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EVALUATION OF PROCEDURES USED IN RECENT STUDIES ON INDUCED RESISTANCE AGAINST SCHISTOSOMIASIS IN MICE

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SUMMARY

This study was concerned with the effects of experimental modifications thought to enhance antibody formation against *S. mansoni*. Specifically it explored the effects of a large number of cercariae exposed to a relatively low radiation dose on the course of an otherwise lethal infection. The results herein presented indicate clearly that preinfection with large numbers of irradiated cercariae had an opposite effect to that hoped for in that it increased the worm population in the portal system and the liver egg accumulation in the host. The uniformity and consistency of this apparently paradoxical reaction indicated that this was not a fortuitous result but, rather, a meaningful facet of the host response to the presence of relatively large numbers of worms from the irradiated cercariae, thus leaving little doubt that an aborted infection with irradiated cercariae is a potential menace to the safety of the host.

INTRODUCTION

Lack of success in conveying protection against schistosomes by the use of irradiated cercariae, as demonstrated in our previous studies [1, 2], has been disappointing but perhaps to be expected in view of the extremely weak antigen developed from these cercariae. It seems obvious that a technique which does not permit survival of the worms in the host does not approximate the conditions under which the immunizing mechanism works and the interpretation of results obtained thereby should be limited accordingly.

In the course of a recent study [2] a relation was found between the number of worms developed from irradiated cercariae and the effects on a challenge infection. The study suggested, in view of failure of cercariae irradiated with doses above 2,000r to affect the course of a challenge infection, that the larger the population developed from irradiated cercariae the smaller certain pathological effects brought about by the challenge infection. Therefore, the Author stressed the need for evaluation of the stimulating antigen in terms of worm survival and persistence rather than by suppression of development and migration alone, as caused by the excessively high radiation doses used in earlier studies [1, 5, 6]. The Author then speculated that radiation doses lower than those used in previous studies might lead to higher survival rates of worms in the host. Specifically, it was predicted that exposure to large numbers of cercariae irradiated with 2,000r would increase the antigenic stimuli and this in turn would enhance the immunizing mechanism. However, attempts to induce resistance in mice by exposure to large numbers of irradiated cercariae was unsatisfactory, as will be seen.

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MATERIAL AND METHODS

The experiments were performed with 290 female white Swiss mice that weighed 18 to 22 g at exposure. Each of the 235 test mice was exposed on 3 occasions on alternate days to 500 mixed cercariae irradiated with 2,000r. The remaining 55 mice served as controls for the infectivity of the normal cercariae used in challenging mice. The infected mice were divided in 3 groups. One was used in a study on protection conveyed by transfered humoral factors produced by irradiated cercariae. An account of this study has been published [3]. In the second group the behaviour of irradiated cercariae was studied by worm counts, by male/female ratios and by liver egg counts which were compared with data from suitable control mice. The third group was used to determine to what extent the prior exposure to 1,500 cercariae irradiated with 2,000r affected a challenge with 70 normal cercariae. The biological and physical methods used have already been described [1, 2].

RESULTS AND DISCUSSION

Table I represents information on the worm population developing from irradiated cercariae. At each interval after exposure worms were recovered from the portal system of the host leaving no doubt that some worms from cercariae irradiated with 2,000r can survive and can migrate to the portal system. As for the male/female ratios, females consistently outnumbered males in

TABLE I

Worms and eggs of *Schistosoma mansoni* recovered from mice exposed to 1,500 irradiated cercariae and from mice challenged two months later with 70 normal cercariae. Figure in parenthesis expresses number of mice examined for eggs and worms. Controls received only the challenge infection

Mice examined	Numbers found at various times after challenge					
	2 months	3 months	4 months	5 months	6 months	7 months
Exposed to irrad. C.	47 (4)	45 (5)	32 (6)	30 (7)	35 (8)	36 (9)
Exposed to irrad. C. and challenged ..	46 (10)	36 (5)	48 (8)	49 (8)	33 (5)	36 (10)
Control	9 (5)	16 (5)	9 (4)	12 (5)	7 (4)	*
Male/female ratios						
Exposed to irrad. C.	0.48	0.42	0.50	0.49	0.44	0.53
Exposed to irrad. C. and challenged ..	0.77	0.73	0.72	0.58	0.53	0.48
Control	1.70	1.23	1.06	1.56	1.90	*
Eggs per g of liver						
Exposed to irrad. C.	0	0.7 (0-1.9)	1.2 (0-2.4)	0.7 (0-1.6)	0.4 (0-1.3)	0.6 (0-1.8)
Exposed to irrad. C. and challenged ..	5.4 (1.3-17.9)	10.9 (8.2-14.0)	13.9 (11.8-19.0)	17.0 (12.6-22.9)	16.9 (6.2-30.3)	25.7 (15.6-42.3)
Control	2.3 (1.6-3.5)	10.7 (8.1-13.1)	11.2 (6.3-15.7)	13.2 (12.1-15.0)	12.4 (11.0-14.4)	*

