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The effect of the tapeworm *Hymenolepis nana* on immunity to tuberculosis in mice

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Some years ago we began a study of BCG-induced immunity to tuberculosis in mice. At first we had some unexpected results, apparently because the animals were naturally infected with the tapeworm *Hymenolepis nana*. Since then a number of papers have appeared which may support the inferences made at that time. These were that the tapeworm may exert a significant effect on the course of experimental tuberculosis by decreasing the survival times of male mice, and thus causing an unequal response between the sexes.

MATERIALS AND METHODS

Tubercle bacilli

The BCG strain was obtained from Dr Mary Ann LeVan, Henry Phipps Institute, Philadelphia. The Vallée strain was obtained from Mr J. Albericci, Porton, Wilts. Both were grown for 7 days in Dubos Tween-albumin medium and were diluted in 0·1 % bovine albumin for intravenous injection.

Mice

Two strains of mice were used. The BALB/c strain was bred from stock supplied by the Laboratory Animals Centre, Carshalton. The FF strain had been obtained many years earlier from Glaxo Laboratories. Mice were given pelleted diet and water *ad lib.*

Tapeworm egg counts

From each mouse faeces were collected and weighed. Five hundred mg. of pellets were placed in 1 ml. of distilled water and roughly broken up by incubating at 37° C. with frequent agitation. Formalin was added to 10 % and incubation was continued to hasten sterilization, in case the faeces were contaminated by tubercle bacilli. The faeces were then thoroughly emulsified on an MSE homogenizer; 3·5 ml. of saturated brine was added and the mixture was thoroughly shaken. Three samples of each mixture were collected, each immediately after shaking, and placed into specially constructed counting chambers. The eggs floated to the surface of the liquid, and were counted over a measured area of the slide. The eggs counted were of course derived from a measured sample of the faecal mixture, and the results could have been converted to eggs per gramme of faeces by multiplying by 83·3. The method gave reasonably reproducible results.

RESULTS

Challenge dose

We wished to find a dose of the Vallée strain which would kill unvaccinated mice in the 20- to 30-day survival range. Since a preliminary test suggested that about 0·2 ml. of week-old culture might be suitable, doses of this order were tested in larger groups of mice.

Three groups of ten mice each (half male, half female; all 8-week-old FF strain) were challenged with different doses (0·4, 0·2 and 0·1 ml.) of 7-day culture of the Vallée strain. The survival time of each mouse after challenge was recorded in days.

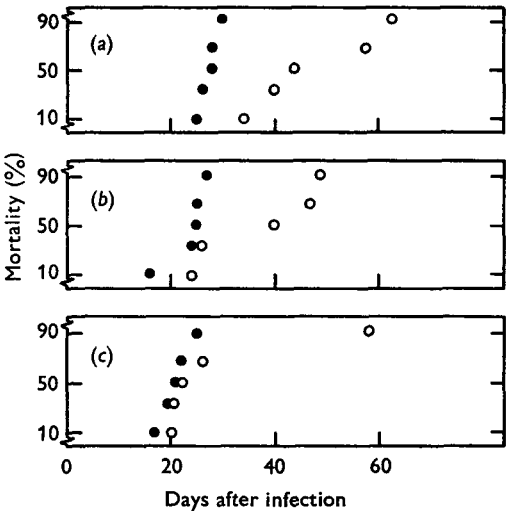

Fig. 1. Survival times of male and female FF strain mice, after intravenous challenge with three different doses of Vallée strain. (*a*) 0·1 ml.; (*b*) 0·2 ml.; (*c*) 0·4 ml. ●, male; ○, female.

Survival times are presented in Fig. 1. With all doses the mean survival time of the females was greater than that of the males—significantly so ($0{\cdot}05 > P > 0{\cdot}02$) at the 0·1 ml. dose.

Now it appears that there is no recorded instance of a mouse strain in which the females are noticeably more resistant to tuberculosis than the males. Hoyt, Moore, Knowles & Smith (1957) and Youmans & Youmans (1959) reviewed the literature and concluded that for most mouse strains the susceptibilities of the sexes was the same, although in some strains the males outlived the females after intravenous challenge. It therefore seemed possible that either we had an unusual strain of mice, or the apparent superiority of the females in this experiment was due to some non-specific factor. This was investigated later.

Apart from this unexpected finding it seemed that 0·2 ml. of Vallée culture should be tried for future experiments on FF strain mice of similar age.

Response to BCG vaccination

Having found a suitable challenge dose of the Vallée strain for the FF mice, we now wished to find whether BCG vaccination significantly prolonged the survival times of mice challenged with this dose. If so, the FF strain would be suitable for further studies on acquired immunity to tuberculosis.

A group of 20 mice (half male, half female, all 6-week-old FF strain) were vaccinated with $0{\cdot}2 \times 10^{-2}$ ml. of BCG. Twenty unvaccinated controls received diluent only. All animals were challenged twenty eight days after vaccination with 0·2 ml. of Vallée strain. The survival time of each mouse after challenge was recorded in days (Fig. 2).

