Comp. Biochem. Physiol. Vol. 93B, No. 1, pp. 33–37, 1989
Printed in Great Britain

0305-0491/89 $3.00 + 0.00

ALLELIC FREQUENCY VARIATIONS AT THE MDH-1 LOCUS WITHIN *SCHISTOSOMA MANSONI* STRAINS FROM GUADELOUPE (FRENCH WEST INDIES): ECOLOGICAL INTERPRETATION

ANDRÉ THÉRON, PHILIPPE BRÉMOND and DANIÈLE IMBERT-ESTABLET

Laboratoire de Biologie Animale, UA CNRS 698, Centre de Biologie et d'Ecologie Tropicale et Méditerranéenne, Université, Av. de Villeneuve, 66025 Perpignan Cedex, France (Tel: 68 51 00 51)

(*Received* 19 *July* 1988)

Abstract—1. An isoenzymatic study on *Schistosoma mansoni* from Guadeloupe has been carried out using isoelectric focusing in polyacrylamide gels.

2. Among the seven systems examined (LDH, MDH, G6PD, PGI, PGM, AcP, HK), only MDH showed variation at the MDH-1 locus and mdh-1a allele frequencies were used to characterise eight strains derived either from human or murine hosts, and representative of different transmission sites.

3. In comparison with criteria previously employed to type these strains (ecological context of their transmission sites, cercarial emergence patterns), mdh-1a frequency variations have been correlated to the degree of participation of the murine host reservoir in the parasite transmission dynamics within the different foci of Guadeloupe.

INTRODUCTION

At the intraspecific level, enzymatic variability in schistosomes from man has been studied for several species: *Schistosoma mansoni*, *S. haematobium*, *S. japonicum* and *S. intercalatum*, as reviewed in detail by Rollinson (1984). This research was mainly concerned with parasite strains from very different geographical origins, about which hardly any data on the ecology of their transmission was available. In most cases, when biochemical variations were observed, they did not correlate with any specific characteristic of the natural history of these schistosomes in their originating environment.

In the Guadeloupean focus of intestinal schistosomiasis, the life cycle of *S. mansoni* is noteworthy by the presence of a murine host (*Rattus rattus*) able in some areas of this insular focus to play the role of reservoir host for the disease, alongside the usual human host.

In 1986, Rollinson *et al.*, from an isoenzymatic study of *S. mansoni* from Guadeloupe, tried to differentiate between schistosomes from rat and those obtained from isolates of human origin. Although they noted that "some regional differences in the MDH-1 alleles appear to exist", they came to the conclusion that "there is no suggestion from the present enzyme data of genetic divergence or separate gene pools; the isolates from rats proved indistinguishable from those of man for the seven enzymes studies". Rollinson *et al.*'s results thus confirmed that *S. mansoni* from Guadeloupe could circulate through *R. rattus* and man in natural habitats (Combes and Imbert-Establet, 1980).

Epidemiological surveys carried out in Guadeloupe and the chronobiological polymorphism study of cercarial emergence in *S. mansoni* (Théron, 1984, 1985) showed however that the part played by rat in the dynamics of parasite transmission was more or less important depending on the ecological context of the different transmission sites found on this island.

Starting from an enzymatic polymorphism study of eight *S. mansoni* strains originating from different transmission sites of Guadeloupe, we sought to find out if there were variations depending no longer on the single criterion of murine or human host ascendance of the parasite but rather on the extent of the rat's intervention in the dynamics of parasite transmission in the different localities.

MATERIALS AND METHODS

Origin and characterization of S. mansoni *strains*

The eight strains of *S. mansoni* studied are representative of the three main types of transmission sites (urbanised, mangrove and sylvatic) existing in Guadeloupe and were chronobiologically typed as a function of their cercarial emergence rhythms: early, intermediate and late (Théron, 1985).

Three strains, GH10, GH20 and GH30 were extracted from eggs obtained from the stools of human patients living respectively in Marigot, Ste Rose and Lamentin (Fig. 2(A)), transmission sites of the urbanized type. All these strains have a cercarial chronobiology with an early pattern.

Five strains, GM40, GM50, GM60, GM70 and GM80 were extracted from eggs obtained in the liver of rats (*R. rattus*) naturally infected and captured respectively in Grand Etang, Dubelloy, Blain, Raizet and Labithie (Fig. 2(A)). GM50, GM60 and GM80 come from mangrove transmission sites; their cercarial chronobiology is of the intermediate type. GM70, also from a mangrove site has a late chronobiological pattern. GM40, representative of strains from sylvatic foci has a late cercarial emergence pattern.

