J. Parasitol., 90(6), 2004, pp. 1329–1331

THE CLEARANCE OF HIDDEN CESTODE INFECTION TRIGGERED BY AN INDEPENDENT ACTIVATION OF HOST DEFENSE IN A TELEOST FISH

Claus Wedekind* and Tom J. Little

Institute for Cell, Animal and Population Biology, Kings Buildings, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland, U.K. *e-mail: claus.wedekind@nat.unibe.ch*

ABSTRACT: Parasites often elude effective recognition or attack (or both) by the host immune system, for example, though a tegument that possesses nonimmunogenic features. However, a general activation of host defense due to independent stimuli may increase immune activity to a level where such disguises are no longer effective, resulting in the clearance of an infection. We experimentally infected three-spined sticklebacks (*Gasterosteus aculeatus*) with the cestode *Schistocephalus solidus*. To independently foster a general immune response a few days later, we cut the tips of spines in some fish and sham-treated other fish. Cutting spines significantly reduced the prevalence of the infection. The injury evoked a physiological reaction that helped to clear a hidden parasite infection.

Parasites of vertebrates often evade detection and expulsion by various countermeasures, including exploitation and suppression of the host immune system (Riffkin et al., 1996; Damian, 1997). Another strategy, exemplified by the cestode *Schistocephalus solidus*, involves a double integument where the outer membrane possesses features with extremely low immunogenicity; antibodies and immune cells recognizing the outer membrane are scarcely produced (Conradt and Schmidt, 1992; Schmidt, 1995). We hypothesized that such immune evasion strategies might fail the parasite if the host's immune system becomes sufficiently activated because of other infections or another source of immune stimulus.

It has been suggested that a general immune activation occurs in response to signals associated with tissue damage (Matzinger, 1990; Hoffmann et al., 1999; Todryk et al., 2000). We, therefore, tested the hypothesis that a slight injury can sufficiently activate the host immune system to clear an otherwise persistent infection. As an experimental model, we used the three-spined stickleback (*Gasterosteus aculeatus*), a teleost fish, and exposed them to the pseudophyllidean cestode *S. solidus*. This cestode grows in the body cavity of the fish and is highly host specific (Bråten, 1966; Orr et al., 1969). The cestode uses the fish only as second intermediate host where it attains infectivity to the final host, a fish-eating bird, 1–3 mo after infection. After the cestodes would normally have been established in their fish host, we cut the tips of spines in some fish (while sham-treating other fish as controls) and subsequently examined the effect on infection. Cutting the tips of spines has routinely been used in behavioral studies on sticklebacks to provide individual markings (Bakker and Sevenster, 1995).

MATERIALS AND METHODS

We bred *S. solidus* in an in vitro apparatus using methods developed by Smyth (1954) and modified by Wedekind (1997). Copepods (*Macrocyclops albidus*), which are the first intermediate hosts of *S. solidus*, were kept in laboratory cultures as described by Orr and Hopkins (1969). We exposed singly kept copepods to 6 freshly hatched parasite larvae each and fed them with freshly hatched *Artemia* sp. Four weeks after exposure to parasites, infection status and the number of procercoids were determined in vivo for each copepod as described by Wedekind et al. (2000). For infecting fish, we used only multiply infected copepods with procercoids that had clearly developed the cercomer stage.

To experimentally reduce some of the genetic and conditional variation among the sticklebacks, we used only fish that were reared in the laboratory. These fish were initially bred from adult sticklebacks caught from a pond during their breeding season in June. In the laboratory, males were separated into 10-L tanks provided with sand in a petri dish and some plant material to allow the fish to build nests. Females of similar size were housed in groups in large tanks and fed ad lib with living plankton. Full-sib groups were produced by introducing a ripe female each into the tank of a single male and letting her spawn. The eggs were removed 1 hr after spawning and reared separately, first in aerated 400-ml beakers until hatching and then as family group in 10-L tanks at 13 C, with a constant supply of springwater and 16-hr illumination per day by a fluorescent 30-W tube. Hatchlings were reared for 9 mo and fed with frozen and living plankton.