* No survivers in the group of mice
Egg counts ×1,000

populations from irradiated cercariae, while the contrary was characteristic among worms from normal cercariae. The fact that this has been already reported [2] makes the hypothesis of an existing sex difference in the susceptibility of cercariae to the radiation effects sufficiently attractive for explanation of results obtained.

A conscientious effort was made to recover all the eggs in the liver digest from mice exposed to irradiated cercariae and the small numbers recorded in the Table were believed to be due to radiation damage to the reproductive mechanism of worms from irradiated cercariae.

When mice were challenged with 70 normal cercariae (35 ♂ + 35 ♀) we observed no protection of the host by the aborted infection with irradiated cercariae. The worm counts from mice that had received irradiated cercariae before challenge were higher than the worm counts from the controls that had received the challenge dose only (Table I). This was to be expected in view of the high numbers of living worms from the irradiated cercariae. Precautions were taken to eliminate the possibility that these worms would be counted and not those from the challenge infection. In the very early preparations all the worms from the irradiated cercariae could be identified and in no case were they included in data referring to the worm counts from the challenge infection. However, with the passage of time difficulty was experienced in distinguishing them from the normal worms. Therefore convincing evidence indicating clearly that the aborted infection did not affect the worm counts from the challenge infection has been gained by comparing worm counts from test mice with those from both, control mice exposed only to the challenge infection and control mice exposed to irradiated cercariae and unchallenged, as shown in the Table.

When the mice were examined for liver egg accumulation, the egg counts from animals that had received irradiated cercariae before challenge tended to be higher than the egg counts from control mice. The simplest explanation for the origin of the increased egg counts in challenged mice seems to be that some of the worms from irradiated cercariae had matured, paired and contributed to the liver egg accumulation. However, the differences in egg counts between challenged and control mice, as seen in the Table, were above those that could be attributed to egg production by paired irradiated worms, thus making the hypothesis above less attractive for explanation of results obtained. Moreover, it was noted that increased egg counts in challenged mice was not an occasional phenomenon, attributable to unusual activity of some of the worms, but a normal consequence of the behaviour of irradiated worms in presence of normal worms from the challenge infection.

The capacity of worms from irradiated cercariae to interact with normal worms from a challenge infection has been tested [4] and some of the data, which seem to be of obvious significance in understanding the results discussed here, are summarized in Fig. 1. The fact that at any period between 46 and 222 days after exposure of mice infected with irradiated single sex cercariae to normal cercariae of the opposite sex, eggs were recovered from the liver digest, leaves little doubt that worms developed from the 1,500 irradiated cercariae were capable to pair with worms from the challenge infection and to produce eggs.

In conclusion it can be stated that results are available which help to clarify the origin of the heavy egg accumulation in mice exposed to 1,500 cercariae, irradiated with 2,000r, prior to challenge with 70 normal cercariae. Thus, it would appear that administration of large numbers of irradiated cercariae was no more effective in changing the course of a challenge infection than exposure to relatively small numbers [2]. The failure of the former procedure to produce better results conceivable has resulted from

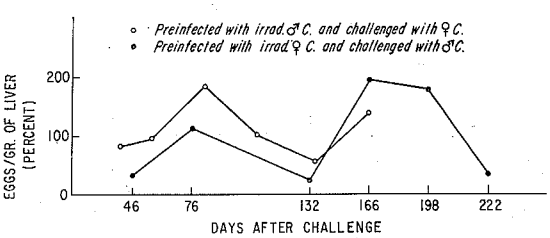

Fig. 1 — Eggs of *S. mansoni* recovered from mice exposed to 500 irradiated single sex cercariae and challenged 2 months later with normal cercariae of the opposite sex. (Percentages, expressed as means from 5 mice, were computed on the basis of egg counts from mice exposed to 70 normal mixed cercariae).

