

Running title: The immune dependence of chemotherapy.

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**The immune-dependence of chemotherapy in
experimental schistosomiasis.**

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(Summary)

Experimental evidence indicates that immune effector mechanisms can enhance the activity of schistosomicidal drugs. Praziquantel, oxamniquine, hycanthone and antimony were less effective against Schistosoma mansoni infections in mice immunosuppressed by T cell-deprivation, than against comparable infections in normal mice. The schistosomicidal activities of praziquantel, oxamniquine and antimony have been experimentally enhanced by the synergistic action of immune sera. In passive serum transfer experiments a S. mansoni antigen of Mr 27kD with non-specific esterase activity was identified as a potentially sensitive target for the antibodies that interact with praziquantel. Indirect immunofluorescence indicated that this antigen was exposed on the worm surface as a result of praziquantel treatment.

Introduction

The idea that the immune response may enhance the action of drugs against infectious agents was first suggested by Ehrlich (1909), but it seems that not until 1948 was a concerted effort made to test the hypothesis experimentally. In the context of treating malaria infections in chickens with quinine Taliaferro concluded that acquired immunity was indeed an important auxiliary to the drug (Taliaferro, 1948). With respect to schistosomiasis it was suggested by McDonagh as early as 1920 that during clinical use antimony might act "indirectly on the parasites by stimulating the oxidizing action of the protective substance - the protein particles in the serum" and Standen (1955) considered that immune mechanisms, and phagocytosis in particular, could play a part in the final in vivo destruction of adult worms that had been weakened by chemotherapy.

Reduced efficacy of drugs in immunosuppressed mice

In an experimental murine model of schistosomiasis mansoni Doenhoff & Bain (1978) formally demonstrated the involvement of immune mechanisms in drug-induced killing of adult worms. Thus, potassium antimony tartrate was found to be less effective against Schistosoma mansoni in mice that had been immunosuppressed by T-cell deprivation (by a combination of adult thymectomy and administration of rabbit anti-mouse thymocyte serum) than in comparably-infected immunologically intact control mice. The efficacy of the antimonial in the immunosuppressed mice could be restored towards the level observed in intact controls by simultaneous administration of immune serum with the drug, the serum having been obtained from mice with heavy S. mansoni infections. The schistosomicidal effect of antimony in immunologically intact mice was also enhanced somewhat by

transfer of antiserum, in this case the serum donors being rabbits that had been immunized with worm antigens (Doenhoff & Bain, 1978).

A further three schistosomicidal drugs, oxamniquine, hycanthone and praziquantel, in addition to antimony, were found to be less effective against S. mansoni in T-cell deprived mice than in intact mice (Sabah et al., 1985). In contrast, amoscanate, which was tested in the same series of experiments as the other four drugs, seemed to be equally effective in T-deprived and normal mice, and results with niridazole were equivocal (unpublished data). When the same six drugs were compared for their efficacy against S. mansoni infections of different ages (Sabah et al., 1986) or against single-sex male worm infections (Sturrock et al., 1986) differences between the compounds were observed, but the results of these experiments did not make it obvious why the actions of oxaminiquine, hycanthone, praziquantel and antimony should be T-dependent, while those of amoscanate, and perhaps also niridazole, were not. In this series of experiments the results obtained with hycanthone and oxamniquine were, however, very similar, which is to be expected since the quinoline oxamniquine is chemically related to compounds of the mirasane series to which hycanthone belongs.

Enhancement of praziquantel activity with immune sera

In view of the role envisaged for praziquantel in large scale chemotherapy control programmes for schistosomiasis recent experimentation has been mainly concerned with this drug. Some other results, (i.e., in addition to those demonstrating that praziquantel was less effective against S. mansoni in T-cell deprived mice than in normal mice,) also implicated acquired immunity in drug action. Firstly, in normal (immunologically intact) mice praziquantel was less effective against 5 week-old than against 6 week-old S. mansoni bisexual infections (Sabah et al., 1986) and 6 week-old single-sex male worm infections were less susceptible than 12 week-old single-sex infections (Doenhoff et al., 1987). Secondly, the cure-rate increased with increasing intensity of S. mansoni infection in normal mice (Doenhoff et al., 1987). It can be argued that levels of acquired immunity are likely to increase both with time after, and with intensity of, infection.