We used 60 fish that had hatched within the same week and stemmed from 2 different full-sib families (30 individuals each). One day before exposure to the parasite-infected copepods, they were placed singly into 1 of 60 tanks (25 × 15 × 15 [high] cm, 18 C, 16-hr illumination per day) and not fed to empty their guts. They were then measured for length to the nearest millimeter and weighed to the nearest 10 mg (methods for weight measurement of small fish in Frischknecht, 1993). Ten hours later, we added 1 living copepod that contained 2–5 infectious procercoids into each tank. To evoke feeding behavior, about 50 uninfected copepods were also added to each tank. By the next morning, all copepods had been eaten. From the second day after exposure until the end of the experiment, the fish were fed ad lib with a mixture of living plankton.

Seven days after exposure to the parasites, groups of 6 fish (3 of each fish sib group) were transferred to 1 of ten 20-L tanks. During transfer, they received the experimental treatment. One of the 6 fish per tank received no spine cut (but was otherwise handled identically), 3 fish received 1 spine cut (1 of the dorsal or the pectoral spines), and 2 fish received an individual combination of 2 spine cuts. The 20-L tanks were constantly supplied with springwater and illumination as described above. One fish died due to an accident, but all other fish survived until the end of the experiment.

Ninety days after exposure, a period that allows the plerocercoids to reach their infective stage (Tierney and Crompton, 1992), the fish were killed by a cerebrospinal cut. The following measurements were taken: length (to the nearest millimeter), total weight (to the nearest 10 mg), weight of gut contents, and number and weight of plerocercoids. The condition factor of each fish was calculated as the fish weight (minus gut content and worm weight) divided by length^b, where b is the slope of the regression between log (weight) against log (length) at the beginning of the experiment (Bolger and Connoly, 1989). Here, b was 3.0.

Most variables were analyzed with simple analysis of variance (ANOVA) models. The exceptions were proportion of fish that became infected and the effect of the number of administered procercoids, which were analyzed in a contingency table using likelihood ratio χ^2. All statistical analyses were performed with the Jmp In statistical package (SAS Institute Inc., 2001).

Received 28 August 2003; revised 13 May 2004; accepted 24 May 2004.

* Division of Behavioural Ecology and Division of Conservation Biology, University of Bern, Baltzerstr. 6, 3012 Bern, Switzerland.

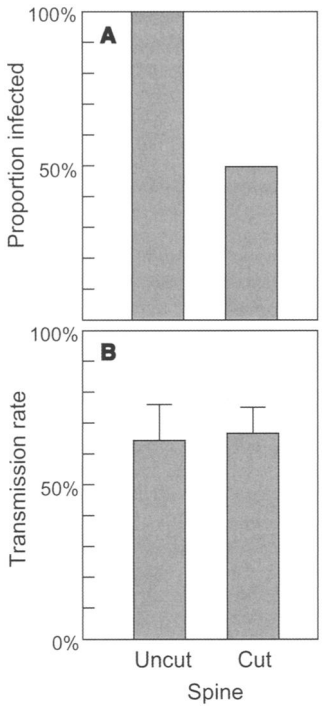

FIGURE 1. (**a**) The prevalence of *Schistocephalus solidus* infection in sticklebacks that received a spine cut or not. (**b**) The transmission rates of parasites in sticklebacks that received a spine cut or not and that were infected 90 days after exposure to the parasite.

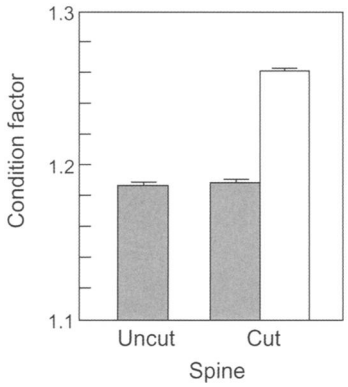

FIGURE 2. The condition factor (calculated by excluding worm weights) of sticklebacks that were infected (filled bars) or uninfected (open bars) at the end of the experiment and that had received a spine cut or not.

RESULTS

Our experiments tested the effect of minor tissue damage (cutting 1 or 2 spines) on the persistence of *S. solidus* infections in *G. aculeatus*. In no case was there a significant difference between receiving 1 versus 2 spine cuts, and therefore, all analyses compared fish that had no cut spine with fish that had at least 1 cut.