All these strains were experimentally maintained for four generations using *Biomphalaria glabrata* from Guadeloupe as intermediate host (exposed each to eight miracidia hatched from eggs collected from the liver of one mouse)

and SWISS OF1 mice as the definitive host (exposed each to 200 cercariae from a pool of 24 shedding snails on average).

Sample preparation

Sexually mature schistosomes were recovered from mice by perfusion at least 12 weeks post infection. Worms were separated according to sex, washed free of excess blood in saline solution and stored in liquid nitrogen.

Individual extracts were prepared by crushing worms on a glass slide in 2–3 μl of distilled water at 1°C. Resulting homogenates were immediately absorbed on 2 × 8 mm (male) or 1 × 10 mm (female) tabs of chromatography paper (Whatman no. 3) that were stored in liquid nitrogen.

Electrophoretic methods

Enzyme analysis were conducted by isoelectric focusing in polyacrylamide gels. Gel solution was prepared according to the Desaga method, with following amounts: Acrylamide (30% in water), 2.81 ml; *N,N'*-methylenediacrylamide (1% in water), 2.48 ml; Ampholytes (Pharmalyte), 1.16 ml; Distilled water, 9.56 ml; Ammonium peroxodisulfate (1.5% in water), 0.50 ml.

0.5 mm thickness polyacrylamide gels were prepared by the LKB capillary casting method, by pouring gel solution on a Gel Bond PAG film between two glass plates with 0.5 mm longitudinal spacers.

Isoelectric focusing was performed at 4°C using a Desaphor HF focusing chamber, a Pharmacia ECPS 3000/150 power supply, a Pharmacia VH1 volthour integrator and a Haake D8-G cryostat.

Enzyme systems included in the study are: Lactate dehydrogenase (LDH: E.C. 1.1.1.27); Malate dehydrogenase (MDH: E.C. 1.1.1.37); Glucose-6-phosphate dehydrogenase (G6PD: E.C. 1.1.1.49); Hexokinase (HK: E.C. 2.7.1.1); Phosphoglucomutase (PGM: E.C. 2.7.5.1); Acid phosphatase (AcP: E.C. 3.1.3.2); Glucose phosphate isomerase (GPI: E.C. 5.3.1.9).

Gels with pH range 3–10 were used for LDH, MDH, G6PD, AcP and GPI, and 4–6.5 for HK and PGM, 50 (male) to 60 (female) samples were applied at 3 cm from the anodal edge of the gel for LDH, MDH, AcP, PGM and GPI, or at 3 cm from the cathodal edge of the gel for G6PD and HK.

Isozyme separation started (without prefocusing of the gel) under 250 V and was conducted with a constant power of 3–5 W. Current decreased of approximately 20 mA during the course of the run and isoelectric focusing was complete when 2400 Vhr were attained.

Enzyme staining

After removing from the cooling plate, gels were rinsed and stained using a 0.5% agar overlay for all systems except MDH and AcP. Recipes used for LDH, G6PD, HK, PGM, AcP and GPI are modified from Wright *et al.* (1979) and can be obtained upon request from the authors. MDH were stained with following solution: 8 ml Tris–HCl 0.5 M pH 7.1 buffer, 38 ml distilled water, 5 ml malic acid solution (10.6 g Na_2CO_3 and 1.34 g D-L malic acid in water, with 100 ml total volume), 25 mg NAD, 15 mg NBT and 1 mg PMS.

Gels were stained at 37°C in the dark until enzyme patterns developed. They were rinsed, fixed in a 7% acetic acid solution and photographed immediately. Isoelectric points were determined by using Pharmacia isoelectric focusing calibration kits.

RESULTS

Enzyme polymorphism in S. mansoni *strains*

Of the seven enzymatic systems tested, five proved invariable (G6PD, HK, PGM, AcP, GPI) and the patterns observed were similar to those obtained by Rollinson *et al.* (1986). Two systems (LDH and MDH) proved polymorphic. LDH gave complex banding patterns and showed little variation which concerned only bands of weak activity; this system was subjected to an important loss of activity under storage conditions and therefore was not taken into account in this study.