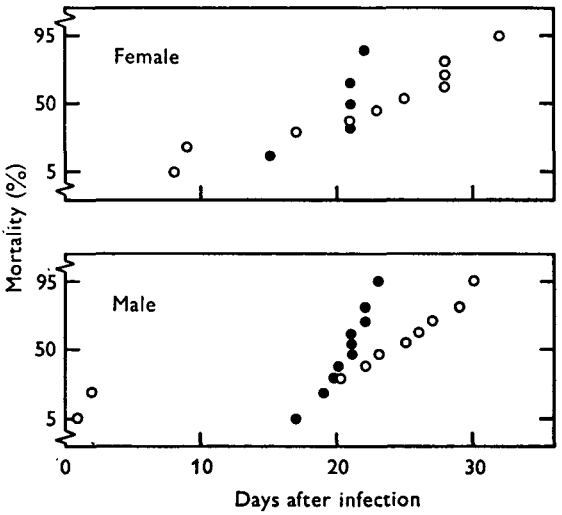

Fig. 2. Survival times of vaccinated and unvaccinated mice of each sex, after intravenous challenge with Vallée strain. ●, Unvaccinated; ○, vaccinated.

These mice were housed in temporary quarters and five control females in one cage died of overheating; their survival times are not recorded. Moreover, four of the vaccinated mice survived only 9 days or less. It was found that these mice, and some others, were suffering from heavy infestations with the tapeworm *Hymenolepis nana*. In view of these accidental interferences, we did not attempt to evaluate the statistical significance of the result. We noted, however, that the average survival time of the remainder of the vaccinated animals was somewhat greater than that of the control.

Tapeworm burdens of mice

In the vaccination experiment above, the deaths of two mice within 2 days of challenge were attributed to heavy infestations with *H. nana*. However, as the animals had previously appeared quite healthy, it seemed that the added stress of challenge was partly responsible for their deaths.

The routine practice in this Department was to segregate the sexes at weaning into different compartments in a battery of cages. The cleaning of the cages in use at

that time may have been inefficient, and, in that case, the cages may have been unequally contaminated with helminth eggs. This suggested a means whereby males and females could be unequally subjected to a non-specific factor. If *H. nana* infestations affected the survival time of tuberculous mice, here was a possible explanation for the sex difference in survival time demonstrated in the first experiment. Therefore an attempt was made to assess the tapeworm burden of each of the remaining mice in the vaccination experiment. This was done by counting the eggs in samples of faeces on day 3 of that experiment.

The mean egg counts for the four groups of mice are illustrated in Fig. 3. The counts were higher in the males than in the females. Hunninen (1935a) noted that female mice tend to be more resistant than males to *H. nana* infestation.

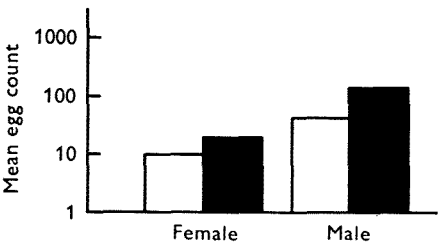

Fig. 3. Mean counts of *Hymenolepis nana* eggs in the faeces of groups of vaccinated and unvaccinated mice of each sex. The values in the figure, when multiplied by 83·3, give the mean numbers of eggs per gramme of faeces for the group. □, Unvaccinated; ■, vaccinated.

It is of further interest that for each sex the vaccinated mice had higher egg counts than did the non-vaccinated. Hunninen (1936) found that *H. nana* infestations become heavier with concurrent paratyphoid infections. It seems that BCG infections similarly may provide sufficient stress to allow *H. nana* populations to increase.

The greatest difference (between vaccinated males and unvaccinated females) illustrated in Fig. 3 is of probable significance ($P = 0{\cdot}05$). Thus it appears that *H. nana* infestation might provide a non-specific stress that would operate unequally in vaccinated and control mice and in the two sexes. Moreover, it appeared that mice with heavy worm burdens died sooner than others. In some cases intussusception caused by massive worm infestation was the obvious cause of death. We appeared to be faced with a non-specific condition which could seriously interfere with tuberculosis immunity studies.

Experimental Hymenolepis nana *infection*

At this stage attempts to test the suitability of the FF strain for acquired immunity studies in tuberculosis had been thwarted by two unexpected findings: an apparent difference in the resistance of the sexes to tuberculosis, and non-specific infestation with *H. nana*. It now seemed probable that the tapeworm infestation was the cause of the apparent sex difference. Possibly our husbandry practices would cause unequal exposure of the sexes to tapeworm infestation after weaning. Alternatively the observed superiority of the female mouse in resistance

to *H. nana* infestation (Hunninen, 1935*a*) could be responsible. This latter possibility was interesting and it was investigated experimentally.