Cutting spines reduced the prevalence among the fish that had been exposed to the cestodes. At the end of the experiment, all fish that had not received a spine cut were infected, whereas only 50% of the exposed fish that had received spine cuts were infected ($\chi^2 = 11.1$, $P < 0.001$; Fig. 1a). Resistance appeared to be an all-or-nothing trait rather than a continuously varying one because transmission rate (the number of plerocercoids per administered procercoid) did not differ between the infected fish of the 2 experimental groups ($F_{1,32} = 0.03$, $P = 0.86$; Fig. 1b). There was also no significant difference between the 2 experimental groups with regard to the final number of parasites among the infected fish ($F_{1,32} = 0.39$, $P = 0.54$). The mean weight of the plerocercoids at the end of the experiment was 184.2 mg (SE = 10.9 mg) and not significantly different between infected fish that had or had not received any spine cuts ($F_{1,32} = 0.50$, $P = 0.48$).

Starting length (overall mean ± SE = 32.1 ± 0.5 mm), weight (412.9 ± 18.9 mg), and condition factor (1.20 ± 0.01) were not significantly different between cut and uncut fish in the infection experiment (ANOVA, P always >0.9). The stress we probably caused through spine cutting did not significantly affect fish growth: infected fish of the 2 experimental groups were similar in length (mean of all infected fish ± SE = 51.7 ± 0.5 mm), net weight (1,657.0 ± 57.2), and condition factor (1.19 ± 0.01) at the end of the experiment (ANOVAs, P always >0.24; Fig. 2). Infections were, however, harmful to the hosts because noninfected fish developed a higher condition factor than infected fish (ANOVA, $F_{1,57} = 13.0$, $P < 0.001$; Fig. 2).

Aside from the experimental treatment, host length, weight, and condition factor were not significant predictors of an infection (ANOVA, $F_{1,57} < 0.47$, P always >0.40). The overall sex ratio, i.e., the percentage of males, was 32% and not significantly different between the 2 experimental groups (Fisher's exact test, $P = 0.25$, 2-tailed). Males were not more often infected than females ($\chi^2 = 0.29$, $P = 0.59$).

DISCUSSION

Although chronic stress is thought to be immunosuppressive for fish (Watts et al., 2001), we have shown that acute stress, a slight injury in this case, may enhance the antiparasite response of the host. In particular, 50% of sticklebacks that had received spine cuts cleared infection of the cestode *S. solidus*, whereas sticklebacks that did not have their spines cut never cleared infection in our experiment. To our knowledge, this is the first evidence that the infection of a vertebrate by a worm can be naturally cleared by stimulation of a defense system in a manner that is unrelated to the infection itself. It could be that, under some circumstances, injury can be beneficial to a host because it acts as a form of immunological priming (Little and Kraaijeveld, 2004).

Our result is compatible with the hypothesis that *S. solidus* establishes itself in the host by not triggering critical components of the immune system. The mechanism by which the worms could elude the immune system has been established by studies demonstrating the presence of a nonimmunogenic second layer of tegument (Conradt and Schmidt, 1992; Schmidt, 1995). Such 'silent' entry by the worms may be disrupted if the fish simultaneously responds to effects associated with injury, for example, the products of damaged cells or invading microorganisms at the wound site (Matzinger, 1990; Hoffmann et al., 1999; Todryk et al., 2000). Although previously unknown in vertebrates, a similar phenomenon has recently been shown in insects. Thus, *Drosophila* sp. infected with the bacterium *Spiroplasma poulsonii* do not naturally show an immune re-

sponse, but if a response is induced ectopically, the result is a reduction of parasite titer (Hurst et al., 2003). That both *Drosophila* sp. and our study fish could still mount effective immune responses when stimulated suggests that these parasites do not actively suppress immune responses but rather that they elude detection.

We cannot, however, rule out the occurrence of active immune suppression, common in many parasitic worms of vertebrates, because the stimulation caused by spine cutting could elevate immune activity above the level that *S. solidus* can suppress or manipulate. At present, we can only speculate on the mechanisms that underlie our observations. One possible explanation for the death of *S. solidus* in injured fish is that the injury led to potent activation of the innate immune system, which is relatively well developed in fish (Watts et al., 2001). Alternatively, there may have been a specific, induced immune reaction. For example, antigen cross-reactivity with organisms infecting the wound site could lead to the generation of antibodies that also recognize the cestode thus activating the complement or other responses that the parasite had previously avoided. What does seem certain is that our fish responded to signals associated with wounding, and it is worth noting that many cells involved in cell repair are also involved in immune responses (Matzinger, 1990). Regardless of the mechanism, however, our results suggest that tipping the balance between susceptibility and resistance to cestode infections may be surprisingly simple.

