HOST-PARASITE RELATIONSHIPS AND IMMUNOLOGY OF *FASCIOLA HEPATICA* L. IN EXPERIMENTAL ANIMALS

Bruce Z. Lang

Department of Biology, Eastern Washington State College, Cheney, Washington
and Department of Veterinary Science, University of Idaho, Moscow, Idaho

Numerous studies on immunity and host-parasite relationships of *Fasciola hepatica* have been published in the past nine years. From this literature, it appears that experimental animals can develop an acquired immunity to *F. hepatica*. Currently, the mechanism of immunity has not been clearly defined, but it appears that young migrating worms, not adults, are responsible for inducing the acquired immunity. Apparently, both cell-mediated immunity and antibody are involved in protecting the host from a secondary, or challenge, infection. The purpose of this article is to review selected papers on *F. hepatica* immunity.

ACQUIRED IMMUNITY TO *FASCIOLA HEPATICA*

It has been demonstrated that various hosts develop acquired immunity to the trematode parasite *Fasciola hepatica* following one or more immunizing infections with normal metacercariae. This has been shown by Lang (1, 2, 3) in mice, Boray (4) and Doyle (5) in cattle, and recently by Hayes *et al.* (6) in rats. Also, Lang and Dronen (7) have demonstrated, using a worm transfer technique (8), that young worms, 8- and 16-days old, when transferred to recipient mice effectively immunized them against a challenge infection. The 16-day-old worms remained in the liver of recipients for 13 to 14 days prior to migration into the common bile duct. Later, it was shown that 20- and 24-day-old transferred worms did not stimulate a significant acquired immunity (9). However, it was not determined if worm age or the duration of liver migration was the critical factor in immunizing the host. Studies of immature worms treated *in vitro* with hyperimmune sera, followed by worm transfer to normal hosts, indicated that most of the 12-, 16-, 18-, 20-, and 24-day-old worms were unable to complete migration in the recipient host (10). Apparently, 20-, and 24-day-old worms were producing antigens involved in stimulating the functional immunity in this system as antibody also interfered with their ability to complete migration. It appears that the duration of liver migration by young worms is the critical event in stimulating acquired immunity. In the above study (10) a precipitate was noted around the anterior end of young worms incubated in immue sera (not heat inactivated). It is possible that the precipitate represented antigen-antibody complexes and that the antigens were regurgitated from the cecae.

MECHANISM OF ACQUIRED IMMUNITY

The mechanism of acquired immunity to *F. hepatica* is not fully understood even though Lang *et al.* (11) demonstrated that peritoneal exudate cells from immune donors conveyed a degree of protection when transferred to recipient mice. They suggested the role of cellular immunity in this system. This work has been corroborated by Corba *et al.* (12) who transferred lymphoid cells from infected rats to isogenic recipients, resulting in a high degree of protection to a challenge infection (66% to 100% reduction). Although Corba *et al.* (12) could not demonstrate that immune sera offered protection to *F. hepatica* in rats, Dargie *et al.* (13) proved that the passive transfer of immune sera to rats conveyed protection against a simultaneously delivered challenge infection. Wikerhauser (14) was unable to demonstrate protection against an oral challenge infection in guinea pigs following intraperitoneal or subcutaneous injection of immune sera. Armour and Dargie (15) conclusively demonstrated that rats were immunized against *F. hepatica* with antiserum if sufficient quantities were used. They suggested that both humoral and cell-mediated mechanisms were implicated in the observed acquired immunity, and young flukes may be responsible for its induction. The role of cell-mediated immunity in *F. hepatica* infections is also

supported by Dodd and O'Nuallain (16), who studied the effects of anti-lymphocytic serum in infected rabbits, Flagstad *et al.* (17) in studies on infections in calves with congenital thymus defects, and Vernes *et al.* (18), who demonstrated macrophage inhibition in infected guinea pigs. Sinclair and Kendall (19) suggested that antigens from the migrating worms were necessary in provoking the immune response, and Erickson and Flagstad (20) provided indirect evidence that the antigen may be a metabolic product of the worm.

Antibodies against *F. hepatica* can be detected and have several types of activities. Certain antibodies may immobilize or kill miracidia (21, 22); are hemocytotropic (23); produce type I and III immediate hypersensitivities (24); are associated with worm migration into the bile ducts (2); and have been used for diagnosis (25, 26, 27). Recently, Lang (10) demonstrated that incubation of young worms in immune sera resulted in a significant decrease in worm burden when transferred into recipient mice. Thus, antibody, in the *in vitro* methods used, followed by worm transfer, has a definite effect on the young worms. The effect of the immune sera was decreased by heat-activation. These results with serum do not negate the role played by cell-mediated immunity in *F. hepatica*-host systems as reported by Lang *et al.* (11) and Corba *et al.* (12) but indicate the complexity of the immune mechanism in the infected host.

Thorpe (28) using immunofluorescent techniques on livers and worms from rats infected with F. *hepatica* demonstrated specific antigenic sites on the cecal lining, excretory ducts, and tegument of worms at 14 and 28 days after infection. This was supported by Cuperlovic (29), who also identified two major antigenic fractions present in the cecal contents of adult flukes. These data, and the fact that mice immunized with normal *F. hepatica* metacercariae have a higher antibody titer 20 days after challenge, when some of the challenge worms have already migrated into the bile ducts, than 40 days after challenge (2) may imply the importance of immune serum in acquired immunity to this parasite. By 25 days after a challenge infection a significant decrease in worm burden was evident in this system (3). Also, 17-day-old worms transferred from immune donors to normal recipients did not complete migration and died (8). Based on the above experiments (7, 10), it is possible to assume that immune serum debilitated some physiological function of the young worms such that most of them could not complete liver migration. Thorpe (28) observed specific immunofluorescence in the worm burrow 14 days after infection which indicated that the regurgitated cecal contents of young worms as well as the adults (29) contained antigens. Antibody against the cecal contents of young worms could have a definite effect on their ability to feed and migrate in the liver. Between 13 and 21 days after infection, worms show an increase in the surface area of the cecal epithelium, which may be associated with physiological changes (30). Immune sera also may have a deleterious effect on worms of a specific age which are producing the functional antigen(s).