the demands on the immunizing mechanism for action against a pooled population of worms from the challenge infection, greatly increased by those developed from the aborted infection with irradiated cercariae. We infer that the requirements placed by the pooled population of worms were too great in relation to the available supply of stimulated antibodies. The proposed explanation is supported by our finding that when the host was supplied directly with the humoral factors produced by 1,500 irradiated cercariae it was found adequate to decrease significantly liver egg accumulation caused by the infection with 70 normal cercariae [3]. Since mice used in the study on protection conveyed by humoral factors produced by 1,500 irradiated cercariae [3] and those used in the experiments herein described were infected simultaneously with the same suspension of normal cercariae, the present findings can be compared with those previously reported. Two experiments were done. One was designed to study induced resistance by comparing of egg counts in the liver of mice infected with irradiated cercariae prior to challenge with corresponding data obtained from mice receiving "immune" serum prior to infection. At intervals from 2 to 6 months after exposure to normal cercariae groups of 5 to 10 mice in both series of test animals were examined for liver egg accumulation. Simultaneously with these determinations eggs were also counted in the livers of 4 to 5 control mice that had received only 70 normal cercariae. In the second experiment evidence of humoral immunity was sought in liver egg counts in mice receiving two months old "immune" serum as compared with results from mice receiving three months old "immune" serum prior to infection. An account of this experiment has been reported [3].

Experiment 1 in Fig. 2 illustrates the two types of changes in the course of an infection with the same pool of normal cercariae caused by the aborted infection with 1,500 irradiated cercariae and by the transferred humoral factors produced by the cercariae. Because of the results given in this Figure attempting to induce resistance by exposure to large numbers of cercariae irradiated with relatively low doses is clearly

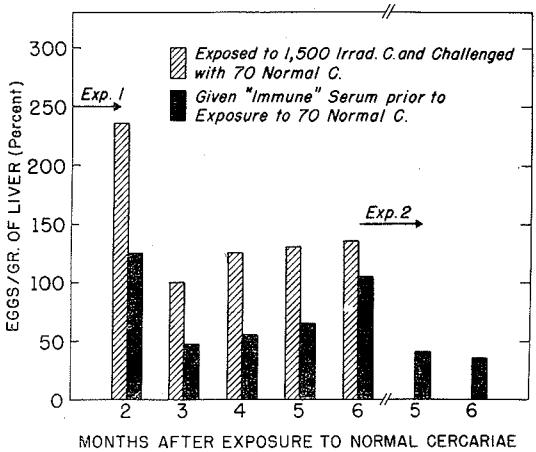

Fig. 2 — Comparison of liver egg accumulation in mice receiving humoral factors, produced by 1,500 irradiated cercariae, prior to infection and of egg counts from mice exposed to the same suspension of irradiated cercariae prior to challenge. (Percentages were computed on the basis of egg counts in mice exposed to 70 normal mixed cercariae).

unsatisfactory. But exposure to cercariae irradiated with higher doses gives no protection [1, 2].

Results have also been available which have helped to elucidate the manner in which the changes induced by the transfered humoral factors affect the normal infection. Generally the male/female ratios in populations from irradiated cercariae were found to be below unity while those in populations from normal cercariae were above unity. The

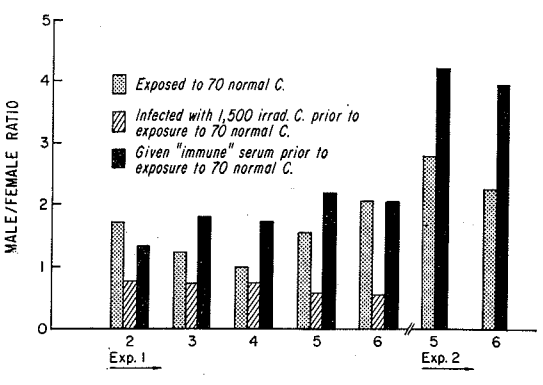

Fig. 3 — Comparison of male/female ratios in worm populations from mice receiving "immune" serum prior to infection, from mice exposed to irradiated cercariae prior to challenge and from controls exposed only to the challenge infection.

finding that the male/female ratios in the worm population from mice receiving "immune" serum prior to infection exceeded those in worm populations from control mice (Fig. 3) suggests that humoral factors lethally affect the female worms. This would mean that the lower liver egg accumulation in mice injected with the "immune" serum prior to infection (Fig. 2) is connected with the mortalities among female worms affected by the factors produced by irradiated cercariae.