For MDH, 2 loci MDH-1 and MDH-2 were recognised (Fig. 1). The MDH-2 monomorphic locus is characterized by a wide band of intensive activity at pH 8.6 with associated, more diffuse activity bands between pHi 8.5 and 7.5. The MDH-1 locus is polymorphic with 2 alleles, mdh-1a and mdh-1b. The homozygotes AA and BB are characterized by an activity at pHi 6.4 and pHi 7.2 respectively. The heterozygote AB shows a three band pattern (pHi 6.4, 6.8 and 7.2) which suggests a dimeric structure for MDH. With experimental cross breedings (Brémond, 1987) we ascertained that the MDH-1 locus located

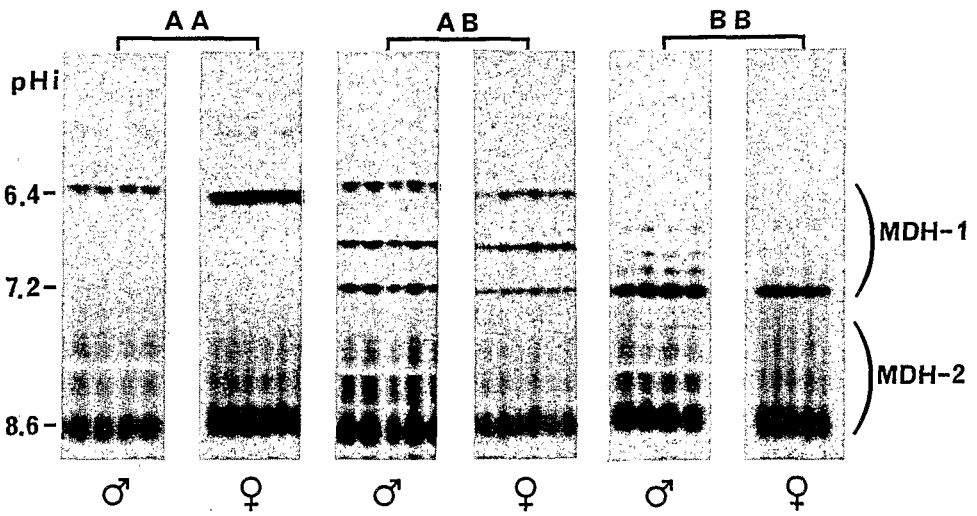

Fig. 1. Malate dehydrogenase patterns in individual extracts of *S. mansoni* from Guadeloupe after separation by isoelectric focusing; three different phenotypes can be distinguished at the MDH-1 locus.

Table 1. Frequencies of mdh-1a allele within *S. mansoni* strains from Guadeloupe

S. mansoni strains	MDH-1 Phenotypes AA	AB	BB	Total sample	mdh-1a frequencies
GH 10	—	—	31	31	0
GH 20	—	—	144	144	0
GH 30	—	—	103	103	0
GM 50	—	23	29	52	0.22 (0.14–0.31)
GM 60	24	70	99	193	0.31 (0.26–0.31)
GM 80	6	117	185	308	0.21 (0.17–0.24)
GM 70	6	79	41	126	0.36 (0.29–0.42)
GM 40	44	40	8	92	0.70 (0.63–0.76)

on the autosomes is maternal-parental twin. Using the technique of individual parasite cloning by intra-molluscan transplantation of sporocysts (Jourdane and Théron, 1980), crossing between AA (male) and BB (female) clones gave only AB phenotype (male and female) at the F1 generation, but AA, AB and BB phenotypes (male and female) at the F2 generation; reverse crossing (female AA × male BB) gave identical results. The MDH-1 locus was therefore selected for the polymorphism study of *S. mansoni* strains from Guadeloupe.

Allozymatic (MDH-1) characterization of S. mansoni *strains*

Details on samples examined, MDH-1 phenotypes and mdh-1a frequencies (f(a)) are reported in Table 1 for the different strains studied. According to these results, these eight strains constitute a heterogeneous complex whose allele (a) frequency varies between 0 and 0.70. With the chi-2 test (at a confidence level of 95%), it is possible to distinguish the degree of affinity of the different strains taken two by two. In

