MIGRATION OF *SCHISTOSOMA MANSONI* SAMBON (TREMATODA, SCHISTOSOMATIDAE) FROM SKIN TO LUNGS IN IMMUNIZED NZ RABBITS (LAGOMORPHA, LEPORIDAE) BY AUTORADIOGRAPHIC ANALYSIS

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ABSTRACT. Skin to lung migration of *Schistosoma mansoni* Sambon, 1907 - (^{75}Se) - selenomethionine-labeled cercariae was tracked by tissue autoradiography on days 1, 4, 6, 8 after challenge, in rabbits immunized with a *S. mansoni* derived saline extract. Either in vaccinated animals or in those of the control unprimed group, the peak of skin schistosomula occurs 24hs after infection. Comparison between peaks of lungs migrating larvae showed that, in control animals, the increase of worm burden in this site, is detected on the 6th day post-infection, differing from immunized rabbits, in which this peak occurs on day 4, when skin and lungs counts are still equivalent, decreasing gradually, showing a different pattern of the *S. mansoni* migration and suggesting that main parasite attrition occurs during the late skin and early lung phases in the immunized group.
KEY WORDS. *Schistosoma mansoni*, migration, autoradiography, immunization, rabbits

Migration of larval *Schistosoma mansoni* Sambon, 1907 has been analyzed by means of autoradiography in a wide range of laboratory animal models, aiming the establishment of migration patterns of the parasites and attrition sites occurring in these experimentally infected hosts (GEORGI 1982; GEORGI *et al.* 1982, 1983; MILLER & WILSON 1978; PEARCE & MCLAREN 1983; DEAN & MANGOLD 1984; WILSON & COULSON 1986; CHANDIWANA 1988; PINTO *et al.* 1990).

Moreover, autoradiographic tracking of *S. mansoni* labeled cercariae is reported as one of the most accurate parameter in the evaluation of induced protection and worm burden quantification in vaccinated animals, compared to their controls.

Previous investigations referring to autoradiographic analysis of migrating *S. mansoni* schistosomula in animal models submitted to different immunizing

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schemes are those of DEAN *et al.* 1984, MANGOLD & DEAN 1984, KNOPF *et al.* 1986, WILSON *et al.* 1986, KARNIJA & MCLAREN 1987, PINTO *et al.* 1987.

Experimental schistosomiasis in the rabbit model has been exaustively investigated in our laboratories and the results obtained so far have demonstrated that an extract containing antigens of adult *S. mansoni*, induces very high levels of protection in rabbits against a challenge of *S. mansoni* cercariae, when immunized animals present a lower parasite burden than that of normal controls (TENDLER *et al.* 1982, 1986, 1991).

The *S. mansoni* migration pattern in the unprimed NZ rabbit was established by autoradiography of tissues (PINTO *et al.* 1990) and the results reported herein are related to the migration occurring in rabbits vaccinated with the same above referred protective *S. mansoni* adult worm extract, compared to normal controls, also by means of autoradiographic analysis.

MATERIALS AND METHODS

PARASITE AND ANIMALS

LE strain of *S. mansoni* is maintained in Swiss Webster mice (*Mus musculus* Linnaeus, 1758) and *Biomphalaria glabrata* Say, 1818. Adult male New Zealand rabbits – *Oryctolagus cuniculus* (Linnaeus, 1758) –, weighting 2kg, were obtained from the Oswaldo Cruz Institute's animal house.

S. MANSONI EXTRACT (SE)

Adult worms were recovered from mice and further processed for the obtainment of the saline extract, as described elsewhere (TENDLER *et al.* 1982, 1986).

IMMUNIZATION SCHEDULE

Eight rabbits received, with 1 wk interval, 2 footpad injections each, of 600 μg of SE emulsified in Complete Freund's Adjuvant (CFA), Difco, containing 1mg/ml *Mycobacterium tuberculosis* Koch, 1882. Twenty-one days after the second injection, the animals received an intraperitoneal injection of the antigen alone, containing 1,000 μg of SE. Control groups consisted of age and number matched unprimed rabbits.

CERCARIAL LABELING

Forty adult *B. glabrata* were infected with 5-7 miracidia each. After 7 wks, infected snails were simultaneously exposed to 20 μCi of (^{75}Se) L-selenomethionine at a specific activity of 20-50 Ci/mmol (Amersham Corporation) for 5hs and labeled cercariae were recovered four days latter.

INFECTION PROTOCOL

Rabbits were infected simultaneously with 1,000 labeled cercariae/animal, percutaneously through the abdominal skin, by the ring method (SMITHERS & TERRY 1965) 90 days after the immunizing booster.

