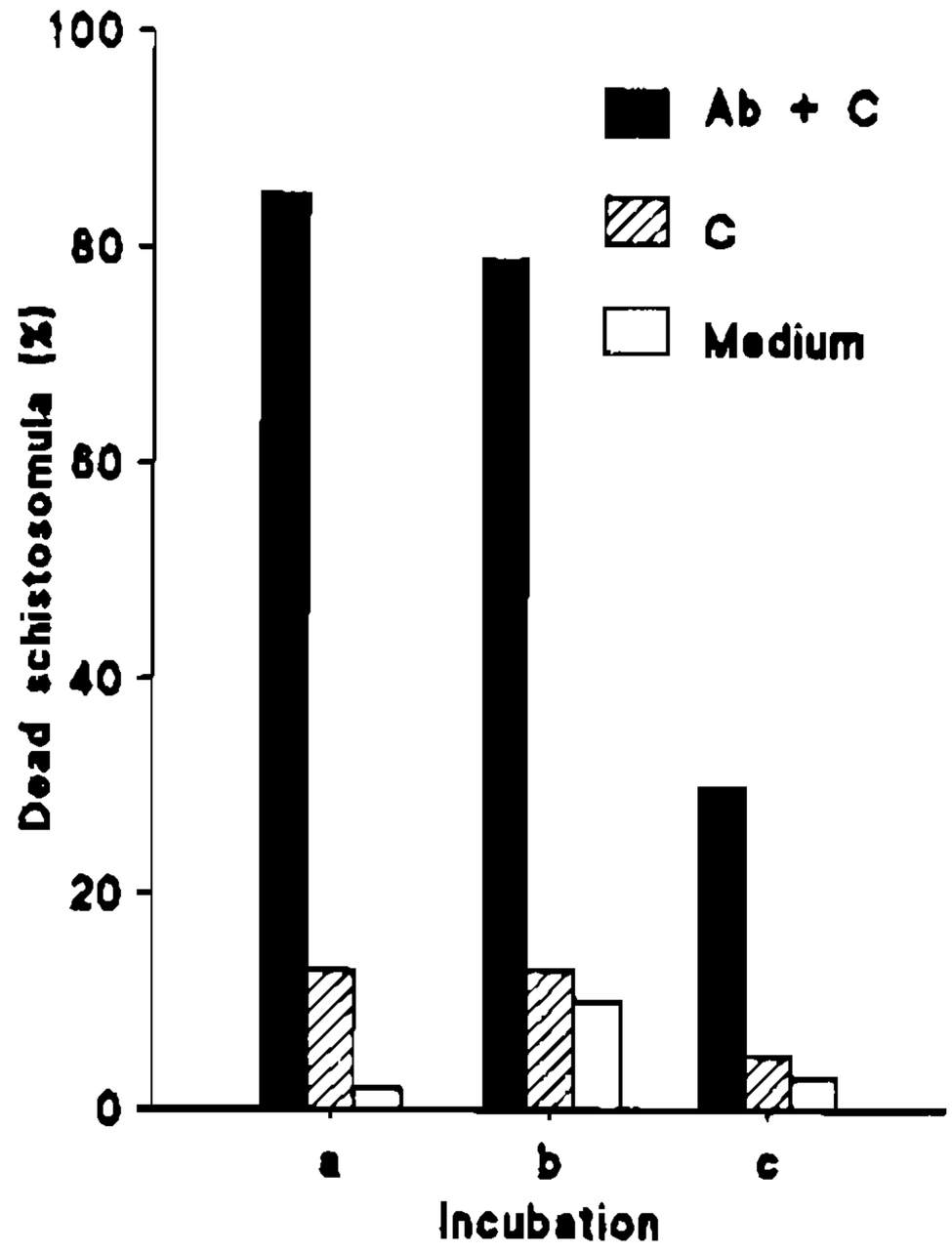

Fig. 3: effect of NHE and FCS in the susceptibility of schla to C-mediated killing. Values shown are the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of 3-h shla (a) or schla previously cultured for 24 h in medium chemically defined (b) or suplemented with NHE (c).