ACKNOWLEDGMENTS

We thank Judith Allen, Victoria Braithwaite, Callum Brown, Tracey Lamb, Sue Mitchell, Andrew Read, Mirjam Walker, and the referees for discussion and comments on an earlier version of the manuscript, and Rolf Eggler and Felicity Jones for assistance. The experiments took place in Switzerland under licence 18/95. C.W. thanks the Swiss National Science Foundation for financial support.

LITERATURE CITED

BAKKER, TH. C. M., AND P. SEVENSTER. 1995. Sticklebacks as models for animal behaviour and evolution. *In* Papers read at the Second International Symposium on Stickleback Behaviour. Behaviour, vol. 132. E. J. Brill, Leiden, The Netherlands, 397 p.

BOLGER, T., AND P. L. CONNOLY. 1989. The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology **34:** 171–182.

BRÅTEN, T. 1966. Host specificity in *Schistocephalus solidus*. Parasitology **56:** 657–664.

CONRADT, U., AND J. SCHMIDT. 1992. Double surface-membrane in plerocercoids of *Ligula intestinalis* (Cestoda. *Pseudophyllidea*). Parasitology Research **78:** 123–129.

DAMIAN, R. T. 1997. Parasite immune evasion and exploitation: Reflections and projections. Parasitology **115:** 169–175.

FRISCHKNECHT, M. 1993. The breeding coloration of male 3-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigor. Evolutionary Ecology **7:** 439–450.

HOFFMANN, J. A., F. C. KAFATOS, C. A. JANEWAY, AND R. A. B. EZEKOWITZ. 1999. Phylogenetic perspectives in innate immunity. Science **284:** 1313–1318.

HURST, G. D. D., H. ANBUTSU, M. KUTSUKAKE, AND T. FUKATSU. 2003. Hidden from the host: *Spiroplasma* bacteria infecting *Drosophila* do not cause an immune response, but are suppressed by ectopic immune activation. Insect Molecular Biology **12:** 93–97.

LITTLE, T. J., AND A. R. KRAAIJEVELD. 2004. The ecological and evolutionary implications of immunological priming in invertebrates. Trends in Ecology and Evolution **19:** 58–61.

MATZINGER, P. 1990. Tolerance, danger and the extended family. Annual Review of Immunology **12:** 129.

ORR, T. S. C., AND C. A. HOPKINS. 1969. Maintenance of *Schistocephalus solidus* in the laboratory with observations on rate of growth of, and proglottid formation in, the plerocercoid. Journal of the Fisheries Research Board of Canada **26:** 741–752.

———, ———, AND G. H. CHARLES. 1969. Host specificity and rejection of *Schistocephalus solidus*. Parasitology **59:** 683–690.

RIFFKIN, M., H. F. SEOW, D. JACKSON, L. BROWN, AND P. WOOD. 1996. Defence against the immune barrage: Helminth survival strategies. Immunology and Cell Biology **74:** 564–574.

SAS INSTITUTE INC. 2001. Jmp In, version 4.04. SAS Institute Inc., Cary, North Carolina.

SCHMIDT, J. 1995. Glycans with N-acetyllactosamine type 2-like residues covering adult Schistosoma mansoni, and glycomimesis as a putative mechanism of immune evasion. Parasitology **111:** 325–336.

SMYTH, J. D. 1954. Studies on tapeworm physiology. VII. Fertilization of Schistocephalus solidus in vitro. Experimental Parasitology **3:** 64–71.

TIERNEY, J. F., AND D. W. T. CROMPTON. 1992. Infectivity of the plerocercoids of *Schistocephalus solidus* (Cestoda: *Ligulidae*) and fecundity of the adults in an experimental definitive host, *Gallus gallus*. Journal of Parasitology **78:** 1049–1054.

TODRYK, S. M., A. A. MELCHER, A. G. DALGLIESH, AND R. G. VILE. 2000. Heat shock proteins refine the danger theory. Immunology **99:** 334–337.

WATTS, M., B. L. MUNDAY, AND C. M. BURKE. 2001. Immune responses of teleost fish. Australian Veterinary Journal **79:** 570–574.

WEDEKIND, C. 1997. The infectivity, growth, and virulence of the cestode *Schistocephalus solidus* in its first intermediate host, the copepod *Macrocyclops albidus*. Parasitology **115:** 317–324.

———, M. CHRISTEN, L. SCHÄRER, AND N. TREICHEL. 2000. Relative helminth size in crustacean hosts: In vivo determination, and effects of host gender and within-host competition in a copepod infected by a cestode. Aquatic Ecology **34:** 279–285.