Immune-dependent thrombocytopaenia in mice infected with *Schistosoma mansoni*

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SUMMARY

As has been shown previously, immunologically intact mice with patent *Schistosoma mansoni* infections had a significantly lower mean platelet number than intact uninfected mice ($P<0{\cdot}0001$). However, platelet numbers in T-cell deprived mice with patent infections were not significantly different from those in uninfected T-cell deprived mice. Also, platelet counts in both the infected and uninfected T-cell deprived groups were not significantly different from those in intact uninfected mice. The *S. mansoni*-induced thrombocytopaenia in mice is thus seemingly immune dependent. Immunologically intact mice with chronic 12-week-old *S. mansoni* infections had IgG antibodies that were reactive in an ELISA-type assay with whole fixed platelets of both mouse and human origin. In Western immunoblots the IgG antibodies from chronically-infected mice reacted in particular against mouse and human platelet antigens of 90, 37 and 30 kDa. Antisera raised from 2 rabbits, immunized respectively with mouse and human platelet antigens, cross-reacted with antigens of the larval, adult worm and egg stages of *S. mansoni*. These results support the hypothesis that an anti-platelet antibody response may be the cause of the thrombocytopaenia observed in mice with patent schistosome infections.

Key words: *Schistosoma mansoni*, schistosome, platelet, thrombocytopaenia, autoimmunity.

INTRODUCTION

A short time after schistosome larvae infect their vertebrate hosts they penetrate a blood capillary or lymphatic vessel and for the rest of their lives they remain intravascular and therefore in constant contact with elements responsible for haemostasis. The interactions of schistosomes with haemostatic defence mechanisms have so far generated considerably less research interest than their interactions with the immunological defence system. We have, however, shown that schistosome eggs interact vigorously with both platelets (Ngaiza & Doenhoff, 1990) and endothelial cells (Ngaiza, Doenhoff & Jaffe, 1993) and that platelet counts are reduced in mice with patent *S. mansoni* infections (Ngaiza & Doenhoff, 1987). The present study investigated whether the schistosome-induced thrombocytopaenia is immune dependent. The results suggest that although it has the semblance of an auto-immune phenomenon the thrombocytopaenia may in fact be due to antibody responses induced by parasite antigens that are cross-reactive with mouse and human platelet antigens.

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MATERIALS AND METHODS

Mice

CBA/Ca mice were bred in-house at the Winches Farm field station of the London School of Hygiene and Tropical Medicine or at the University of Wales, Bangor.

Schistosome infections

An isolate of *Schistosoma mansoni* from Puerto Rico was used throughout this work. Infections of cercariae were applied percutaneously as described by Smithers & Terry (1965).

T-cell deprivation

Following surgical removal of the thymus from 6 to 8-week-old CBA/Ca strain mice, each mouse received 4 subcutaneous injections of 0·25 ml of rabbit anti-mouse thymocyte serum on alternate days starting 2 days after thymectomy. This procedure results in a permanent 90% depletion of circulating T cells (Doenhoff & Leuchars, 1977).

All procedures on experimental animals were performed strictly under the provision of licences

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granted under the UK Animals (Scientific Procedures) Act 1986. All mice were checked for health daily. Results from any animals displaying overt symptoms of schistosomiasis (anaemia, weight loss, cachexia, bloody diarrhoea) were excluded.

Preparation of schistosome antigens and antisera

Schistosome-derived material was prepared as described. Cercarial transformation fluid was obtained by mechanical transformation of cercariae to schistosomula in culture medium *in vitro* as described by Darani *et al*. (1997) and soluble extracts of cercariae, adult worms, and eggs as described by Doenhoff *et al*. (1981).

Chronic infection serum (CIS) was obtained from intact CBA/Ca strain mice that had been infected 12 weeks previously with 25 *S. mansoni* cercariae.

Platelet preparation and anti-platelet antisera

Suspensions of normal mouse and human platelets were obtained from blood collected in citrated anticoagulant solution (0·1 M) and separated from erythrocytes and nucleated blood cells by differential centrifugation as described by Polley & Nachman (1981). For storage, platelets in suspension were centrifuged, the supernatants removed and the platelet pellets stored at −20 °C.