Red cells from different origins present variable sensitivity to lysis by acidic plasma, the Ham test, a phenomenon which correlates with the presence of DAF on those surfaces. Thus while NHE are extremely resistant to the Ham test, sheep red blood cells (SRBC) and erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PHNE), which are naturally DAF-defective, present a marked sensibility in this assay. To explore the possibility that DAF was conferring protection to the schistosomula, we took advantage of the fact that pronase is capable of removing DAF from NHE membrane. The treatment with pronase removes DAF rendering the cells sensitive to lysis in acidic plasma, mimicking the behavior of PNHE (Pangburn et al., 1983), as shown in Fig. 4. On the other hand, treatment of NHE with trypsin removes glycophorin (Marchesi et al., 1972) but does nor interfere with DAF activity on that surface (Sugita et al., 1986), as shown in Fig. 4. Schistosomula incubated with pronase-treated NHE are as sensitive to complement damage as those incubated in defined medium, whereas trypsin treatment of NHE does not interfere with their abilility to confer protection to schistosomula against complement lesion (Horta & Ramalho-Pinto, 1991). Along the same line, when schistosomula are incubated for 24 h with SRBC or PNHE, cells that are naturally deficient in DAF, and then assayed for sensitivity to complement damage, the percentage of dead parasites after the lethal antibody assay was similar to that of the larvae incubated in medium with 10% FCS (Horta & Ramalho-Pinto, 1991). This result further supported the notion that DAF was participating in the protective activity to complement lesion conferred by NHE.

