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# DECAY ACCELERATING FACTOR (DAF) AS THE HOST ANTIGEN WITH PROTECTIVE ACTIVITY TO COMPLEMENT KILLING OF SCHISTOSOMULA

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*The acquisition of host antigens by* Schistosoma mansoni *was studied by evaluating the resistance of schistosomula to the complement attack mediated by lethal antibody. Schistosomula cultured for 24 hours with intact human erythrocytes (N-HuE) or ghosts of any type of ABO or Rh blood group, showed a marked resistance to complement damage. Sheep red blood cells, pronase-treated N-HuE or erythrocytes from patients with paroxysmal nocturnal hemoglobinuria, which are complement-sensitive cells, were unable to protect schistosomula. Schistosomula protected by N-HuE became again susceptible to complement killing after incubation with a monoclonal antibody anti-DAF. These results indicate that,* in vitro, *host DAF from N-HuE can be acquired by schistosomula surface in a biological active form that protects the parasite from the complement lesion.*

Schistosomes are well-adapted parasites that can present a remarkable longevity in their vertebrate hosts in spite of the presence of a vigorous immune response known to be lethal to newly invading larvae. This ability of the adult worm to survive in a lethal environment requires a highly sophisticated and succesful mechanism of evasion to immune effectors. One of the proposed mechanisms to account for this survival is presented by the host antigen hypothesis (S. R. Smithers et al., 1969, *Proc. Roy. Soc. London B, 171:* 483-494). It suggests that, during their development, the young schistosomula acquire molecules from the host that mask the epitopes which are targets to the immune effectors. Accordingly, no reaction would be mounted directly against the adult worms, interpreted by the host immune system as self. However, it is difficult to envisage such masquerading mechanism as allowing the worm to evade every single mechanism of membrane lesion mediated by the immune system, unless this coat of host origin is so complete that the host becomes tolerant to the parasite. A problem is apparent in this reasoning when one considers that although the adult worms are invulnerable to immune damage, anti-schistosome antibodies can recognize and bind to their surface (D. J. McLaren, 1984, *Parasitology, 88:* 597-611). On the other hand, a series of *in vitro* studies have suggested that the contact of schistosomula with serum as well as erythrocytes renders them protected to immune attack (J. A. Clegg et al., 1972, *Int. J. Parasitol., 2:* 79-98). In addition, antigens of host origin have been detected on the surface of the adult worm (J. A. Clegg et al., 1971, *Nature, 232:* 653-654; D. Dean, 1974, *J. Parasitol., 60:* 260-263). Therefore, an argument arises on the actual role of host antigens on the surface of schistosomes. An attractive hypothesis is that host molecules partially covering the worm would provide a specific functional protection against the lethal immune effector.

Because the protective mechanism that enables the parasite to survive in the immunocompetent host deals with the immune attack mechanism itself, the definition of the mechanism of schistosome killing seems an essential component to solve this puzzle. In this brief review, I will provide some experimental evidence, *in vitro* and *in vivo*, on the major lethal device used by the host to promote the death of the young parasite and then present circumstantial evidence to indicate that acquired erythrocyte surface proteins may provide a functional protective armour for the adult schistosome.

A few years ago we were able to demonstrate that schistosomula are killed *in vitro* by rat eosinophils in the presence of either heat-inactivated rat immune serum or fresh rat normal serum as source of complement (S. R. Smithers et al., 1977, *Am. J. Trop. Med. Hyg., 26;* (suppl), 11-19; F. J. Ramalho-Pinto et al., 1978, *J. Exp. Med., 147:* 147-156). However, in

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later studies using immune serum transfer into irradiated animals, we demonstrated that radiosensitive cells did not play any important role in the protective immunity in rats and mice *in vivo* (A. M. Góes & F. J. Ramalho-Pinto, 1980, *Ciência e Cultura, 32:* 613). Therefore, we looked for another possible effector of the antibody-dependent killing of schistosomula *in vivo.*

By using purified cobra venom factor (M. B. Pepys et al., 1979, *J. Immunol. Meth., 30:* 105-109), we were able to deplete rats and mice of their serum complement haemolytic activity for more than 96 h and show that this treatment completely abolished protective immunity, as measured by the lung recovery assay (A. M. Góes & F. J. Ramalho-Pinto, 1980, *Ciência e Cultura, 32:* 613). These results indicated that in these experimental models an intact complement system is essential to promote the death of schistosomula mediated by antibody *in vivo.*

