

## SITES AND MECHANISMS OF SCHISTOSOME ELIMINATION

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Progress in identifying sites and mechanisms of schistosome elimination has depended on advances in understanding basic schistosome migration, which in turn has been closely tied to the development of techniques for locating migrating larvae. We are indebted to a number of people for the development of the tools needed for these studies.

The "lung chop" technique, used to recover migrating schistosomula from rodent lungs by mincing and incubation, was described by Olivier (1952) and used to compare the lung schistosomulum populations of resistant (previously infected) and control mice after a challenge infection by Olivier and Schneidermann (1953). The technique was re-introduced by Clegg (1965) and popularized as a means of studying resistance by Sher et al. (1974). In 1980, Smithers and Gammage introduced a method for recovering schistosomula from the minced, collagenase-treated, incubated skins of infected mice. In addition to others, Smithers also contributed refinements to the adult schistosome recovery procedure based on hepatic portal perfusion, generally considered to be the most reliable assay of resistance to schistosome infection.

In general, recovery techniques have provided reliable information about the relative sizes of schistosome populations in the skin, lungs and liver of normal and resistant hosts. Ironically, they have sometimes provided misleading information in the model with which some of them were first used, the chronically infected mouse. It is now clear that the difficulties encountered in recovering live larvae from the lungs and livers of previously infected mice, and much of the resistance induced in mice by a previous infection, are consequences of egg-induced pathological changes in host tissues and hemodynamics. Because of the complications arising from immunologically nonspecific mechanisms of worm elimination, this model will not be discussed further.

There have been many contributors to the methods used for the histopathological evaluation of schistosome migration and elimination, including a number from Brazil. In recent years, those who have influenced the way we use light and electron microscopy to study schistosome include Lichtenberg and various collaborators at Harvard University, McLaren and coworkers at Mill Hill, London, and Wilson and others at the University of York.

Recently an autoradiographic tracking method has become widely used in studies of migration and immunity. Knight et al. (1968) introduced the standard molecular label used in such studies, <sup>75</sup>Se-selenomethionine, and Nansen et al. (1976) and Christensen et al. (1977) contributed to the development of methods for labeling the larvae of *Fasciola hepatica* and *Schistosoma mansoni* respectively. After re-evaluating a number of labeling procedures, Georgi (1982) developed a practical technique for the macroautoradiographic detection and counting of migrating schistosomes in tissue squashes of rodents, and in collaboration with our laboratory first used this technique to compare migration kinetics and elimination in immunized hosts.

The combined efforts of these and many other people have made it possible to obtain the information about routes of schistosome migration which serves as our road map in the search for sites of elimination.

As Georgi et al. (1987) pointed out, much of the available evidence concerning schistosome migration can be taken as support for any of the three major migration models, the active vascular model (according to which larvae migrate against the direction of blood flow from the lungs to the liver via the vena cava), the transdiaphragmatic model (according to which larvae pass directly from the lungs through the pleural cavity and diaphragm into the liver), and the passive vascular model

(according to which larvae leaving the skin are distributed randomly around the body in the direction of blood flow, successful migrators arriving in the liver by chance). Rightly or wrongly, we are most influenced by proponents of the passive vascular model. In 1912, Miyagawa first proposed that schistosome larvae are carried passively around the body with the blood. In recent years, R. A. Wilson and various collaborators have provided a variety of experimental data to support this hypothesis.

What we have found out by a combination of histopathologic, recovery and autoradiographic methods is that migration in normal (non-immune) hosts is similar in a variety of host-schistosome species combinations. The same three major sites of accumulation, skin, lungs and liver, are seen in mice, rats, hamsters and guinea pigs, using *S. mansoni*, *S. Japonicum*, *S. haematobium* and *Schistosomatium douthitti* (See Table I references). A few larvae accumulate in lymph nodes during the skin-to-lung phase of migration, and a few in all other tissues of the body during the lung-to-liver phase. The pattern is essentially the same for all schistosome and host species studied, although the rates of migration differ considerably between schistosome species.