For preparation of rabbit anti-whole platelet antisera, pellets of approximately 10^{10} mouse or human platelets were thawed in 2 ml of HEPES-buffered modified Tyrode's solution, sonicated and emulsified with an equal volume of Freund's adjuvant. Ten 0·1 ml volumes of the respective emulsions were injected in separate subcutaneous sites. The injections were repeated 6 times at 2-week intervals, after which the rabbits were exsanguinated and their sera stored at −20 °C.

Blood platelet counts

Counts on platelets in blood samples from control and infected mice were performed using phase-contrast microscopy as described by Ngaiza & Doenhoff (1987), adapted from Brecher & Cronkite (1950).

Enzyme-linked immunosorbant assay (ELISA)

Washed platelets in suspension were fixed in 4% paraformaldehyde and used as antigen to coat the wells of microtitration plates for detection of anti-platelet IgG antibodies from the serum of CBA/Ca mice chronically infected with *S. mansoni*. Indirect ELISA was performed as described (Voller, Bidwell & Bartlett, 1976).

Electrophoresis and Western immunoblotting

Washed platelet antigens or schistosome antigens were subjected to SDS–PAGE and electrotransferred onto nitrocellulose paper (NCP) as described (Laemmli, 1970; Towbin, Staehelin & Gordon, 1979).

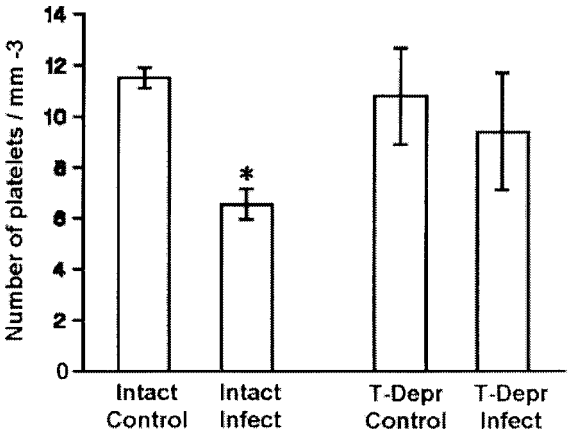

Fig. 1. The effect of *Schistosoma mansoni* infection on blood platelet counts in immunologically intact and T-cell deprived CBA/Ca mice. Intact Control = immunologically intact uninfected mice; Intact Infect = immunologically intact mice infected 45 days previously with 150 *S. mansoni* cercariae, T-Depr Control = uninfected T-cell deprived mice; T-Depr Infect = T-cell deprived mice infected 45 days previously with 150 cercariae. $n = 5$ in each of the 4 groups. $^*P < 0{\cdot}0001$ when compared with Intact Control.

RESULTS

Fig. 1 shows that 45 days after percutaneous exposure to 150 *S. mansoni* cercariae a group of immunologically intact mice had a lower mean number of blood platelets than a group of intact, but uninfected, mice ($P < 0{\cdot}0001$). However, the group mean number of blood platelets in T-cell deprived mice infected with the same number of *S. mansoni* cercariae was not statistically different from that in the uninfected deprived mice, or in the intact control uninfected mice.

The results in Fig. 1 suggested experiments to investigate whether antibodies were responsible for the reduction in platelet number in intact infected mice. Both mouse and human platelets which had been fixed and allowed to adhere to the surface of microtitration plates were used as targets to detect antibodies in an ELISA-type assay and mice carrying infections of lower intensity but greater chronicity were used because initial experiments indicated that antibody activity could not readily be displayed in sera of mice infected for only 6–7 weeks.

Fig. 2 shows that IgG antibodies that reacted against both species of platelet were detected in sera of mice with 12-week-old *S. mansoni* infections derived from 25 cercariae.

