Host-parasite Relationships of *Fasciola hepatica* in the White Mouse. III. Worm Transfer

BRUCE Z. LANG[1], Department of Zoology,
University of Oklahoma, Norman

INTRODUCTION

Fasciola hepatica will infect and mature in certain strains of mice, and the mouse offers an ideal host for studies dealing with host-parasite interactions (Dawes, 1962). Lang (1965) established *F. hepatica* in an inbred strain of Swiss albino mice (Larsh and Kent, 1949). Techniques for maintenance of the life cycle in the laboratory have been described (Lang, 1966a). In this *Fasciola*-mouse system only two worms could be administered per large male mouse at one time. Various host responses were measured at intervals after infection, and the course of infection in mice was separated into three phases: incubation (0-17 days after infection), acute (18-35 days), and chronic with repair (36-160 days or longer) (Lang, 1965, 1966a). It was determined that the parasites migrated from the liver into the common bile duct from 30-40 days postinfection, and that the worms grew at a rapid rate to over 22 mm in length by 160 days of infection (Lang, 1966a).

On the basis of these data, it was considered feasible to attempt to induce an acquired immunity in mice by administering an immunizing (stimulating) infection, followed by a challenge infection given after the mice recovered from the initial acute phase of infection (Lang, 1966b, 1967). In these studies, a significant acquired immunity was demonstrated (decreased challenge worm burden) by 40 days after the challenge infection. At 20 days after challenge, a significant decrease in worm burden was not apparent; however, a significant shift in the location of challenge worms in immunized mice as compared to nonimmunized mice was demonstrated. Histopathologic studies were carried out on tissues from immunized and nonimmunized mice after challenge, and suggested a delayed hypersensitivity reaction in the liver of immunized mice eight to 10 days after challenge (Lang, 1966b, 1967). It seemed possible that a delayed hypersensitivity reaction contributed to the decrease in

[1]Present address: Department of Biology, Eastern Washington State College, Cheney, Wash.

worm burden in immunized mice, either directly or indirectly (Lang, 1967), and in the severe necrosis seen in mice during a primary infection (Dawes, 1961; Lang, 1966a, 1966b). The possible role of a delayed hypersensitivity reaction in the mechanism of immunity to *F. hepatica* has been discussed (Lang, 1967) and partially tested (Lang et al., 1967). Results indicated that the transfer of peritoneal exudate cells from immunized donor mice to normal recipient mice, followed by a challenge infection, effected a significant decrease in worm burden. Larsh et al. (1966) showed that delayed hypersensitivity played an important role in acquired immunity to *Trichinella spiralis*. Other workers have indicated that humoral antibodies alone cannot impart acquired immunity in experimental animals infected with *F. hepatica* (Urquhart et al., 1954; Dawes and Hughes, 1964; Pantelouris, 1965; Thorpe, 1965a, 1965b).

The purpose of this study was to determine the effects on worms after migration through the livers of immunized mice by re-establishment in normal mice via worm transfer. Immature worms can be successfully transferred from one host to another (Dawes and Hughes, 1964), and this technique formed the basis of this experiment.

Materials and Methods

Lang (1966a, 1966b) described the maintenance of the experimental animal, life cycle in the laboratory, storage of metacercariae, and techniques of animal infection and necropsy. Immunized mice used in this experiment had been infected with two *F. hepatica* per mouse for 20 weeks. Metacercariae used for the following infections were 85-110 days old at infection. Two immunized mice and two nonimmunized (uninfected) male mice (18 weeks old), were administered *(per os)* 25 metacercariae per animal. At the same time, 12 uninfected male mice (18 weeks) were administered two metacercariae per mouse. These mice served as infected controls (group III). Four uninfected male mice (18 weeks) were designated as uninfected controls (group IV). Seventeen days after infection, the two immunized mice and the two nonimmunized mice were necropsied and the immature worms, recovered from the livers, were washed in sterile 0.85% saline at 37 C. The 17-day-old worms collected from the immunized mice were injected (16-gauge needle) intraperitoneally, two per mouse, into each of eight recipient mice. This group constituted the experimentals (group I). The same procedure was followed for worms collected from nonimmunized mice, and these recipients were worm-transfer controls (group II). Recipient mice were uninfected males 20 weeks old at transfer. All mice were necropsied 18 days after transfer (35 days after the initial infection), and mice from groups I, II, and III were examined for worms. Spleens were weighed (wet weight) from all groups on a Roller-Smith tissue balance.

Results

Two group-I mice died (25% mortality) 10 and 16 days after transfer (27 and 33 days after initial infection). Two group-II mice died (25% mortality) 10 and 13 days after transfer (27 and 30 days after initial infection). Three mice from group III (25% mortality) died 25, 27, and 31 days after infection.