Evidence from other experiments [4] also suggested that the presence of male worms from irradiated cercariae may not be necessary in the development of resistance against schistosomes. Some of the results from these experiments, presented in Fig. 4, seem to imply that there is no chance whatsever of the male worms acting as a stimulating antigen. The liver egg counts were essentially the same in control mice exposed to the challenge infection as in mice exposed to irradiated male cercariae prior to challenge. However, the number of eggs in the liver of mice that received equally treated female cercariae prior to challenge were significantly lower than those from control mice.

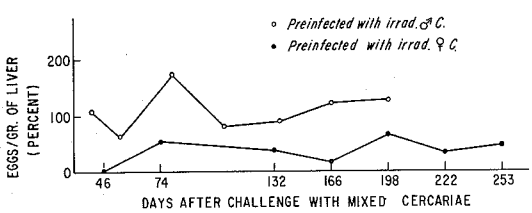

Fig. 4 — Egg accumulation per g of liver in mice exposed to 500 single sex cercariae irradiated with 2,000r and challenged 2 months later with 70 normal mixed cercariae. (Percentages, expressed as means from 5 mice, were computed on the basis of egg counts from mice exposed only to the challenge infection).

The apparent efficacy of 500 female cercariae irradiated with 2,000r in causing lower egg accumulation, when in experiments here described the 750 female cercariae, present in the pool of 1,500 mixed cercariae used, failed to do this, is not readily explained. It is again tempting to interprete these differences as reflecting interference of worms from irradiated cercariae with the defense mechanism produced by them. To put it in another way, the inhibitory factors, produced by the irradiated females only, were inadequate to cope with the demands posed by the challenge population, greatly increased by the males from the irradiated cercariae, which were found to be unsuitable candidate antigens in the defense mechanism against schistosomes. If this line of reasoning is adopted, a corollary follows: If the antigenic structure of the males were strictly analogous to that of females, the net effect of the aborted infection with 1,500 irradiated cercariae, consisting of equal numbers of males and females, would probably exceed that given by the aborted infection with 500 equally treated female cercariae. This concept, however, requires substantiation and its significance remain not clear at this time.

Although the possibility that irradiated cercariae may act as an inducer of resistance against schistosomiasis has not been excluded, the results obtained in transfering humoral factors produced by irradiated cercariae indicate the likelihood of more success. The best present conjecture based both on the experiments reported here and on those described elsewhere [3, 4] is that the role of irradiated cercariae in induced resistance against schistosomiasis will be evaluated in terms of transferable humoral factors produced by irradiated female cercariae.

RESUMO

Avaliação de processos usados em estudos recentes sôbre a resistência induzida contra a esquistossomose, em camundongos

Êste estudo foi dedicado aos efeitos das modificações experimentais capazes de estimular mais eficientemente o mecanismo imunológico contra o *S. mansoni*. Especìficamente, testou-se o efeito de grande número de cercárias expostas a uma relativamente baixa dose de radiação no decurso de uma infecção letal.

Os resultados obtidos indicaram claramente que a infecção com grande número de cercárias irradiadas tinha efeito contrário ao esperado. Observou-se aumento da população de vermes no sistema porta e elevação do número de ovos acumulados no fígado do hospedeiro. A uniformidade e consistência desta reação, aparentemente paradoxal, indicaram que o resultado não é fortuito,

revelando uma faceta significativa da resposta do hospedeiro à presença de relativamente grande número de vermes evoluídos, a partir de cercárias irradiadas, deixando assim pouca dúvida sôbre o fato de que uma infecção abortiva com cercárias irradiadas torna-se ameaça potencial para a segurança do hospedeiro.

ADDENDUM

After this paper has been accepted for publication, a report by D. G. Erickson and W. L. Caldwell, entitled "Acquired resistance in mice and rats after exposure to gamma-irradiated cercariae", appeared in *Am. J. Trop. Med. & Hyg. 14*:566-573, 1965. While it seems evident from this study that the aborted infection with irradiated cercariae changes the course of a challenge infection, during the first 8-9 weeks after exposure to normal cercariae, the exact nature of the changes produced by worms irradiated with 4,000r or with 8,000r, cannot be deduced from this work, and their relationship to specific acquired resistance to schistosomes continues uncertain. It remains to be determined, using still more extended periods of observations, whether the reduced worm yeilds and deaths among "immunized" animals would continue to diverge from those among control animals or would approximate each other as the infection became older [1, 2]. It is regrettable, therefore, that data, related to worm yeilds and mortalities among mice and rats, were not presented for the period beyond 8-9 weeks for comparative purposes.

It would appear that the Authors were more interested in discrediting our previous studies [1, 2] through distortion and mispresentation of our results, than in trying to resolve differences in experimental findings [4].

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