In Western immunoblots antisera from mice of another experiment with chronic (15-week-old) *S. mansoni* infections were found to react against a series of antigens present in extracts of both mouse and human platelets: the reactions given by sera from

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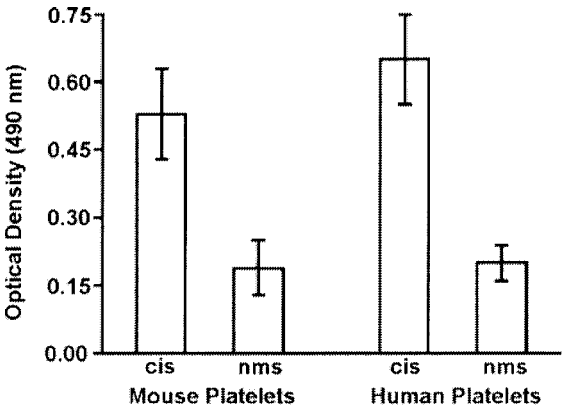

Fig. 2. Enzyme-immunoassay of anti-platelet antibody reactivity in CBA/Ca mice infected with *Schistosoma mansoni*. Reactivity of chronic infection sera from mice infected 12 weeks previously with 25 *S. mansoni* cercariae (cis) or from strain-, age- and sex-matched uninfected normal mouse controls (nms) against paraformaldehyde-fixed mouse and human platelet antigens. Results are from 6 mice in each group.

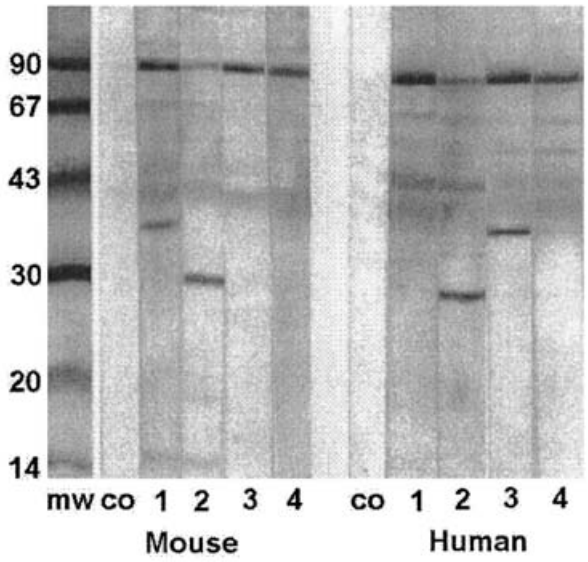

Fig. 3. Western immunoblot reactivity of antisera from mice infected with *Schistosoma mansoni* against electrophoresed mouse and human platelet antigens. Antigenic activity in, respectively, whole mouse platelet (left panel) and whole human platelet (right panel) extracts were probed with sera from 4 representative CBA/Ca mice with 15-week-old *S. mansoni* infections (lanes 1–4) or from a strain-, age- and sex-matched uninfected control mouse (co). mw, Molecular weight standards in kDa.

4 representative infected mice and an uninfected control animal are shown in Fig. 3. Heterogeneity in the IgG antibody responses of the infected animals is evident, but reactivity against a 90 kDa molecule, the antigenicity of which appears to be conserved in both mouse and human platelets, was present in the serum of each of the infected animals tested. The antigenicity of a 30 kDa molecule reacted against by 1 infected mouse serum seems similarly conserved between mouse and human platelets (Fig. 3, lane 2), although heterogeneity in this regard was found with respect to a 37 kDa molecule (Fig. 3, lanes 1 and 3).

Platelet counts were not performed on the mice from which the results in Figs 2 and 3 were obtained, but in a separate comparable experiment in which infections had been established 15 weeks previously with 25 cercariae, the uninfected control group had a mean count of $948 \pm 82 \times 10^3$ platelets/mm^3, while the infected group had a mean count of $384 \pm 329 \times 10^3$ platelets/mm^3 ($P < 0{\cdot}01$).

Fig. 4 shows the reactivity of IgG antibodies in sera of 2 rabbits immunized with homogenates of, respectively, whole mouse platelets and whole human platelets, against *S. mansoni* larval, adult worm and egg antigens in Western immunoblots. A complex of molecules of between 37 and 50 kDa are reacted against in extracts of the 3 different schistosome stages.

DISCUSSION

The present results indicate that the thrombocytopaenia that is induced by *S. mansoni* infections in mice (Ngaiza & Doenhoff, 1987) is immune response-dependent in so far as an infection-induced reduction in platelet number did not occur in T-cell deprived (i.e. immunosuppressed) mice. The phenomenon has some resemblance to an auto-immune antibody response. However, antibodies produced by 2 rabbits immunized with, respectively, mouse or human platelets, were reciprocally reactive against larval, adult and egg schistosome antigens. The antibodies with specificity for platelets generated during the course of infection in mice could thus have been induced by parasite antigens because of their inherent antigenic cross-reactivity with the host, but the result is suggestive of an autoimmune response.