TISSUE AUTORADIOGRAPHY

Tissue specimens (skin and lung) were removed on days 1, 4, 6, 8 after infection, from two animals/ group/day and processed for autoradiography as previously described (PINTO *et al.* 1990). Labeled schistosomula appear as distinct foci of reduced silver. Results are presented as percentage of foci counted for each site/day per group.

RESULTS

Results of migration tracking (Fig. 1), show that by day 1, the peak skin of schistosomula could be detected in immunized and control groups, the first presenting 13.6% more foci than the latter, at this time.

By day 4, 49.1% of the foci could be counted in the skin and 50.9% in the lungs of vaccinated rabbits, compared to the 57.8 and 42.2%, respectively, of the control group.

Peak value for schistosomula counts in lungs of control animals were observed on the 6th day after infection, when 79% were detected in this site, whereas in the immunized group, the foci were reduced to 36.3% at this time.

By day 8, counts were quite similar in both groups, when lung foci represented 27.2 and 36.8% in immunized and control groups, respectively.

Statistical analysis showed that the expressed differences regarding foci counts in the comparison between immunized and control groups are significative ($tc > 1,0\%$).

DISCUSSION

Previous experiments demonstrated that in rabbits, very high levels of protection are conferred by vaccination with SE in CFA. The mean reduction in worm burden recovery is significant with respect to normal controls, resulting in a mean protection of 88.0% (TENDLER *et al.* 1982, 1986, 1991).

The present findings show that the migration pattern in immunized rabbits also differs from that observed in unprimed animals. Days 1 and 6 post-infection are, respectively, the times of peak skin and lung schistosomula accumulation in control groups compared to immunized animals. In the later, the same pattern of migration regarding peak skin 24h after infection is maintained, but with the peak lung accumulation occurring at day 4, when the number of skin foci is almost identical to that shown in the lungs. Pulmonar foci in immunized animals decrease gradually from day 4 onward to become equivalent to those of controls by day 8.

The results presented herein confirm previous data on the *S. mansoni* migration pattern in naive rabbits (PINTO *et al.* 1990). Moreover, they suggest that attrition and worm elimination mostly occur in the late skin and early lung phases in immunized rabbits, differing from the elimination of schistosomes in unprimed rabbits, when most of the loss is detected after lung passage.

A different pattern was also screened, by means of autoradiographic

analysis, in naive and vaccinated Swiss Webster mice, when SE immunized animals presented a delayed arrival of schistosomes in the liver, although major attrition occurred after the lung phase in both groups (PINTO *et al.* 1987).

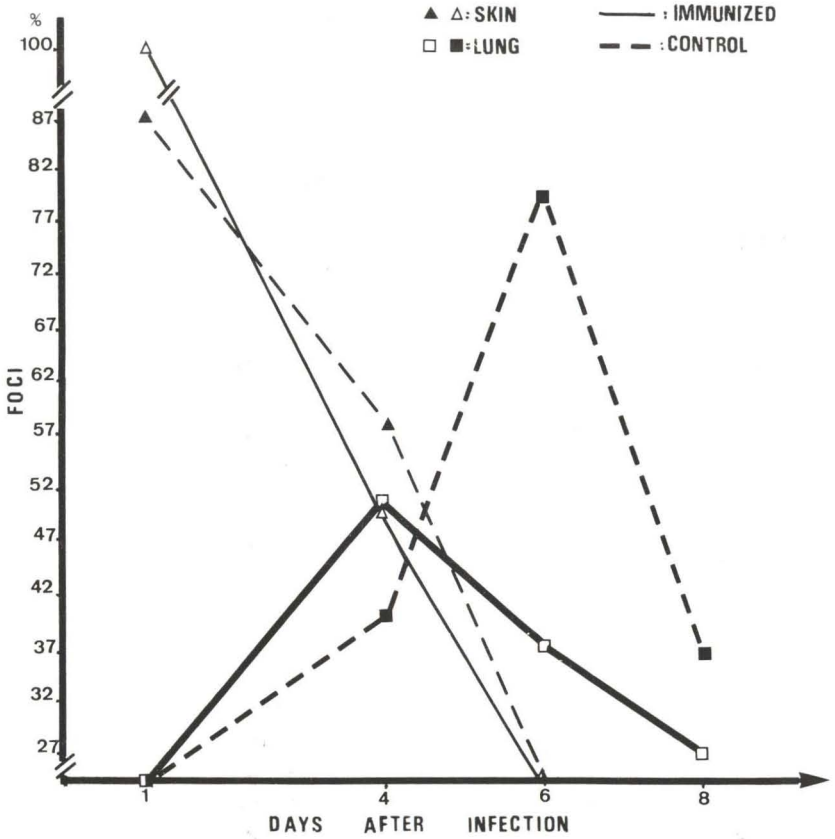

Fig. 1. Pattern of *S. mansoni* migration, from skin to lungs in the immunized and control NZ rabbits.