With respect to elimination, we will first ask *WHEN* it occurs, since this turns out to be much easier to answer than *WHERE*. In normal (non-immune) hosts, autoradiographic tracking studies have indicated that a similar pattern may exist in all host-schistosome species combinations (Table I). In all cases studied, nearly all larvae which enter the skin eventually reach the lungs. This is a surprising finding, since it was thought for many years that skin characteristics were important factors in determining host species differences in susceptibility to schistosome infection (Standen, 1953; Stirewalt & Hackey, 1956). Also, in all cases studied essentially all elimination of larvae, as indicated by disappearance of autoradiographic foci from either the whole body or from the three major sites of accumulation, can be attributed to failure of larvae to migrate from the lungs to the liver. Since the missing larvae do not appear in post-lung pre-liver migration sites, it is unlikely that they ever successfully pass through the capillaries of the lungs. The duration of the normal elimination phase varies greatly between parasite species (Georgi, personal communication). *Schistosomatium douthitti* and *Schistosoma japonicum*

migrate rapidly, passing through the lungs between 3 and 7 days after infection, and essentially all of the normal elimination from the body occurs during this period. With *S. haematobium*, the period of migration through the lungs and elimination from the body is much longer, extending over a number of weeks. With *S. mansoni*, this period is intermediate, occurring between 5 and 21 days after infection. When the total numbers of larvae eliminated are compared for the different schistosome species, a direct relationship is seen between rate of passage through the lungs and chance of surviving to adulthood. This observation indicates that the overall risk of elimination of schistosomes from non-immune hosts may be a function of the total time spent in the lungs.

TABLE I

Sites of normal (non-immune) elimination of schistosomes  
Evidence from autoradiographic tracking data

Species	Parasite	Host	Apparent sites of elimination			Ref.**
			Skin	Lungs	Liver	
<i>mansoni</i>		mouse	±	+++	-	(1)
		rat	±	+++	-*	(2)
		hamster	+	+++	-	(3)
		guinea pig	±	+++	-	(4)
<i>japonicum</i>		mouse	±	+++	-	(3)
		hamster	±	+++	-	(3)
<i>haematobium</i>		mouse	±	+++	-	(5)
		hamster	±	+++	-	(3)
<i>douthitti</i>		mouse	±	+++	-	(3)
		hamster	±	+++	-	(3)

\* Autoradiographic analysis was not performed past day 21 of infection, so the self-cure which takes place at about day 28 in the livers of rats infected with *S. mansoni* was not observed.

\*\* References: (1) Georgi, 1982; Georgi, J. R., personal communication; Georgi et al., 1983; Mangold & Dean, 1983; Dean et al., 1984; Wilson et al., 1986; Kamiya et al., 1987; Hsü, S. Y. L., personal communication. (2) Georgi, J. R., personal communication; Knopf et al., 1986. (3) Georgi, J. R., personal communication. (4) Kamiya & McLaren, 1987. (5) Georgi, J. R., personal communication; Georgi et al., 1986.

Against this background of normal elimination, we may next ask when elimination occurs in immunized hosts. With one important exception, a surprisingly consistent pattern of challenge infection migration and elimination is revealed by autoradiographic tracking data (Table II). Rats immunized by a previous *S. mansoni* infection, and rats, guinea pigs, and in some laboratories mice immunized with irradiated *S. mansoni* cercariae all show an absence of elimination in the skin, delayed

migration from skin to lungs, delayed exit from the lungs, and disappearance of larvae at some point after arrival in the lungs and before appearance in the liver. In the guinea pig, an additional phase of immune elimination occurs in the liver. In all of these models (with the same exception), and in irradiated *S. japonicum* cercaria-immunized mice as well, additional lines of evidence confirm these results (Table III); post-skin resistance mechanisms have been demonstrated by showing that (1) lung stage schistosomula injected into the lung vasculature of immune animals are vulnerable to elimination, (2) immune serum antibody can confer protection to naive hosts harboring lung stage infections, (3) peak recoveries of lung stage schistosomula are delayed but not reduced in immunized hosts, and (4) in most cases histopathological examination fails to reveal sufficient numbers of damaged or dead larvae in the skin to account for the levels of immunity observed.

From the above evidence, a strong case can be made that, with the notable exception to be discussed later, and possibly other models such as the rhesus monkey (Hsü et al., 1971; Hsü et al., 1975) about which less is known, most immune elimination takes place after arrival of migrating larvae in the lungs. Since, as in normal hosts, larvae disappear from the body throughout the time they are in the lungs and

do not seem to accumulate in large numbers in post-lung pre-liver sites, it can be hypothesized that the eliminated larvae never successfully migrate from the lungs. The prolonged and coincident periods of residence in the lungs and disappearance of larvae from the body in immunized hosts provide support for the idea that larval elimination, whether normal or immune, may be a function of the total time spent in the lungs. Although specific antibody may be required for immunity in these models, the effect of the required antibody-parasite interaction may be to increase the period of vulnerability to the same, presumably nonspecific, mechanism of elimination that takes place in the lungs of non-immune hosts.