At necropsy, no worms were recovered from group I (six mice); however, recent worm damage was present in the livers of two mice. From the six mice in group II, nine of 12 (75.0%) worms were recovered. All worms had migrated to the common bile ducts after 18 days in recipient mice (group II). The nine infected controls from group III yielded 13 of 18 worms (72.2%), and all worms were recovered from the common bile ducts (35 days postinfection). The following average spleen weights were recorded: group I, 240.6 mg; group II, 418.6 mg; group III, 282.1 mg; group IV, 83.1 mg.

Discussion

It had been suggested that the mortality (22-33 days postinfection) described in mice responding to a primary infection with *F. hepatica* was due primarily to the accumulation of acute liver damage (Dawes, 1961, 1962; Lang, 1966a, 1966b). The results from this study, although they involved a limited number of animals, do not support this. Mortality in recipient mice began 10 days after transfer. Dead animals from groups I and II, especially at 10 days, had little apparent liver damage. It appeared that 10 days was not enough time for the transferred worms to cause the severe damage normally associated with mortality (Lang, 1966a, 1966b). From these data, it appeared that mortality was not entirely due to severe liver damage, but was, in part, a function of the age of the worm. Transfer worms were 27 days old at the initiation of mortality in recipient mice. This corresponded to the onset of mortality in infected controls (group III, 25 days postinfection). It is possible that immature worms, 20 days or slightly older, produced some toxic excretion or secretion that was primarily responsible for mortality in this system. After the worms migrated to the common bile ducts, this toxic material was no longer produced or was released in the lumen of the duct to be expelled, no longer contacting the hepatic parenchyma. Mortality, then, was terminated.

It is apparent from the worm recovery data that migration through the livers of immunized mice prevented the final establishment of the worms in the common bile ducts of group I mice (none were recovered). The worms were able to continue liver migration (after transfer) as demonstrated by mortality at 10 and 16 days after transfer. Just how these worms were affected is not known. Death must have occurred rapidly, as one mouse died two days prior to necropsy and both worms were recovered. The critical period for these worms appeared to be between 16 and 18 days after transfer. In group II, 75% of the transferred worms were recovered from the common bile ducts. This was consistent with the recovery from group III mice, and recoveries in earlier experiments (Lang, 1966a, 1966b).

The difference in average spleen weights between groups II and III (418.6 mg and 282.1 mg, respectively) was not anticipated. At the present time the reasons for this greater splenomegaly in recipient mice is not known. Generally, splenomegaly of this extent has been recorded only in immunized and then challenged mice with two or more infections (Lang, 1967).

Literature Cited

Dawes, B. 1961. On the early stages of *Fasciola hepatica* penetrating into the liver of an experimental host, the mouse: a histological picture. J. Helminthol. (R. T. Leiper suppl.): 41-52.

_____. 1962. On the growth and maturation of *Fasciola hepatica* in the mouse. J. Helminthol. 36: 11-38.

_____ and D. L. Hughes. 1964. Fascioliasis: the invasive stages of *Fasciola hepatica* in mammalian hosts. *In* Ben Dawes (ed.), *Advances in Parasitology*, Vol. 2, Academic Press, Inc., London, p. 97-168.

Lang, B. Z. 1965. *Fasciola hepatica* in the laboratory white mouse. J. Parasitol. 51 (suppl.):24.

_____. 1966a. Host-parasite relationships of *Fasciola hepatica* in the white mouse. I. Response to a primary infection. J. Elisha Mitchell Sci. Soc. 82: 195-203.

.....1966b. Studies on host-parasite relationships of *Fasciola hepatica* in the laboratory mouse. Ph.D thesis, Dep. Parasitol., Univ. N. C., Chapel Hill.

.....1967. Host-parasite relationships of *Fasciola hepatica* in the white mouse. II. Studies on acquired immunity. J. Parasitol. 53: 21-30.

....., J. E. Larsh, Jr., N. F. Weatherly and H. T. Goulson. 1967. Demonstration of immunity to *Fasciola hepatica* in recipient mice given peritoneal exudate cells. J. Parasitol. 53: 208-209.

Larsh, J. E., Jr. and D. E. Kent. 1949. The effect of alcohol on natural and acquired immunity of mice to infection with *Trichinella spiralis*. J. Parasitol. 35: 45-53.

....., G. J. Race, H. T. Goulson and N. F. Weatherly. 1966. Studies on delayed (cellular) hypersensitivity in mice infected with *Trichinella spiralis*. III. Serologic and histopathologic findings in recipients given peritoneal exudate cells. J. Parasitol. 52: 146-156.

Pantelouris, E. M. 1965. *The Common Liver Fluke*. Pergamon Press, London, 259 pp.

Thorpe, E. 1965a. Liver damage and the host-parasite relationship in experimental fascioliasis in the albino rat. Res. Vet. Sci. 6: 498-509.

..... 1965b. An immunocytochemical study with *Fasciola hepatica*. Parasitol. 55: 209-214.

Urquhart, G. M., W. Mulligan and F. W. Jennings. 1954. Artificial immunity to *Fasciola hepatica* in rabbits. J. Inf. Dis. 94: 126-133.