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Influence of mechanical tail-detachment techniques of schistosome cercariae on the production, viability, and infectivity of resultant schistosomula: a comparative study

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Abstract Two decaudation methods used by *Schistosoma mansoni* cercariae for the production of schistosomula by in vitro transformation were comparatively tested. Both low-pressure syringe passage and vortex mixing of the cercariae produced schistosomula of similar percentages of viability, and the levels of worm recovery following subcutaneous injection in mice were also comparable, though low. By all criteria tested, including simplicity and time requirement, the two methods are equally efficient and are well applicable for mass production of schistosomula.

Introduction

The schistosomulum has frequently been used for assessment of the antischistosome activity of humoral and cell-mediated immune-response components and for immunization experiments (Anwar et al. 1979; Chung et al. 1982; James et al. 1982; Dessein et al. 1983; Cottrell et al. 1989; Gold et al. 1994). Many of the methods used to transform schistosome cercariae into schistosomules are unsuitable for large-scale production. Simulation of natural skin penetration by cercariae using rodent skins, the method most prevalently employed in the past (reviewed by Stirewalt and Uy 1969), can be used to produce relatively small numbers of schistosomula.

For the production of large numbers of schistosomula, two different methods employ mechanical tail detachment followed by maximal separation of the detached tails from the cercarial bodies. One of these methods involves vortex mixing (Ramalho-Pinto et al. 1974), whereas the other applies repeated syringe-needle passage for the same purpose (Colley and Wikel 1974). Centrifugation in discontinuous Percoll gradients, a method developed more recently, efficiently separates bodies from the tails of vortexed cercariae (Lazdins et al. 1982). The present study compares vortex mixing and syringe passage for tail detachment, which are followed by identical treatment in the ensuing steps for production of schistosomula. Methods for the determination of schistosomule viability have recently been comparatively assessed (Gold 1997). In the present study, schistosomula were also assessed for infectivity via the use of a mouse model.

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Materials and methods

Maintenance medium

Medium employed for culture of schistosomules, DSM, consisted of an equal-parts mixture of RPMI 1640 and F-12 (Gibco-BRL, Gaithersburg, Md.) supplemented with 2 m*M* glutamine, 20 m*M* HEPES, 100 U penicillin/ml, and 100 μg streptomycin/ml, with gentamycin being added at 50 μg/ml when necessary.

Cercariae and schistosomula

An Egyptian strain of *Schistosoma mansoni*, kept in Puerto Rican *Biomphalaria glabrata* snails and outbred albino ICR mice, was used throughout this work. We induced cercaria shedding by subjecting infected, water-immersed snails to light for 2 h. Cercariae (5×10^4/ml) were subjected to one of the following tail-detachment procedures:

1. Vortex mixing for 1–2 min as described by Ramalho-Pinto et al. (1974) and later applied by Lazdins et al. (1982); the Vortex Genie 2 Mixer (Scientific Instruments, Inc., Bohemia, N.Y.) was used at the highest speed setting.
2. Syringe-needle passage as modified from the method of Colley and Wikel (1974), using an 18-gauge double-headed needle interconnecting two 12-ml Luer-lock syringes; cercariae were passed through the needle seven times.

Syringe passage was done by one individual in an attempt to ensure a consistent level of pressure on the syringe plungers, and vortex mixing was always performed on the same vortex mixer at the highest speed setting.

Cercariae were checked under a microscope for tail detachment, placed upon 60% isopycnic Percoll (Pharmacia, Uppsala, Sweden) prepared with DSM medium, and treated as described by Lazdins et al. (1982). The resultant cercarial body pellet was washed five times with DSM and further incubated in DSM at 37 °C at a concentration of 10^4 cercariae/ml for 3 h to enable conversion of the cercariae to schistosomula. A proportion of the washed schistosomula was then assessed immediately for viability, whereas the remainder were incubated at 37 °C for an additional 16 h in DSM in an atmosphere consisting of 7.5% CO_2 in air.

Approximately 50 schistosomula that had been incubated for either 3 or 19 h (3 h initially and 16 h subsequent to washing) were introduced into the wells of 96-well flat-bottomed microtiter plates (Sterilin, Stone, UK).