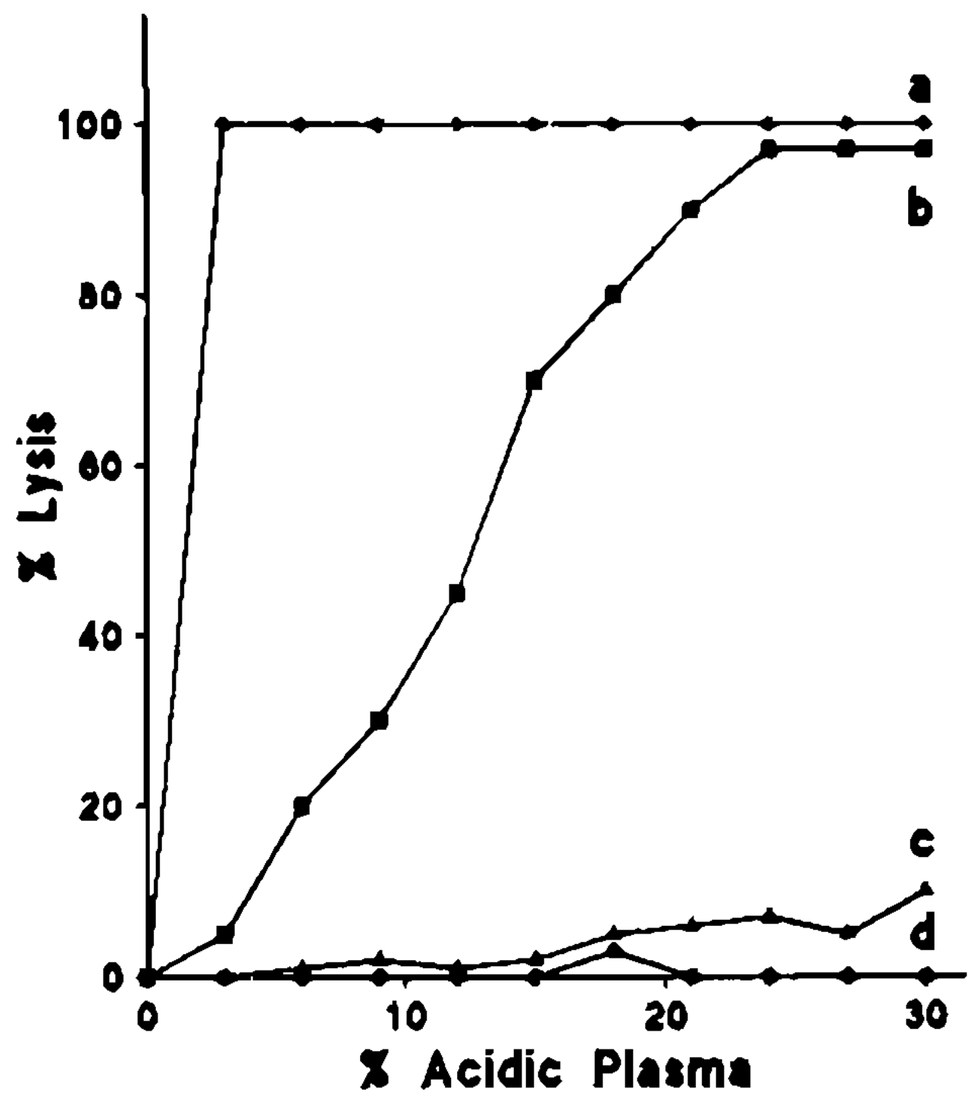

Fig. 4: effect of trypsin- and pronase-treated NHE in the sensibility of NHE to lysis by acidic plama. NHE were treated with trypsin or pronase before incubation with acidic plasma. Each point represents the mean percent of NHE lysated by acidic plama from duplicate samples of SRBC (a), NHE treated with pronase (b) or with trypsin (c) or normals NHE (d).

Further evidence for this was obtained by immunoprecipitation. Schistosomula were incubated with NHE for 24 h, labeled with ^{125}I and extracts were immunoprecipitated with either polyclonal or monoclonal antibodies anti-DAF and subjected to PAGE. In both experiments a radioactive band of 70-kDa was detected by autoradiography, indicating the presence of DAF on the surface of the protected parasites (Horta & Ramalho-Pinto, 1991). To confirm this finding, parasites incubated with NHE were then treated with the monoclonal antibody anti-DAF and assayed for protective activity to lethal antibody. Our findings demonstrated that most of the parasites incubated with Mab anti-DAF were unable to escape

complement death (Horta & Ramalho-Pinto, 1991).

The finding that DAF could be transferred *in vitro* to the surface of schistosomula allowing these parasites to escape lethal antibody damage led us to propose that this molecule could be an important host antigen and account for *S. mansoni* survival in the immunological competent vertebrate.

Considering that *in vitro* DAF was relocating from the NHE to the schistosomula membrane a number of mechanisms had to be considered. As DAF is inserted in the NHE membrane by a glycosilphosphatidylinositol (GPI) anchor, this relocation could be effected by membrane fusion or the insertion of the this hydrophobic moiety into parasite membrane. Another possibility was the cleavage of DAF from the NHE surface by a phosphatidylinositol specific enzyme (PIPL C or D) followed by binding to the parasite surface. This could be accomplished either by covalent binding promoted by an specific enzyme or simply by combination to a specific receptor.

The direct insertion of human DAF by the GPI anchor to a different substrate was observed in experiments by Medof and colleages that detected incorporation of partially purified human DAF in the membrane of SRBC (Medof et al., 1984). The suggestion that the same mechanism of DAF transference was occurring in schistosomes was considered by Alan Sher and co-workers. They observed that schistosomes recovered from guinea-pigs and treated with PIPLC were considerably more sensitive to complement lysis effected by the alternative pathway (Pearce et al., 1990). This view, however, was not supported by our experiments. Evidence collected in our laboratory indicated that the supernatant obtained after centrifugation at 100,000x g of NHE or pink ghosts incubated in culture medium devoid of FCS contained a factor is capable of protecting schistosomula to complement damage (Fig. 5). Although most of the protective activity could still be found in the pellet, the supernatant of 100,000 g retained the protective activity. This result indicates that the protective factor was not associated to membrane structures and was present in a water soluble form. When analyzed by Western blotting with an IgG1 Mab anti-human DAF (SW-10) a band of 70-kDa was detected in the supernatant, indicating that a soluble form of DAF was present (data not shown) and possibly mediating the protective effect. The result with the 100,000 x g supernant of pink ghosts of NHE indicated that the presentation of DAF in the parasite surface was not occurring simply by insertion of the GPI anchor to the schistosomule membrane. On the other hand, the binding of DAF to the surface of schistosomula was strong enough to allow repeated washings of the protected parasites without removal of resistance.