A group of BALB/c strain mice (half male, half female) were reared tapeworm-free by Hunninen's method (Hunninen, 1935*a*). When 8 weeks old, ten of each sex were given about 2000 eggs of *H. nana* by mouth (Hunninen, 1935*b*). The eggs were recovered from the faeces of infested mice and washed by repeated centrifugation in water. Control mice, ten of each sex, were given supernatant from the same suspension. Subsequent examination of faecal samples showed that *H. nana* infestation had been successfully induced in the test groups. The controls remained free. Twenty-one days after infestation five mice of each sex-treatment group were

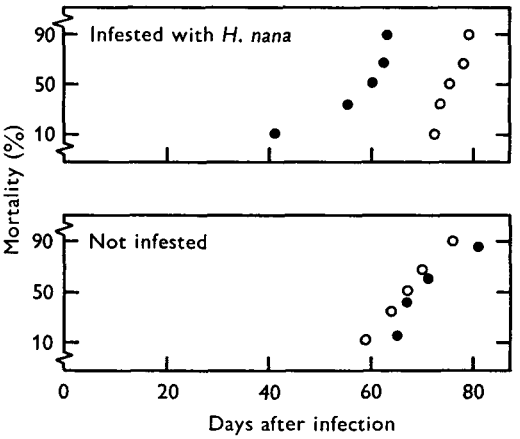

Fig. 4. Survival times of mice infested with *Hymenolepis nana* and of uninfested controls, after intravenous challenge with Vallée strain. ●, Male; ○, female.

challenged with 0·2 ml. of 50-fold dilution of Vallée culture. The rest were kept as unchallenged controls. The survival times were recorded in days.

All mice not challenged with tubercle bacilli, whether infested with *H. nana* or not, survived indefinitely and were killed in good condition at 150 days. The survival times of the rest—those challenged with tubercle bacilli—are shown in Fig. 4.

Whereas, in the absence of *H. nana* infestation the resistance of the sexes to tuberculosis was similar, in the tapeworm-infested group the males died significantly earlier than the females ($0{\cdot}01 > P > 0{\cdot}001$). This and previous findings suggested that the influence of *H. nana* should be avoided in tuberculosis immunity experiments. Accordingly, for further studies it was decided to use mice from another source, to change the diet, and to use only one sex. When this was done no further trouble was experienced with *H. nana*.

DISCUSSION

We have presented evidence that *H. nana* may influence the course of experimental tuberculosis. First, intussusception and acute enteritis killed some tuberculous, but not non-tuberculous, mice which harboured the tapeworm. Second, the experimental infestation showed that the tapeworm caused male mice to die earlier

than females following challenge with tubercle bacilli. Conversely, BCG infection seemed to alter resistance to *H. nana* as judged by faecal egg counts.

Bailenger, Roger & Pautrizel (1964) showed that female mice were more resistant than males to *H. nana*, as judged by the number of worms they harboured, and that this difference was accentuated by injecting males with testosterone proprionate or females with oestradiol benzoate. Beck (1951*a*) found that the production of eggs by individual *Hymenolepis diminuta* was greater in male rats than in females, even when the worms were approximately the same size. Later (1951*b*) Beck showed that egg production was lowered by castration of male hosts; it could be restored to the previous level by daily injections of testosterone. Addis (1946) demonstrated that worms of *H. diminuta* were smaller in immature or castrated male rats, and that this effect was overcome by daily injections of testosterone. On the other hand, immaturity or castration of females did not affect the size of the *H. diminuta* they harboured. If one assumes that carriage of large worms, infestation by a large number of worms, and high output of worm eggs are all indices of lowered resistance of the host, then these reports suggest that the resistance of the male rodent may be lessened by these tapeworms.

Similarly, our experimental infestation lowered the resistance of male mice to tuberculosis. This effect might be explained by a direct effect of *H. nana* on the non-specific resistance mechanisms of the host, similar to those reported by the other authors just mentioned.

We have not attempted to investigate further the precise mechanisms involved. However, it seems likely that the interaction is mediated by cellular rather than humoral mechanisms. There is now general agreement that immunity to mycobacteria is cell-mediated, and evidence is accumulating that cellular factors are concerned in resistance to helminths (Larsh, 1967). Indeed transfer of immunity to *H. nana* by spleen cells has been demonstrated by Friedberg, Neas, Faulkner & Friedberg (1967). However, since the cells concerned would include both macrophages and lymphocytes, their work did not demonstrate that the transferred immunity was not mediated by humoral antibody.

SUMMARY

Mice which carried the tapeworm *Hymenolepis nana* gave anomalous results when they were infected with tubercle bacilli. Males died earlier than females. The cause of death in some was intussusception or acute enteritis. More tapeworm eggs were excreted in the faeces of male mice, and, for each sex, egg counts were higher in BCG-vaccinated animals.

This work forms part of the studies on immunity to tuberculosis for a Ph.D. thesis, and was carried out while I was in receipt of a Harrison-Watson Studentship of Clare College and a Wellcome Fellowship of the Animal Health Trust.

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