ACKNOWLEDGEMENTS. To Dr. Nicolau Maués da Serra Freire, Universidade Federal Rural do Rio de Janeiro (UFRRJ), for the graphic transposition of the results.

BIBLIOGRAPHIC REFERENCES

DEAN, D.A. & B.L.MANGOLD. 1984. Autoradiographic analysis of resistance to reinfection with *Schistosoma mansoni* in mice. Evidence that the liver is major site of worm elimination. **Am. J. Trop. Med. Hyg. 33**: 97-103.

DEAN,D.A.; B.L. MANGOLD; J.R. GEORGI & R.H. JACOBSON. 1984. Comparison of *Schistosoma mansoni*_migration patterns in normal and irradiated cercaria-immunized mice by means of autoradiographic analysis. **Am. J.**

Trop. Med. Hyg. 33: 89-96.

CHANDIWANA,S.K. 1988. Use of ^{75}Se tracer and autoradiographic techniques in the study of schistosomiasis. **Parasitology 97**: 480-502.

GEORGI, J.R. 1982. *Schistosoma mansoni*: quantification of skin penetration and early migration by differential external radioassay autoradiography. **Parasitology 84**: 263-281.

GEORGI, J.R.; D.A. DEAN & S.K. CHANDIWANA. 1982. Quantification of *Schistosoma mansoni* in mouse lungs by radioassay and autoradiography of ^{75}Se-labeled schistosomula. **J. Parasitol. 68**: 1092-1095.

GEORGI, J.R.; D.A. DEAN & B.L. MANGOLD. 1983. *Schistosoma mansoni*: temporal distribution of radioselenium-labelled schistosomula in lungs of mice during the first two weeks of infection. **Parasitology 86**: 31-36.

KARNIJA, H. & D.J. MCLAREN. 1987. *Schistosoma mansoni*: migration potential and radiation attenuated parasites in naive guinea pigs. **Exp. Parasitol 63**: 98-107.

KNOPF, P.M.; D. CIOLI; B.L. MANGOLD & D.A. DEAN. 1986. Migration of *Schistosoma mansoni* in normal and passively immunized laboratory rats. **Am. J. Trop. Med. Hyg. 35**: 1173-1184.

MANGOLD, B.L. & D.A. DEAN. 1984. The migration and survival of gamma-irradiated *Schistosoma mansoni* larvae and the duration of host-parasite contact in relation to the indution of resistance in mice. **Parasitology 88**: 249-266.

MILLER, P. & R.A. WILSON. 1978. Migration of the schistosomula of *Schistosoma mansoni* from skin to lungs. **Parasitology 77**: 281-302.

PEARCE, E.J. & D.J. MCLAREN. 1983. Reappraisal of the guinea pig as an experimental host for studies of schistosomiasis mansoni. **Parasitology 87**: 455-464.

PINTO, R.M.; D. NORONHA; M.S.S. ALMEIDA; M. TENDLER; N. KATZ & S.S. PALMA. 1987. *Schistosoma mansoni*: migration patterns in normal and immunized Swiss Webster mice by means of autoradiographic analysis. **Mem. Inst. Oswaldo Cruz 82**: 231-232.

PINTO, R.M.; D. NORONHA; M.S.S. ALMEIDA; M. TENDLER & N. KATZ. 1990. Autoradiographic analysis of *Schistosoma mansoni* migration in the NZ rabbit. **Mem. Inst. Oswaldo Cruz 85**: 91-94.

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

TENDLER, M.; A.O. LIMA; R.M. PINTO; M.Q. CRUZ; H.M. BRASCHER & N. KATZ. 1982. Immunogenic and protective activity of an extract of *Schistosoma mansoni*. **Mem. Inst. Oswaldo Cruz 77**: 275-283.

TENDLER, M.; R.M. PINTO; A.O. LIMA; G. GEBARA & N. KATZ. 1986. *Schistosoma mansoni*: vaccination with adult worm antigens. **Int. J. Parasitol. 16**: 347-352.

TENDLER, M.; M.S.S. ALMEIDA; R.M. PINTO; D. NORONHA & N. KATZ. 1991. *Schistosoma mansoni* - New Zealand rabbit model: resistance induced by

infection followed by active immunization with protective antigens. **J. Parasitol. 77**: 138-141.

WILSON, R.A.; P. COULSON & B. DIXON. 1986. Migration of the schistosomula of *Schistosoma mansoni* in mice vaccinated with radiation-attenuated cercariae and normal mice: an attempt to identify the timing and site of parasite death. **Parasitology 92**: 101-116.

Recebido em 01.II.1994; aceito em 08.VIII.1994.