The apparent difference in the relative susceptibilities of 5 week-old and 6 week-old bisexual infections was exploited in experiments aimed at identifying the schistosome antigens relevant to the immune-dependent action of praziquantel. Thus, 5 weeks after infection with S. mansoni mice were treated orally with subcurative doses of praziquantel and simultaneously with intravenous injections of immune polyspecific rabbit serum. (The rabbits had been immunized with a crude preparation of antigens

released by intact S. mansoni worms maintained for 3 to 4 hours at high density in culture medium in vitro - Doenhoff et al., 1987). The rabbit antisera had no effect on worm numbers when administered alone to mice with 5 week-old infections, but simultaneous administration of such sera with praziquantel improved the cure-rate of the drug significantly in comparison with drug-alone treated controls (Doenhoff et al., 1987; 1988). Synergistic interaction has also been demonstrated between serum from S. mansoni-infected mice and praziquantel in mice that were immunosuppressed by treatment with anti- μ serum (Brindley & Sher, 1987), and more recently immune rabbit sera of the type that synergized with praziquantel were also shown to enhance the activity of oxamniquine against S. mansoni in mice (Lambertucci et al., 1989).

Identification of targets of synergistically-active antibody

The rabbit antisera which were initially shown to interact with praziquantel were polyspecific, and their synergistic qualities varied depending on the batch of worm antigen used for immunization. Attempts were made to raise monospecific antisera against antigens recognized by the reactive sera, and not by the inactive sera, but these were unsuccessful (unpublished results).

It was subsequently found that serum from rabbits that had been repeatedly infected via the ear with unattenuated S. mansoni cercariae (rabbit infection sera) also enhanced the action of praziquantel, and when their immunoprecipitating activity was compared with the polyspecific sera raised against worm culture supernatants, the infection sera were considerably less polyspecific (Doenhoff et al., 1988). A series of monospecific antisera were raised to a predominant cationic antigen recognized by the rabbit infection sera (by the methods described in Dunne et al., 1986), and these sera were in turn found to react synergistically with praziquantel (Doenhoff et al., 1988).

The antigen immunoprecipitated by reactive monospecific sera was identified as an esterase by virtue of its ability to hydrolyze the non-specific esterase substrate beta-naphthyl acetate, and in 'Western blots' of worm extracts run on non-reducing SDS-PAGE these antisera recognized a molecule of Mr 27kD (Doenhoff et al., 1988). A second 200kD antigen has been implicated in the immune-dependent action of praziquantel after a panel of monoclonal antibodies were screened for drug-antibody synergistic activity against worms from athymic (nude) mice (Brindley et al., 1989).

The mode of action of praziquantel and antibody

The pharmacological action of praziquantel is not completely understood, but lipid membranes on the worm surface may become destabilized as a consequence of the drug's lipophilicity and large molecular area (Schepers et al., 1988). One of the early morphological results of praziquantel-induced damage seen by scanning electron microscopy is a disruption of the integrity of the membrane over tubercles on the S. mansoni male worm dorsal surface (Shaw & Erasmus, 1987), and an increase in parasite-specific antigenicity is detectable after drug-treatment of worms in vitro (Harnet & Kusel, 1986). In agreement with these observations it was found that antisera which killed worms synergistically with praziquantel in vivo reacted most intensely in indirect immunofluorescence with the dorsal tubercles of drug-treated male worms (Doenhoff et al., 1988; Brindley & Sher, 1987). Scanning and transmission electron microscopy has confirmed that treatment with drug and antiserum together result in greater morphological damage to the worm surface than either reagent alone (Modha, Lambertucci, Doenhoff, & McLaren, submitted).

It is clear that antibodies against more than one S. mansoni antigen may be synergizing with praziquantel (Doenhoff et al., 1988; Brindley & Sher 1989), but the relationship between them and with those antibodies which are involved in the immune-dependent actions of oxamniquine (Lambertucci et al., 1989) and antimony (Doenhoff & Bain 1978) are not known. On the other hand other monospecific antisera have been found to react in indirect immunofluorescence against antigens exposed on praziquantel-treated worms, but these sera did not synergize well in vivo with praziquantel (Modha et al, unpublished), indicating that only selected antigens which are exposed through drug damage may be sensitive to immune attack.

Relevance to human disease

It remains to be seen whether these experimental observations can be exploited to improve the treatment of human schistosomiasis. Results from experiments with mice indicating that duration of infection and infection intensities affect the outcome of chemotherapy (Doenhoff et al., 1987) may have clinical parallels in the observation that infected children respond less well to treatment than adults (Lambertucci et al., 1982), and schistosomicides are only poorly effective against acute human disease (Lambertucci et al., 1988). Effective immunotherapeutic measures to complement chemotherapy should result from continuing the work to identify, characterize and isolate the different antigens implicated in the immune-dependence of drug action.