TABLE II

Sites of immune elimination of schistosomes:  
a. Evidence from autoradiographic tracking data

Immuni- zation	Species		Apparent sites of elimination			Ref.***
	Parasite	Host	Skin	Lungs	Liver	
Irrad. cerc.	<i>mansoni</i>	mouse	-	+++	-	(1)
			guinea pig	+++	+	(2)
			-	+++	+	(3)
Previous infection	<i>mansoni</i>	rat*	nd**	+++	-	(4)

\* Immune groups in these experiments were normal recipients of immune serum.

\*\* Not done.

\*\*\* References: (1) Dean et al., 1984. (2) Kamiya et al., 1987; Hsü, S. Y. L., personal communication. (3) Kayima, H., and McLaren, D. J., personal communication. (4) Knopf et al., 1986.

TABLE III

Sites of immune elimination of schistosomes:  
b. Evidence from assays other than autoradiographic tracking

Immunization	Parasite	Species		Worm recovery	Elimination sites (references*) indicated by:		
		Host			Serum transfer	Worm transfer	Histopath. exam.
Irrad. cerc.	<i>mansoni</i>	mouse		lung (1) skin (2)	lung (3) skin (4)	lung (5) skin, lung ( $\pm$ ) (6)	lung (7) skin (8)
		rat		lung (9)	lung (9)	lung (10)	lung (4)
		guinea pig			lung, liver (4)	lung, liver (10)	
	<i>japonicum</i>	mouse rhesus			lung (11)		skin (12)
Previous infection	<i>mansoni</i>	rat		skin (13) lung (14)	skin (15) lung (16)	lung (17)	

\* References: (1) Minard et al., 1978. (2) Miller & Smithers, 1980. (3) Mangold & Dean, 1986. (4) McLaren, D. J., personal communication. (5) Dean et al., 1981; Mangold et al., 1986. (6) Miller et al., 1981; McLaren et al., 1985. (7) Mastin et al., 1983; Lichtenberg et al., 1985; Crabtree & Wilson, 1986. (8) McLaren, D. J., personal communication; Hsü et al., 1983. (9) Ford et al., 1984. (10) McLaren et al., 1985. (11) Moloney et al., 1987. (12) Hsü et al., 1971, 1975. (13) Perez et al., 1974. (14) Mangold & Knopf, 1978. (15) Phillips et al., 1977. (16) Mangold & Knopf, 1981. (17) Knopf et al., 1986.

But what about the exceptions? In the most studied model, the irradiated *S. mansoni* cercaria-immunized mouse, the generality of the simple hypotheses presented above is threatened by the fact that two sets of laboratories have obtained completely different patterns of results for immune elimination (Tables II and III). At both the University of Iowa and the National Institute for Medical Research at Mill Hill, London, several lines of evidence, including autoradiographic tracking data, indicate that, unlike normal elimination, most immune elimination occurs in the skin. Histopathological observations confirm this conclusion in both laboratories, and at Mill Hill consistent support for skin killing is provided by the timing of effects seen in antibody transfer, worm transfer and cell ablation (D. J. McLaren, personal communication) experiments. Most striking is the observation made at Mill Hill that, while approximately 90% of skin penetrating larvae are still detectable 6 days after challenge infection, only about 50% are detectable on day 8. In contrast, in our experiments and in those carried out at the University of York, nearly all skin penetrants are still detectable (most in the lungs) at day 10, with 3 weeks being required for a drop to the 50% level.

This is a true difference! Can it really be that lung but no skin elimination occurs in two laboratories while skin but very little lung elimination occurs in two others? Initial experiments at Mill Hill and in our lab indicate that the differences observed are not attributable to differences in strain of mouse, dose of irradiation of immunizing cercariae, or skin sites used for immunization or challenge. The possibility that parasite strain differences are responsible has not been ruled out and is being examined.

