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Schistosoma mansoni: ACQUIRED RESISTANCE IN MICE BY IMPLANTATION OF YOUNG IRRADIATED WORMS INTO THE PORTAL SYSTEM (1)

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SUMMARY

In two distinct experiments, immature **S. mansoni** worms (LE strain, Belo Horizonte, Brazil), aged 20 days, obtained from the portal system of white outbred mice, were irradiated with 14 and 4 Krad, respectively. Afterwards, the worms were directly inoculated into the portal vein of normal mice. Inoculation was performed with 20 irradiated worms per animal. Fifty days after inoculation, the mice that received 4 and 14 Krad-irradiated worms and their respective controls were infected with **S. mansoni** cercariae (LE strain), by transcutaneous route. Twenty days after this challenge infection, the animals were sacrificed and perfused for mature irradiated (90-day-old) and immature (20-day old) worm counts. Analysis of the results showed that statistically significant protection against cercariae occurred in both groups with irradiated worms.

KEY WORDS: Schistosoma mansoni — irradiated worms — immunoprotection — transplantation of worms.

INTRODUCTION

Acquired resistance in mice, which is always partial and never absolute, has been the subject of various reviews[8, 11, 12, 13]. Recently, and more closely related to the matter of the present study, is the comprehensive review elaborated by DEAN[3]. According to this review, the most consistent data with corroborative results were obtained by three major systems of immunization: 1. Through normal bisexual infection — the so called concomitant immunity; 2. Through infection with irradiated cercariae and schistosomules, and 3. Through heterologous antigens obtained from **Fasciola hepatica**. On the other hand, the pertinent literature shows different works that achieved to induce acquired immunity, through other approaches. However, these works have been discussed by several authors, that were not able to corroborate the same results. In this way, immunoprotection has been obtained by unisexual infection, inoculation of antigens obtained from evolutive stages such as cercariae, schistosomules, worms and eggs, and inoculation of antigens from bacteria, snails, etc. Acquired immunity has been also obtained

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through heterologous infections with other species of the genus **Schistosoma**. Nevertheless, the number of works carried out under this last research line is still very reduced to allow more definite conclusions.

Concomitant immunity — the most effective and studied so far — presents different factors that contribute to its higher or lower manifestation, as follows: worm burden, duration of infection, infection route, and genetics of the host. The system involving irradiated cercariae and schistosomules shows also different variables that interferes with the response. Thus, the resistance so produced depends on the immunizing worm burden, irradiation dose, immunization route, strain of mice, and strain of parasite used[2, 4].

In the present study, an attempt to obtain immunization through young irradiated worms, by using a strain of **S. mansoni** that was not employed for this purpose as yet, and a stage of the parasite rarely used in irradiated schemes for immunization, has been made.

MATERIAL AND METHODS

The LE strain (Belo Horizonte, Brazil) of **S. mansoni** was used in this research. This strain has been maintained at the laboratories of the "Grupo Interdepartamental de Estudos sobre Esquistossomose — GIDE" (Schistosomiasis Research Unit), Federal University of Minas Gerais, Brazil, for more than twenty five years, and it has been used by different authors, as stated in hundreds of original articles published for the same period.

Twenty-day-old **S. mansoni** worms (LE strain, Belo Horizonte, Brazil) obtained from the portal system of mice, as described by BARBOSA et al.[1], were irradiated by means of a Co-60 reactor, at the dose levels of 14 and 4 Krad (not used to this purpose up till now), in two different experiments. About 20 irradiated worms were inoculated into the portal system of white outbred mice, according to the technique reported by ROCHA & COELHO[10]. Fifty days after inoculation, the animals with irradiated worms and respective controls of the same cohort were infected with normal cercariae of **S. mansoni**, transcutaneously. The mice that were inoculated with worms irradiated with 14 Krad were infected with 50 cercariae, whereas the group infected with 4 Krad-irradiated worms were infected with 100 cercariae. Twenty days after the challenge infection, all the animals were sacrificed and perfused for worm recovery, according to the technique prescribed by PELLEGRINO & SIQUEIRA[7]. The difference in the development of the worms, related to their age (irradiated = 90 days; challenge infection = 20 days), allowed the identification of the individuals from the two populations, easily.