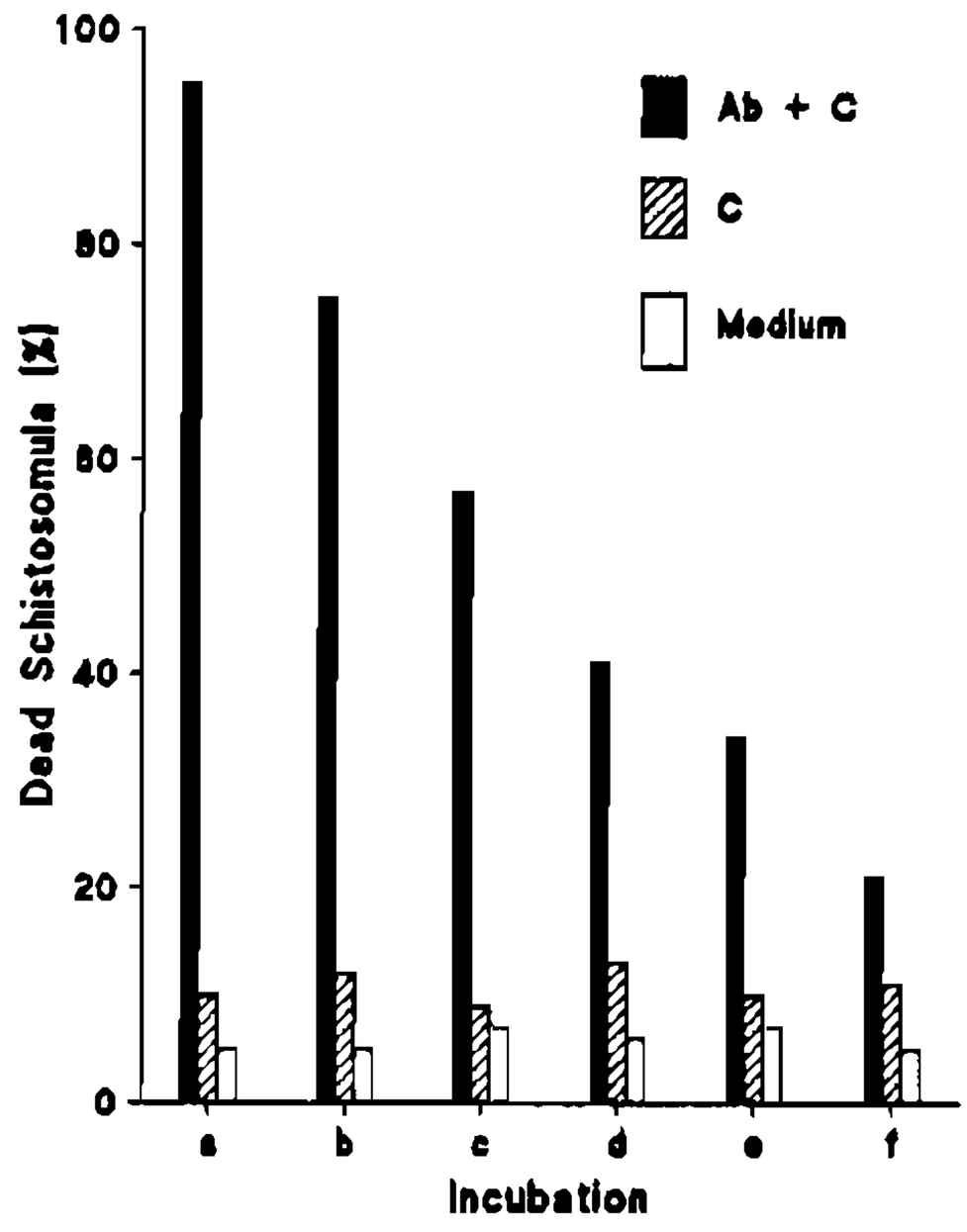

Fig. 5: effect of supernatant of NHE in the susceptibility of schla to C-mediated killing. Values shown are the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of schla previously cultured for 24 h in medium chemically defined (a) or suplemented with 1% (b), 5% (c) or 10% supernatant of NHE (d), pellet (e) or NHE (f).

To investigate the nature of molecule(s) participating in the anchorage of DAF to the parasite surface, we treated recently transformed schistosomula with different proteolytic enzymes previous to the incubation with NHE. When schistosomula were treated with trypsin and then incubated with either pink ghosts of NHE the protective activity was considerably decreased (Fig. 6). This result indicated that trypsin was interfering with the development of protection possibly by removing polypeptide(s) on the surface of schistosomula involved in the binding of DAF to this membrane. On the other hand, pre-treatment with chymotrypsin did not interfere with the acquisition of resistance by the schistosomula (data not shown).