We have been carrying out additional studies on the mechanism of lung-stage immune elimination. As a first attempt to detect damaged or dead *S. mansoni* larvae in irradiated cercaria-immunized mice, we looked for *qualitative* changes in the autoradiographic foci produced by lung schistosomula. Our approach was to measure the optical densities of individual foci by determining the loss in intensity of a narrow beam of light passed through them, and to compare these optical densities with those obtained for known dead (heat-killed, intravenously injected) and known live (liver) larvae

of the same age and from the same batch of radiolabeled cercariae. The results were clear-cut and surprising. Foci produced in the lungs of immunized mice by larvae killed by gentle heat treatment (50 °C for 5 min) produced very low optical densities two days after injection and then disappeared over the next few days. In contrast, lung schistosomula resulting from a challenge infection with cercariae produced optical density frequency distribution curves identical to those produced by known live (recoverable) larvae in the livers of the same mice, and identical to those produced by lung and liver schistosomula in non-immune mice. Thus, at 21 days after challenge, when larvae were disappearing from the lungs of immunized mice at a maximum rate, no disintegrating larvae as indicated by fading autoradiographic foci could be detected.

The next step was to directly examine the larvae producing autoradiographic foci in the lungs 21 days after challenge infection. Serial sections of lungs were coated with photographic emulsion and processed for autoradiography. Microscopic examination confirmed the optical density findings. All foci examined contained apparently undamaged larvae. These data confirm the results of a previous histopathological study (Lichtenberg et al., 1985). In contrast, none of the autoradiographic foci produced by heat-killed larvae contained an intact schistosomulum 2 days after injection of the larvae into the lungs.

An interesting finding in this study was that nearly half of the larvae present in the lungs 21 days after a cercarial challenge were in alveoli. Review of the literature revealed several earlier reports of schistosomula in air spaces (Miyagawa & Takemoto, 1921; Koppisch, 1937; Kagan & Meranze, 1958; Sadun et al., 1958; Lin & Sadun, 1959; Magalhães-Filho, 1959; Lichtenberg & Ritchie, 1961; Wilks, 1967), and Crabtree & Wilson (1986) recently made similar observations in an electron microscopic study. Coulson & Wilson (1988) compared the efficiency of lung chop recoveries performed before and after the entry of 45-50% of lung schistosomula into air spaces (7 and 17 days after infection, respectively), and found that recoveries were similar at the two times. In addition, they found that similar proportions of 7 and 17 day larvae matured when injected into the mesenteric veins of normal mice. These two observations indicated that the prolonged

retention of schistosomula in the lungs of immunized mice, and even their entry into air spaces, does not result in irreversible damage to the larvae.

The combined results of the autoradiographic tracking, optical density, and viability studies discussed above raise the possibility that schistosomula may be eliminated from the lungs of both normal and immunized hosts while still alive. This possibility has been discussed by Georgi et al. (1987) and circumstantial evidence provided in the form of autoradiographic foci in the trachea, esophagus, and gastric and intestinal luminal rinses of mice. It seems likely that the eventual site of death of larvae expelled from alveoli while alive would be the trachea or alimentary canal, although exit of live larvae from the body remains a possibility.

In spite of striking differences, it may be possible that a common immunological process is responsible for schistosome elimination in the skin and lungs. It can be hypothesized that in both cases an antibody-mediated delay in migration sets the stage for the eventual killing of larvae by nonspecific processes. Although delayed migration from the skin of immunized mice, rats and guinea pigs does not result in permanent damage in some laboratories, and there are no "black holes" such as alveoli to fall into in the skin, it can be imagined that the inflammatory reactions elicited by schistosomula as they move through the skin would be more destructive against some schistosome strains or under some experimental conditions than others. If only the fastest migrators escape the skin of mice at Mill Hill or in Iowa, then perhaps this select population continues to migrate quickly through the lungs, as indeed they have been shown to do at Mill Hill (Kamiya et al., 1987), thus resembling the population that escapes the lungs at the University of York and in our laboratory. In other words, in immunized hosts the separation of survivors and victims may take place in the skin under some conditions and in the lungs under others, while in normal hosts selection may generally occur in the lungs.

Though we have learned a lot about the elimination of schistosomes in recent years, we still do not have a clear idea about the molecular events which are responsible for protective immunity. It can be expected that the many

studies on the molecular basis of host-schistosome interaction currently being carried out will provide valuable insights.

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