RESULTS

As can be seen in Table 1, the immature worms irradiated with 14 and 4 Krad were able to induce protection in mice against cercarial infection. Statistical analysis by means of the Student's t test showed a significant difference between the worm recovery mean of the groups previously inoculated with irradiated worms and that one of their respective controls (4 Krads, $p<0.05$; 14 Krad, $p<0.001$).

DISCUSSION

Considering the data presented in Table 1, it can be seen that the animal groups which received a prime infection with 20 immature worms, irradiated with 4 and 14 Krad, were able to acquire protection against cercarial challenge.

In this way, DEAN et al.[5] using the Puerto Rican Strain of **S. mansoni** achieved immunoprotection through the inoculation of 105 twenty-day-old worms (irradiated with 50 Krad) into the mesenteric veins of inbred mice. Nevertheless, the present paper and the Dean's mentioned paper differ one of the other significantly. The strains of the parasite used were different, and the inocula employed by DEAN et al.[5] were five times higher than those used in the present paper, whereas they used an irradiation dose three times higher.

On the other hand, FORD et al.[6] obtained protection against **S. mansoni** cercariae, by inoculating 400 irradiated (20 Krad) worms, 11 days old, into the mesenteric veins of rats (**Rattus norvegicus**). These findings showed that young

TABLE 1

Immunization of mice by inoculation of 20 young irradiated worms, twenty-day-old, into the portal vein

	Number of cercariae used in the challenge infection	Mean and standard deviation of worm recovery from the challenge infection		Means and standard deviation of irradiated worm recovery	Statistical analysis by the Student's t test P<
		Group of mice previously inoculated with irradiated worms (Exp. I - 14 Krad Exp. II - 4 Krad)	Control group		
Exp. I	50	18.93 ± 4.97 n = 14	12.64 ± 4.95 n = 14	0	0.001
Exp. II	100	25.00 ± 9.42 n = 10	35.20 ± 9.40 n = 10	0.1* ± 0.32	0.05

* only 1 atypical adult worm.

irradiated worms are very effective, when used as immunizing material. However, the rat is considered a "non permissible host"[9] and, therefore, it differs widely from the mouse, which is believed to be a "permissible host". Furthermore, the amount and the age of the irradiated worms used for inoculum differred significantly from those ones used in the present study, as well as from those used by DEAN et al.[5].

Finally, the results presented here, and the findings obtained by other authors engaged in this research line, showed that young irradiated worms inoculated into the portal system of the vertebrate host could promote immunoprotection against cercarial challenge. Thus, further researches will be necessary to clear up some basic aspects related to the immunizing process. Moreover, considering the pertinent literature, it is evident that more detailed studies should be carried out in order to find out how long the immunity induced by the use of irradiated parasites endures in the host.

RESUMO

Schistosoma mansoni: **resistência adquirida em camundongos pela implantação de vermes jovens irradiados no sistema porta.**

Em dois experimentos distintos, vermes imaturos de **Schistosoma mansoni**, com 20 dias de idade, obtidos do sistema porta de camundongos albinos, foram irradiados com 14 e 4 Krad e posteriormente inoculados diretamente na veia porta de camundongos normais. Cada animal, de cada experimento específico, recebeu 20 vermes irradiados. Decorridos 50 dias de inoculação, os camundongos com os vermes irradiados com 4 e 14 Krad e seus respectivos controles foram infectados pela via transcutânea, com cercárias da cepa LE de **Schistosoma mansoni**. No 20º dia após esta infecção desafio, os camundongos foram sacrificados e perfundidos para as contagens dos vermes maduros irradiados (90 dias de idade) e imaturos (20 dias de idade).

A análise dos resultados mostrou que ocorreu proteção, estatísticamente significativa, contra cercárias nos grupos previamente inoculados com vermes irradiados com as doses de 4 e 14 Krad.

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