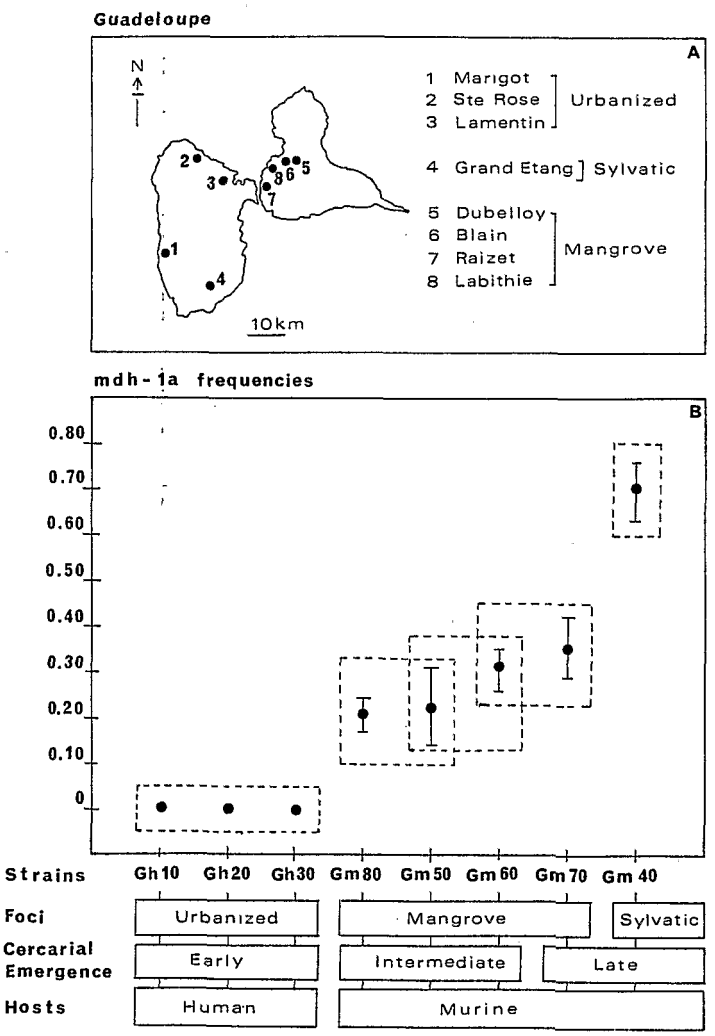

Fig. 2. (A) origin of the eight strains of *S. mansoni* from Guadeloupe and their transmission sites; (B) Correlations between the mdh-1a frequencies of the *S. mansoni* strains studied and their eco-epidemiological characteristics.

this way, three distinct groups with significantly different allelic frequencies are found (Fig. 2(B)).

The first group concerns GH10, GH20, GH30 strains for which f(a) = 0. All these strains are from urbanized foci and have a cercarial emergence of the early pattern.

The second group more heterogenous, concerns GM80, GM60 and GM70 strains for which f(a) ranges between 0.21 and 0.36. These four strains all come from mangrove foci; the first three have an intermediate chronobiological pattern, GM70 has a late cercarial emergence pattern.

The third group is represented by the GM40 strain for which f(a) = 0.70. It is the only strain from a sylvatic focus and its cercarial chronobiology is of the late pattern.

DISCUSSION

The polymorphism study of *S. mansoni* from Guadeloupe carried out on seven enzymatic systems enables us to use the MDH-1 locus to characterize the eight strains of schistosomes from different localities in this caribbean focus.

On the basis of frequency variation in the mdh-1a allele, the *S. mansoni* strains studied fall into three significantly different groups. An analysis of mdh-1a frequency variations in terms of the two criteria used to define the strains, one ecological (i.e. the feature of the strain's natural transmission site) and the other biological (i.e. the cercarial emergence rhythms), show that f(a) always increases when one passes (Fig. 2(B)): (1) from *S. mansoni* from urbanized foci to *S. mansoni* from mangove foci and thence to *S. mansoni* from a sylvatic focus; (2) from *S. mansoni* strains with early cercarial chronobiology to strains with intermediate and then with late chronobiology.

Epidemiological surveys carried out in Guadeloupe, mainly based on the relative importance of human and murine infection prevalences, have shown that the intervention of the murine reservoir host in the dynamics of parasite transmission was always very low in urbanized foci (Théron *et al.*, 1978), more significant in mangrove swamps (Pointier and Théron, 1979) and predominant in sylvatic focus (Combes *et al.*, 1975). This peculiarity of the transmission dynamics of the parasite was later confirmed by a study of polymorphism in the cercarial emergence rhythms of the schistosome populations of these different foci. The early patterns characterize schistosome populations passing preferentially through man whereas intermediate and late chronobiological patterns characterize schistosome populations in whose rat is regularly present in the life cycle of the parasite (Théron, 1985).

Under these conditions, given the correlations previously established (Fig. 2(B)), it seems logical to interpret MDH-1 allelic frequency variations in terms of the degree of participation by murine reservoir host in the life cycle of *S. mansoni* in the different foci of the strains studied: the greater the part taken by the rat in maintaining the parasite cycle in a transmission site, the higher the mdh-1a frequency among *S. mansoni* populations of this focus.

Whereas chronobiological polymorphism, for which differential selection of early and late genotypes can be explained by the adaptative value of these phenotypes (late cercarial emergence favours the rat infection, their activity being mainly crepuscular), the correlation between high frequencies of the mdh-1a allele within schistosome populations and the part taken by the murine host in the life cycle remains to be clarified.