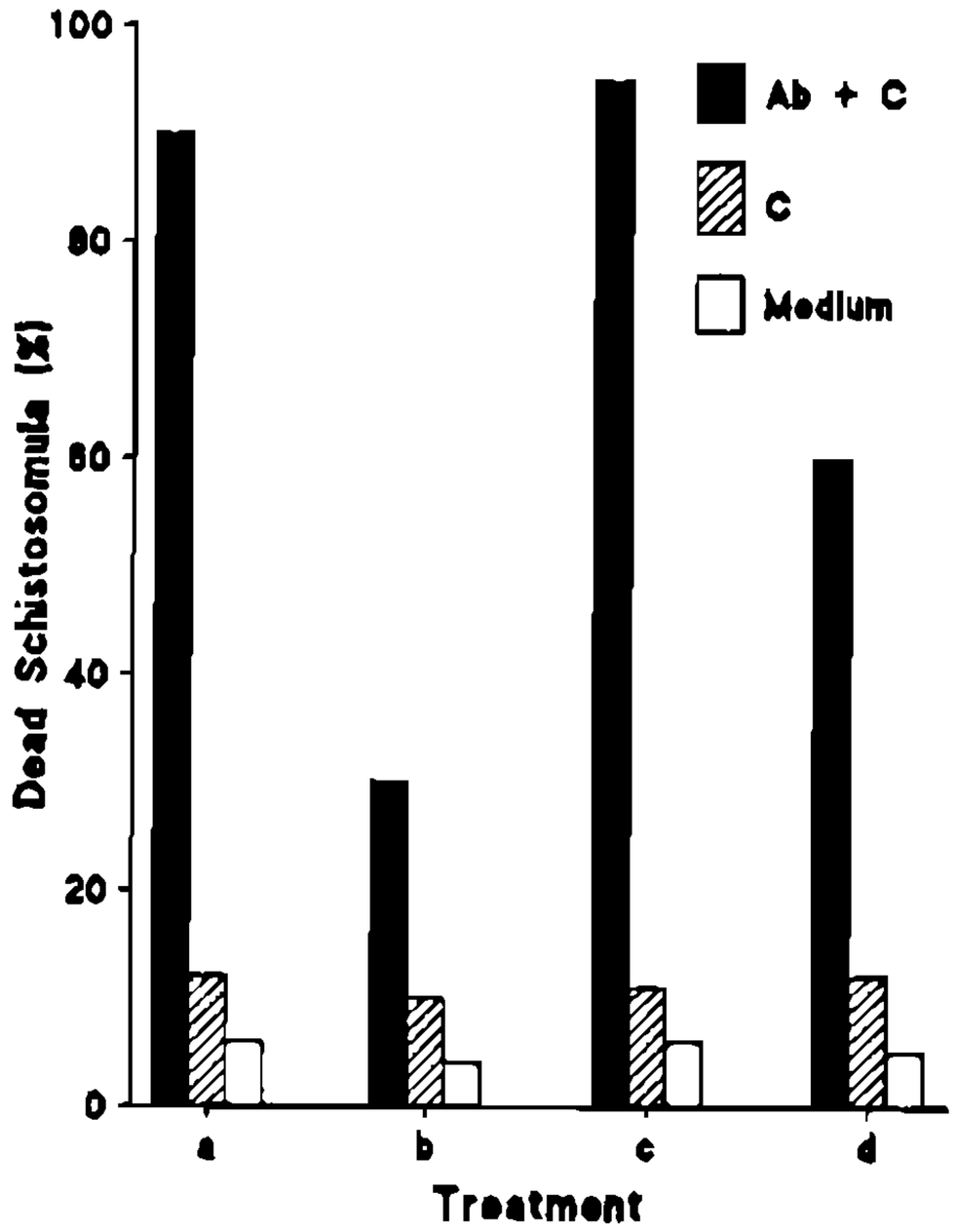

Fig. 6: effect of trypsin in the susceptibility of schla to C-mediated killing. Schla were treated with trypsin before incubation with NHE. Each point represents the mean percent of parasite killing mediated by C or Ab + C from duplicate samples of schla non treated previously cultured for 24 h in medium chemically defined (a) or suplemented with NHE (b) or schla treated previously cultured for 24 h in medium chemically defined (c) or suplemented with NHE (d).

In conclusion, our current study suggests that the NHE DAF can be transferred to schistosomula in a soluble form and that the binding of this molecule to the parasite surface is dependent upon trypsin-sensitive chymotrypsin-insensitive polypeptide(s) present on the surface of the worm. In our view, this polypeptide that seems to function as acceptor for DAF on the surface of the schistosomule may constitute a good candidate as target for protective immunity.

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