Further circumstantial evidence for the participation of an antibody-dependent complement mediated cytotoxicity in protective immunity was provided by a time course study of the levels of lethal antibody during schistosome infection in both rats and mice. By using serum titration we could observe a fair correlation between the levels of lethal antibody and protective immunity (M. F. M. Horta & F. J. Ramalho-Pinto, 1987, *Mem. Inst. Oswaldo Cruz,* this issue). Along this line, we also observed that the same fraction of rat IgG that was lethal to schistosomula *in vitro,* containing IgG (2a + 2b), was shown to confer protective immunity to normal recipients (M. F. M. Horta & F. J. Ramalho-Pinto, 1984, *J. Immunol., 133:* 3326-3332).

As our results pointed to an antibody-dependent complement-mediated killing of young schistosomula *in vitro* and *in vivo,* we decided to reinvestigate the mechanism of host antigen protection of *S. mansoni* to complement damage *in vitro.* Our approach was to look for a host antigen that could protect schistosomula by blocking the activation of complement cascade at the surface of the parasite. As the parasite is in close contact with red blood cells during its development, we had our attention focused on the decay accelerating factor (DAF), that protects normal human erythrocytes from complement destruction in the body. DAF is a membrane-associated inhibitor of complement C3 and C5 convertases and is present in all cell types in intimate contact with the complement system. The absence of DAF in red cells renders them highly susceptible to the lytic action of complement, a situation observed in patients with paroxismal nocturnal hemoglobinuria (PNH), an acquired haemolytic disease (M. K. Pangburn et al., 1983, *Proc. Natl. Acad. Sci. USA, 80:* 5430-5034; A. Nicholson-Weller et al., 1983, *Proc. Natl. Acad. Sci. USA, 80:* 5066-5070). The DAF isolated from human erythrocytes is a polypeptide of apparent molecular weight of 70K, and when added to DAF-deficient red cells *in vitro* reincorporates into their membranes and functions by intrinsically blocking complement activation in these cell surfaces (M. E. Medof et al., 1984, *J. Exp. Med., 160:* 1558-1578). If transferred to the schistosomula surface, the host DAF would be an appropriate candidate to perform the functional role of protecting the worm from the membrane attack by complement.

To investigate the hypothesis of participation of DAF as a functional host antigen, we used an updated version of the *in vitro* experiments by Clegg & Smithers (1972, *Int. J. Parasitol., 2:* 79-98). Our system involved two steps: 1) a 24 h incubation of mechanically transformed schistosomula in plain defined medium (NCTC-135), or supplemented with 5% foetal calf serum (FCS) and 1% normal human erythrocytes (N-HuE), to allow the acquisition of host antigens by the parasites, followed by extensive washing to remove non-adherent materials; and 2) an antibody-dependent complement mediated cytotoxicity assay, to evaluate the degree of protection afforded to the schistosomula by the culture components during the first step. Our results showed that 96-100% of 3 h mechanically transformed schistosomula (F. J. Ramalho-Pinto et al., 1974, *Exp. Parasitol., 36:* 360-372) and about 90% of the parasites incubated for 24 h in plain defined medium were sensitive to the lethal antibody effect of complement in the cytotoxic assay. On the other hand, less than 30% of the schistosomula cultured in NCTC-135 supplemented with FCS and N-HuE were still susceptible to complement damage. Schistosomula incubated with only N-HuE or FCS presented an intermediary degree of protection to complement lesion. The observation that the binding of anti-schistosomula IgG to the surface

of protected schistosomula, as measured by an indirect immunofluorescent technique, was equivalent to the binding to non-protected parasites indicated that the protection was occurring at the level of complement activation, not interfering with antibody binding.

To determine which portion of the erythrocytes was contributing to protect the parasites, N-HuE cells were lysed in hypotonic medium and separated by centrifugation into the hemoglobin-rich and the membranous ghosts fractions, and then assayed for their protective activity. Only the fraction containing erythrocyte ghosts protected schistosomula from the lethal effect of complement, indicating the participation of a membrane-associated molecule. This protection did not seem to depend on a particular blood group substance, since N-HuE of any ABO or Rh specificity confered protection to the parasite (F. J. Ramalho-Pinto et al., 1986, *Brazilian J. Med. Biol. Res., 19:* 652A).