Several hypotheses can be discussed: (a) the correlation observed is purely accidental. This hypothesis seems unlikely; (b) the correlation observed results from selective pressure exerted by the murine host on the parasite; either the allele (a) is neutral to adaptation but is associated to a gene selected by rat, or the allele (a) gives a better adaptative value to schistosomes developing within rat.

Concerning the last point, Bout *et al.* (1978) and Rotmans (1978a, b) suggest that the schistosomes' MDH could have an immunogenic action in mice. So, one might suppose that the immune response of the murine host differs according to whether the schistosomes posses the (a) or the (b) allele. It is however difficult to envisage such a hypothesis since it is hard to see how the immune response, generally induced by surface proteins, could involve MDH which are intracellular, cytoplasmic or mitochondrial.

While the causes which favour the selection of the mdh-1a allele are still to be discovered, our study confirms on the biochemical level the existence in Guadeloupe of separate *S. mansoni* populations in transmission sites which are geographically fairly close together but ecologically and epidemiologically different. Henceforth mdh-1a frequencies appear as valuable markers to appreciate the importance of the reservoir host in the parasite's cycle in this endemic area, and they should now be taken into account in control programs for this parasite.

Acknowledgements—This work received financial support from the UNDP World Bank WHO Special programme for Research and Training in Tropical Diseases, the CNRS (Sciences de la Vie) and from the ORSTOM (Département Santé).

REFERENCES

Bout D., Dupas H., Capron M., El Gazawi A., Carlier Y., Delacourte A. and Capron A. (1978) Purification, immunochemical and biochemical characterization of malate dehydrogenase of *Schistosoma mansoni*. *Immunochemistry* **15**, 613–638.

Brémond P. (1987) Contribution à la biologie des populations de schistosomes: Polymorphisme enzymatique de *S. mansoni* de Guadeloupe; caractérisation électrophorétique (IEF) d'hybrides expérimentaux. *Thèse, Univ. Montpellier.*

Combes C., Léger N. and Golvan Y. J. (1975) Le rôle du rat dans la dynamique de l'endémie schistosomienne en Guadeloupe. *C. R. Acad. Sci. Paris* **281**, 1059–1061.

Combes C. and Imbert-Establet D. (1980) Infectivity in rodents of *Schistosoma mansoni* cercariae of human and murine origin. *J. Helminthol.* **54**, 167–171.

Jourdane J. and Théron A. (1980) *Schistosoma mansoni*: cloning by microsurgical transplantation of sporocysts. *Exp. Parasitol.* **50**, 349–357.

Pointier J. P. and Théron A. (1979) La Schistosomose intestinale dans les forêts marécageuses à *Pterocarpus* de Guadeloupe (Antilles françaises). Ecologie du Mollusque vecteur *Biomphalaria glabrata* et de son parasite *Schistosoma mansoni*. *Ann. Parasitol.* **54**, 43–56.

Rollinson D. (1984) Recent advances in the characterisation of schistosomes of man and their intermediate host. *Trop. Dis. Res. Series* **5,** 401–441.

Rollinson D., Imbert-Establet D. and Ross G. C. (1986) *Schistosoma mansoni* from naturally infected *Rattus rattus* in Guadeloupe: identification, prevalence and enzyme polymorphism. *Parasitol.* **93,** 39–53.

Rotmans J. P. (1978a) *Schistosoma mansoni*: purification and characterization of malate dehydrogenases. *Exp. Parasitol.* **46,** 31–48.

Rotmans J. P. (1978b) *Schistosoma mansoni*: antigenic characterization of malate dehydrogenase isoenzymes and use in the defined antigen substrate spheres (DASS) system. *Exp. Parasitol.* **46,** 49–58.

Théron A. (1984) Early and late shedding patterns of *Schistosoma mansoni* cercariae: ecological significance in transmission to human and murine hosts. *J. Parasitol.* **70,** 652–655.

Théron A. (1985) Le polymorphisme du rythme d'émission des cercaires de *Schistosoma mansoni* et ses relations avec l'écologie de la transmission du parasite. *Vie Milieu* **35,** 23–31.

Théron A, Pointier J. P. and Combes C. (1978) Approche écologique du problème de la responsabilité de l'homme et du rat dans le fonctionnement d'un site de transmission de *Schistosoma mansoni* en Guadeloupe. *Ann. Parasitol.* **53,** 223–234.

Wright C. A., Southgate V. R. and Ross G. C. (1979) Enzymes in *Schistosoma intercalatum* and the relative status of the lower Guinea and Zaire strains of the parasite. *Int. J. Parasitol.* **9,** 523–528.