S. mansoni is, of course, primarily a parasitic infection of humans and it is of interest that the IgG antibody reactivity in the ELISA assay against human platelets was as intense as that against mouse platelets. In Western immunoblots of mouse and human platelet antigens probed with the same mouse infection sera there was some heterogeneity between mice with regard to the reactivity of their IgG antibodies, but antibody reactivity against a 90 kDa molecule present in both mouse and human platelets was found in sera of all the mice. The identity of this antigenic molecule, and of the 37 kDa and 30 kDa molecules that reacted most intensely with antibodies in the sera of individual mice, remains to be determined. The mouse with antibody specific for the 30 kDa platelet antigen had a less intense reactivity against the 90 kDa molecule than the other 3 mice, but it is not yet known whether this apparent inverseness is of any significance.

These results may help explain the cause of the thrombocytopaenia that has been observed in

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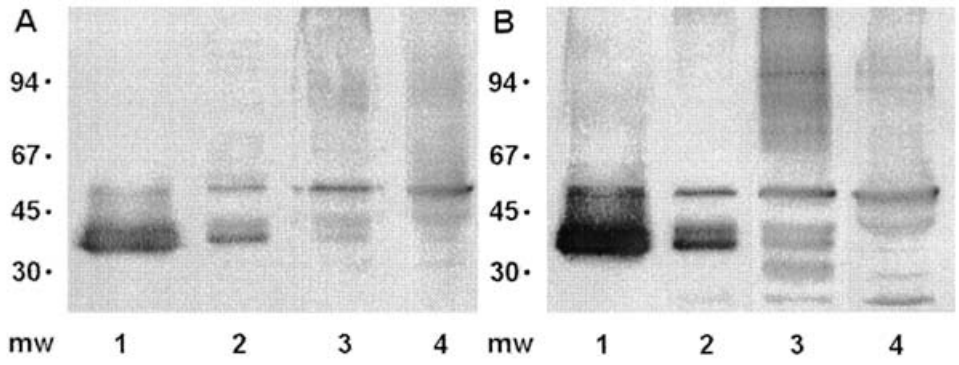

Fig. 4. *Schistosoma mansoni* larval, adult worm and egg molecules that are antigenically cross-reactive with mouse and human platelets. Western immunoblots of *S. mansoni* cercarial transformation fluid (lane 1), soluble antigens from whole cercarial homogenate (lane 2), soluble antigens from whole adult worm homogenate (lane 3), and soluble egg antigens (lane 4) were probed with rabbit antisera raised against (A) whole human platelet homogenate, or (B) whole mouse platelet homogenate. mw, Molecular weight markers in kDa.

S. mansoni-infected mice (Ngaiza & Doenhoff, 1987) and humans (Souza, de Toledo & Borges, 2000). However, further work is required to characterize the platelet antigens that are the targets of the putative autoantibody responses induced by schistosome infections and to determine whether the autoantibodies cause any alteration of platelet function. In this regard preliminary experiments indicate that IgG purified from the serum of infected mice inhibited thrombin-induced, but not epinephrine-induced aggregation of normal mouse platelets *in vitro* (Ngaiza, 1988) and further work on this observation and on antigen characterization is underway.

Anti-platelet antibodies may not be the sole cause of the thrombocytopaenia: other factors may be operating, particularly very early after patency when antibody reactivity is not so readily detected. Schistosome eggs are potently thrombogenic (Doenhoff & Ngaiza, 1990) and this may cause platelets to be consumed.

The adaptive advantages of schistosome-induced thrombocytopaenia need further investigation. We have unpublished evidence to indicate that platelets can kill schistosomula both *in vivo* and *in vitro* and thus may act as an innate defence mechanism against schistosome infection. The reduction in platelet number and modulation of their function that is induced by established patent infections may thus be a mechanism for reducing the effectiveness of the platelet activity against incoming larvae.