Assessment of viability

The viability of incubated schistosomula was assessed in the wells of microtiter plates by microscopy (Olympus inverted microscope, 100× or 200× magnification; Olympus Co., Tokyo, Japan) in the presence or absence of methylene blue staining as described by Hsü and Hsü (1975), Hsü et al. (1977), and Gold (1997). Viability assessment was based upon granularity, body motility, and methylene blue uptake. Unstained schistosomula were considered dead when they lacked motility and/or were characteristically granular.

Assessment of infectivity

Age-matched male outbred ICR albino mice (n = 5/group) were used. Two groups of mice were inoculated with 3-h schistosomula (one group, with vortexed cercariae; and one group, with syringe-passaged cercariae), two groups of mice were dosed on the following day with 19-h schistosomula (one group, with vortexed cercariae; and one group, with syringe-passaged cercariae), and one group of mice, serving as the control, was inoculated with cercariae that had been suspended for 3 h in water at room temperature. For each inoculation, 400 organisms were injected subcutaneously, and the worm burden was determined after 7 weeks (Duvall and Dewitt 1967).

Results

After both repeated syringe passage and vortexing of cercariae, approximately 90% of the cercariae were recovered as cercarial bodies. Following separation on a 60% Percoll gradient the cercarial body yield ranged between 65% and 80%. The resultant preparations showed complete tail loss in syringe-passaged cercariae when strong pressure was applied, whereas among the vortex-mixed organisms and those passed through syringes at weak pressure application, up to 10% had retained their tails.

Viability data recorded for syringe-transformed and vortexed cercariae are presented in Tables 1 and 2. Our data clearly indicate that vortexing of the cercariae produced markedly higher rates of schistosomule survival than did forceful syringe transformation (Table 1). Low-force syringe passage (up to ten passages) resulted in only a small proportion of cercariae retaining their tails, similar to the proportion observed in the vortexed cercariae. As assessed at 19 h after tail separation, the mortality of these schistosomules had also dropped to levels comparable with those of vortexed cercariae (Table 2). Subsequently, low-pressure syringe passage was employed for the infectivity experiments. The viability of 3-h schistosomula was only 5% higher than that of 19-h worms.

Table 1 Comparison of the viability of overnight (19 h)-incubated schistosomula obtained from cercariae following tail detachment by forceful syringe passage or vortexing[a]

Experiment number	% Dead after syringe passages	% Dead after vortexing
1	35.1 ± 5.2 (±2.1)	18.0 ± 2.8 (±1.2)
2	21.8 ± 8.6 (±3.5)	14.0 ± 4.5 (±1.8)
3	27.6 ± 3.7 (±1.4)	15.8 ± 2.8 (±1.1)
4	18.2 ± 10.3 (±4.2)	10.9 ± 5.2 (±2.1)
5	24.6 ± 8.4 (±4.2)	15.2 ± 5.3 (±2.6)
6	25.2 ± 5.3 (±2.6)	7.7 ± 3.6 (±1.8)

[a] Results are expressed as mean values ± SD. *Numbers in parentheses* indicate the SEM recorded for 4–7 repetitive readings of duplicate schistosomule samples taken from the incubation flask in each of the viability experiments performed at weekly intervals

Table 2 Comparison of the viability of overnight-incubated schistosomula obtained from cercariae following gentle syringe passage or vortexing[a]

Experiment number	% Dead after syringe passages	% Dead after vortexing
1	22.7 ± 5.5 (±2.3)	16.1 ± 8.6 (±3.5)
2	15.5 ± 10.1 (±4.1)	19.4 ± 11.6 (±4.7)
3	7.0 ± 3.7 (±1.5)	3.8 ± 1.4 (±0.6)
4	21.0 ± 9.1 (±3.7)	19.0 ± 8.0 (±3.3)
5	17.0 ± 7.1 (±2.9)	26.8 ± 6.2 (±2.5)
6	15.4 ± 1.5 (±0.6)	16.6 ± 2.2 (±0.9)

[a] Details concerning readings are essentially the same as those described in the footnote to Table 1

With respect to 3-h-old schistosomula, both viability and infectivity assessments demonstrated no marked difference between syringe transformation and vortexing (Fig. 1), and in many instances the data were almost identical. However, both vortexed and syringe-transformed schistosomula produced markedly lower percentages of adult worm recovery than did the control cercariae injected in parallel with the in-vitro-transformed schistosomula. Furthermore, no correlation could be found between the percentage of viability of the schistosomula and the adult worm recovery produced by either decaudation method in the respective experiments. The same applied for the viability of 19-h-old schistosomula (data not shown).