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References.

- Brindley, P.J. & Sher, A., 1987. The chemotherapeutic effect of praziquantel against Schistosoma mansoni is dependent on host antibody response. J. Immunol., **139** : 215-220.
- Brindley, P.; Strand, M; Norden, A.P. & Sher, A., 1989. Role of host antibody in the chemotherapeutic action of praziquantel against Schistosoma mansoni: identification of target antigens. Mol. Biochem. Parasitol., **34** : 99-108.
- Doenhoff, M.J. & Bain, J., 1978. The immune-dependence of schistosomicidal chemotherapy: relative lack of efficacy of an antimonial in Schistosoma mansoni-infected mice deprived of their T-cells, and the demonstration of drug-antiserum synergy. Clin. Exp. Immunol., **33** : 232-238.
- Doenhoff, M.J.; Sabah, A.A.A.; Fletcher, C.; Webbe, G. & Bain, J., 1987. Evidence for an immune-dependent action of praziquantel on Schistosoma mansoni in mice. Trans. Roy. Soc. Trop. Med. Hyg., **81** : 947-951.
- Doenhoff, M.J.; Modha, J. & Lambertucci, J.R., 1988. Antischistosome chemotherapy enhanced by antibodies specific for a parasite surface esterase. Immunology, **65** : 507-510.
- Dunne, D.W.; Agnew, A.M.; Modha, J. & Doenhoff, M.J., 1986. Schistosoma mansoni egg antigens: preparation of rabbit antisera with monospecific precipitating activity, and their use in antigen characterization. Parasite Immunol., **8** : 575-586.
- Ehrlich, P., 1909. Chemotherapie von Infektionskrankheiten. Zeitschr. Arztl. Fortbildung, **6** : 721-733.
- Harnet, W. & Kusel, J., 1986. Increased exposure of parasite antigens at the surface of adult male Schistosoma mansoni exposed to praziquantel in vitro. Parasitol., **93** : 401-405.

Lambertucci, J.R., 1988. Treatment of the acute (toxaemic) phase of schistosomiasis with oxamniquine. Trans. Roy. Soc. Trop. Med. Hyg., **82** : 350-351.

Lambertucci, J.R.; Modha, J. & Doenhoff, M.J., 1989. Schistosoma mansoni: the therapeutic efficacy of oxamniquine is enhanced by immune serum. Trans. Roy. Soc. Trop. Med. Hyg., In Press.

Lambertucci, J.R.; Graco, D.B.; Pedroso, E.R.P.; Rocha, M.O.C.; Salazar, H.M. & Lima, D.P., 1982. A double blind trial with oxamniquine in chronic schistosomiasis mansoni. Trans. Roy. Soc. Trop. Med. Hyg., **76** : 751-755.

McDonagh, J.E.R., 1920. The treatment of bilharziasis with antimony. J. Trop. Med. Hyg., **23** : 165-170.

Sabah, A.A.; Fletcher, C.; Webbe, G. & Doenhoff, M.J., 1985. Schistosoma mansoni; reduced efficacy of chemotherapy in infected T-cell deprived mice. Exp. Parasitol., **60** : 348-354.

Sabah, A.A.; Fletcher, C.; Webbe, G. & Doenhoff, M.J., 1986. Schistosoma mansoni: The effect of chemotherapy on infections of different ages. Exp. Parasitol., **61** : 294-303.

Schepers, H.; Brasseur, R.; Goormaghtigh, E.; Duquenoy, P & Ruysschaert, J-M., 1988. Mode of insertion of praziquantel and derivatives into lipid membranes. Biochem. Pharmacol., **37** : 1615-1623.

Shaw, M.K. & Erasmus, D.A., 1987. Schistosoma mansoni: structural damage and tegumental repair after in vivo treatment with praziquantel. Parasitol., **94** : 243-254.

Standen, O.D., 1955. The progress of degenerative changes in schistosomes following the treatment of experimental infections with 1:7 bis(p-dimethylaminophenoxy)heptane. Trans. Roy. Soc. Trop. Med. Hyg., **49** : 416-423.

Sturrock, R.F.; Doenhoff, M.J. & Webbe, G., 1986. "Schistosomiasis". In Experimental Models in Antimicrobial Chemotherapy., vol 3, ed. by M.A. Zande a O. Zak, Academic Press, San Francisco, pp 241-279.

Taliaferro, W.H., 1948. The role of the spleen and the lymphoid-macrophage system in the quinine treatment of gallinaceum malaria. J. Infect. Dis., **83** : 164-180