To determine the biochemical nature of the molecules being transfered from the erythrocytes to the parasite, we extracted lipids from the ghost fraction of N-HuE with N-butanol and assayed both lipidic and proteic fractions. Only the proteic fraction rendered schistosomula non-susceptible to complement damage, indicating the participation of erythrocyte membrane proteins in the protection of parasites.

To further characterize the N-HuE surface proteins that facilitates the escape of schistosomes from immune damage by complement, intact human erythrocytes were treated with proteolytic enzymes and assayed for their protective activity towards parasites. Two enzymes were used: pronase, which removes DAF from the cell surface, rendering them as susceptible to lysis by complement as PNH erythrocytes (M. K. Pangburn et al., 1983, *Proc. Natl Acad. Sci. USA, 80:* 5430-5434), and trypsin which removes glycophorin, a major erythrocyte surface glycoprotein (V. T. Marchesi et al., 1972, *Proc. Natl Acad. Sci. USA, 69:* 1445-1449), without affecting DAF (Y. Sugita et al., 1986, *J. Biochem., 100:* 143-150). The treatment of intact N-HuE with affinity-purified trypsin for 2 h at 37 °C depleted N-HuE from glycophorin as shown by PAS staining after SDS-PAGE. Trypsin-treated N-HuE still retained all the ability to confer protection from complement damage. This result indicated that glycophorin was not responsible for the protection of schistosomula afforded by human erythrocytes. On the other hand, treatment of N-HuE with pronase abolished the ability of N-HuE to protect schistosomula from the lethal antibody activity. These findings indicated that DAF, an erythrocyte membrane protein resistant to trypsin but sensitive to pronase, could be involved in the protection of schistosomula from the complement damage.

In order to validate the hypothesis that DAF might be involved in protecting schistosomula from complement injury, we took advantage of the occurrence of two erythrocytes naturally devoid of DAF or expressing this molecule in very small quantities: the sheep red blood cells (SRBC) and the erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH-HuE). All DAF-deficient red blood cells were unable to protect schistosomula from the lethal antibody damage (F. J. Ramalho-Pinto et al., 1986, *Brazilian J. Med. Biol. Res., 19:* 652A).

These results strongly supported our hypothesis that host antigen protection to schistosomula, upon contact with N-HuE, is afforded by DAF, a specific complement inhibitor protein present on the surface of N-HuE, which is pronase-sensitive, trypsin-resistant and absent from SRBC and PNH erythrocytes.

To demonstrate that N-HuE molecules were being transferred to the parasite, we used a rabbit anti-HuE antiserum, able to block the acquired protection from complement damage. We showed that parasites incubated with N-HuE membranes or a semi-purified fraction of the membrane proteins, known to contain DAF (A. Nicholson-Weller et al., 1982, *J. Immunol., 129:* 184-189) acquired a band of about 70K, as detected by immunoblotting.

Another molecule present in N-HuE, the homologous restriction factor (HRF) (L. S. Zalman et al., 1987, *J. Exp. Med., 165:* 572-577) have been shown to interfere with complement lytic activity, being also absent in PNH-HuE. Accordingly, to eliminate the possibility that HRF could be the protective molecule, and ascertain the participation of DAF in this protection, we have used a monoclonal anti-DAF antibodies (kind gift of Dr

Victor Nussenzweig) to block protective activity. We demonstrated that the monoclonal antibody directed against membrane DAF completely abolished the complement resistance of schistosomula provided by N-HuE in the lethal antibody assay.

In summary, our results demonstrate that schistosomula of *S. mansoni* can escape complement damage *in vitro* by incubation with intact N-HuE or ghosts but not with erythrocytes naturally (PNH or SRBC) or artificially (pronase-treated HuE) devoid of DAF. The protected parasites acquire a 70K protein from N-HuE origin, which is not glycophorin, and lose their protection when treated with a monoclonal antibody that specifically complex with DAF. These results indicate that DAF is an important host antigen acquired by schistosomula, and could represent the major escape mechanism of schistosomes to immune damage by antibody and complement *in vivo*.

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