Discussion

Descriptions of two methods for the mechanical detachment of the tails of cercariae from their bodies were published in 1974 by Colley and Wikel and by Ramalho-Pinto et al. Whereas the former investigators relied on repeated syringe passage through a 22-gauge needle as the shearing force, the latter used vortexing for tail detachment. The ability to separate cercarial bodies from tails using Percoll gradients (Lazdins et al. 1982) provided a means of obtaining almost pure schistosomule cultures for biochemistry, immunology, and molecular-biology

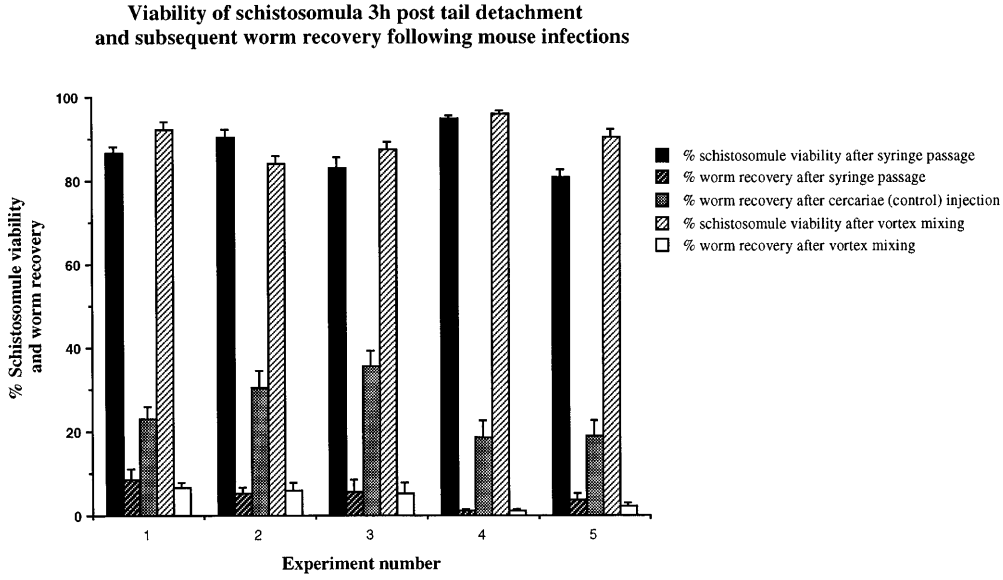

Fig. 1 The viability of schistosomules was assessed by 4–6 duplicate viability counts in each of the 5 experiments depicted. Recovery of adult worms was assessed in 5 mice/group per experiment. Data are given as average values with SEM

studies. Using these techniques, we tried to establish which of the two mechanical separation methods would prove superior according to the following criteria: yield, viability of schistosomula, and schistosomula infectivity.

Prolonged incubation (19 h) in the growth medium without serum reduced the viability to some extent, but when identical numbers of the viable schistosomules were injected into mice the percentages of adult worm recovery were very similar for both incubation times, which indicates that the damage sustained by these viable schistosomules through the mechanical shearing forces did not increase with incubation time. James and Taylor (1976) also demonstrated a similarly low percentage of recovery with schistosomules obtained by syringe transformation followed by incubation. These authors did not have the body-tail separation method of Lazdins et al. (1982) at their disposal. Thus, vortex mixing and the ensuing tedious separation of tails from bodies according to the method of Ramalho-Pinto et al. (1974) produced a very low yield of motile organisms (19.4%), far lower than the 100% or 96.1% yields that they achieved with syringe transformation.

Our results indicate that the two tail-separation methods combined with identical, sequential steps are equally effective in all aspects tested and can be used interchangeably for the mass production of pure suspensions of viable schistosomules for a variety of immunology and biochemistry studies.

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