Successful completion of the life cycle of *F. hepatica* in the vertebrate host may be dependent on good recognition of parasite antigens and a subsequent immune response. This can be supported by the following: when previously immunized mice were given a challenge infection, worms migrated to the common bile ducts by 20 days (3) instead of the normal 35 to 40 days for that strain (1, 2, 3); on a first infection in mice there was an association between migration of the worms to the common bile duct and the development of a specific immune response as characterized by cell-mediated immunity, significant histological changes in the liver, and increased antibody titer (1, 2, 11); when mice were rendered incapable of a good host response through cortisone treatment, the worms remained in the liver until the host died (Lang, unpublished). It is possible that during liver migration, development of the host-immune response is used by the worms as a stimulus to initiate movement into the common bile duct, where they are removed from most of the host's response mechanisms. This may explain why Hughes and Harness (31, 32) were unable to demonstrate the "host antigen" effect for *F. hepatica*.

The role of immune serum and cell mediated immunity in acquired immunity to *F. hepatica* infection must be re-evaluated. It seems probable that cell-mediated immunity

and immune serum function together in the infected host to produce damage to both the parasite and host, resulting in acquired immunity and some host mortality due to an immunologic-based disease.

ACKNOWLEDGMENTS

Published with the approval of the Director of the Idaho Agricultural Experimental Station, Moscow, Idaho, as research project No. 7583.

REFERENCES

1. B. Z. LANG, J. Elisha Mitchell Sci. Soc. 82: 195-203 (1966).
2. B. Z. LANG, J. Parasit. 53: 21-30 (1967).
3. B. Z. LANG, Am. J. Trop. Med. Hyg. 17: 561-567 (1968).
4. J. D. BORAY, Vet. Med. Rev. pp. 84-96 (1967).
5. J. J. DOYLE, Res. Vet. Sci. 12: 527-534 (1971).
6. T. J. HAYES, J. BAILER, and M. MITROVIC, J. Parasit. 58: 1103-1105 (1972).
7. B. Z. LANG and N. O. DRONEN, J. Parasit. 58: 84-87 (1972).
8. B. Z. LANG, Proc. Okl. Acad. Sci. 47: 81-84 (1968).
9. B. Z. LANG, J. Parasit. 60: 90-92 (1974).
10. B. Z. LANG, J. Parasit. 60: 925-929 (1974).
11. B. Z. LANG, J. E. LARSH, N. F. WEATHERLY, and H. T. GOULSON, J. Parasit. 53: 208-209 (1967).
12. J. CORBA, J. ARMOUR, R. J. ROBERTS, and G. M. URQUHART, Res. Vet. Sci. 12: 292-295 (1971).
13. J. D. DARGIE, J. ARMOUR, and G. M. URQUHART, Parasitology 67 (Suppl.):XXV (1973).
14. T. WIKERHAUSER, Vet. Archiv. 31: 71-80 (1961).
15. J. ARMOUR and J. D. DARGIE, Exp. Parasit. 35: 381-388 (1974).
16. K. DODD and T. O'NUALLAIN, J. Pathol. 99: 335-337 (1969).
17. T. FLAGSTAD, S. ANDERSON, and K. NIELSEN, Res. Vet. Sci. 13: 468-475 (1972).
18. A. VERNES, J. BIGUET, F. FLOCH, and M. N. LEFEBVRE, Pathol. Biol. 20: 23-29 (1972).
19. I. J. SINCLAIR and S. B. KENDALL, Res. Vet. Sci. 10: 483-485 (1969).
20. L. ERICKSON and T. FLAGSTAD, Exp. Parasit. 35: 411-417 (1974).
21. E. J. L. SOULSBY, J. Helminth. 31: 161-170 (1957).
22. M. MOVSESIJAN, J. Comp. Path. 78: 421-425 (1968).
23. J. J. DOYLE, Int. Arch. Allergy Appl. Immunol. 45: 744-751 (1973).
24. J. J. DOYLE, Int. Arch. Allergy Appl. Immunol. 45: 572-758 (1973).
25. E. J. L. SOULSBY, J. Comp. Path. 64: 267-274 (1954).
26. P. H. EVERALL, J. Clin. Path. 23: 630-636 (1970).
27. R. TAILLIEZ and B. KORACH, An. Ins. Pasteur (Paris) 118: 61-78 (1970).
28. E. THORPE, Parasitology 55: 209-214 (1965).
29. K. CUPERLOVIC, Project No. E30-ADP-2, Institute for the Application of Nuclear Energy in Agriculture, Veterinary Medicine and Forestry, Yugoslavia (1972).
30. B. DAWES, J. Helminth. 36: 11-38 (1962).
31. D. L. HUGHES and E. HARNESS, Res. Vet. Sci. 14: 151-154 (1973).
32. D. L. HUGHES and E. HARNESS, Res. Vet. Sci. 14: 220-222 (1973).