On the other hand, a schistosome-induced reduction in platelet number and/or function could have relevance for host well-being. Thus, platelets have atherogenic potential (Farstad, 1998) and atherosclerosis, a disease of coronary and cerebral arteries, is the underlying cause of 50% of deaths in westernized societies (Lusis, 2000). An atherogenic pathway involving thrombin-activated platelets has been described (Gawaz, 2000). We have described elsewhere how *S. mansoni* infections exert an inhibitory effect on atherogenesis in apoE gene knock-out mice (Doenhoff *et al.* 2002) and the seemingly autoimmune, anti-platelet effect of schistosome infections described here may in part explain that observation. The extent to which these experimental results can be extrapolated to human diseases needs, however, to be investigated more fully.

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REFERENCES

BRECHER, G. & CRONKITE, E. P. (1950). Morphology and enumeration of human blood platelets. *American Journal of Physiology* **3**, 365–377.

DARANI, H. Y., CURTIS, R. H. C., McNEICE, C., PRICE, H. P., SAYERS, J. R. & DOENHOFF, M. J. (1997). *Schistosoma mansoni*: anomalous immunogenic properties of a 27 kDa larval serine protease associated with protective immunity. *Parasitology* **115**, 237–247.

DOENHOFF, M. J. & LEUCHARS, E. (1977). Effects of irradiation, anti-thymocyte serum and corticosteroids on PHA and LPS responsive cells of the mouse. *International Archives of Allergy and Applied Immunology* **53**, 505–514.

DOENHOFF, M. J., PEARSON, S., DUNNE, D. W., LUCAS, S., BAIN, J., MUSSALLAM, R. & HASSOUNAH, O. (1981). Immunological control of hepatotoxicity and parasite egg excretion in *Schistosoma mansoni* infections: stage specificity of the reactivity of the immune serum in T-cell deprived mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 41–53.

DOENHOFF, M. J., STANLEY, R. G., GRIFFITHS, K. & JACKSON, C. L. (2002). An anti-atherogenic effect of *Schistosoma mansoni* infections in mice associated with a parasite-induced lowering of blood total cholesterol. *Parasitology* **125**, 415–421.

FARSTAD, M. (1998). The role of blood platelets in coronary atherosclerosis and thrombosis. *Scandinavian Journal of Clinical and Laboratory Investigation* **58**, 1–10.

GAWAZ, M., BRAND, K., DICKFIELD, T., POGATSAMURRAY, G., PAGE, S., BOGNER, C., KOCH, W., SCHOMIG, A.

& NEUMANN, F. J. (2000). Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. *Atherosclerosis* **148**, 75–85.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *London* **227**, 680–685.

LUSIS, A. J. (2000). Atherosclerosis. *Nature*, *London* **407**, 233–241.

NGAIZA, J. R. (1988). Platelet studies in the host–parasite relationship of *Schistosoma mansoni* infections in mice. Ph.D. thesis, University of London.

NGAIZA, J. R. & DOENHOFF, M. J. (1987). *Schistosoma mansoni*-induced thrombocytopenia in mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 655–656.

NGAIZA, J. R. & DOENHOFF, M. J. (1990). Blood platelets and schistosome egg excretion. *Proceedings of the Society for Experimental Biology and Medicine* **193**, 73–79.

NGAIZA, J. R., DOENHOFF, M. J. & JAFFE, E. A. (1993). *Schistosoma mansoni* egg attachment to cultured human umbilical vein endothelial cells: an in vitro model of an early step of parasite egg excretion. *Journal of Infectious Diseases* **168**, 1576–1580.

POLLEY, M. J. & NACHMAN, R. L. (1981). The human complement system in thrombin mediated platelet function. In *Platelets in Biology and Pathology – 2* (ed. Gordon, J. L.), pp. 309–319. Elsevier/North Holland Biomedical Press, Amsterdam, Netherlands.

SMITHERS, S. R. & TERRY, R. J. (1965). The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. *Parasitology* **55**, 695–700.

SOUZA, M. R. D., DE TOLEDO, C. F. & BORGES, D. R. (2000). Thrombocytemia as a predictor of portal hypertension in schistosomiasis. *Digestive Diseases and Sciences* **45**, 1964–1970.

TOWBIN, H., STAEHELIN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences*, *USA* **76**, 4350–4354.

VOLLER, A., BIDWELL, D. E. & BARTLETT, A. (1976). Enzyme immunoassays in diagnostic medicine: theory and practice. *Bulletin of the World Health Organization* **53**, 55–65.