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Immune depression induced by acanthocephalan parasites in their intermediate crustacean host: Consequences for the risk of super-infection and links with host behavioural manipulation

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ABSTRACT

Parasite survival in hosts mainly depends on the capacity to circumvent the host immune response. Acanthocephalan infections in gammarids are linked with decreased activity of the prophenoloxidase (ProPO) system, suggesting an active immunosuppression process. Nevertheless, experimental evidence for this hypothesis is lacking: whether these parasites affect several immune pathways is unknown and the consequences of such immune change have not been investigated. In particular, the consequences for other pathogens are not known; neither are the links with other parasite-induced manipulations of the host. Firstly, using experimental infections of *Pomphorhynchus laevis* we confirmed that the lower immune activity in parasitised *Gammarus pulex* is induced by the parasite infection. Second, using natural infections of three different parasites, *P. laevis*, *Pomphorhynchus tereticollis* and *Polymorphus minutus*, we showed that acanthocephalan infection was associated with reduction of the activity of the ProPO system and the haemocyte concentration (two major parameters of crustacean immunity) suggesting that immune depression is a phenomenon affecting several immunological activities. This was confirmed by the fact that acanthocephalan infection (whatever the parasite species) was linked to a lower efficiency to eliminate a bacterial infection. The result suggests a cost of parasite immune depression. Finally, acanthocephalans are also known to induce behavioural alterations in the intermediate host which favour their transmission to definitive hosts. We did not find any correlation between behavioural and immunological alterations in both experimentally and naturally-infected gammarids. Overall, this study suggests that whilst immune depression might be beneficial to acanthocephalan survival within the intermediate gammarid host, it might also be costly if it increases host mortality to additional infections before transmission of the parasite.

1. Introduction

A critical condition for internal parasites to successfully accomplish their life-cycles is to survive within the host, which mainly depends on the parasite capacity to circumvent the host immune response (Loker, 1994). Immune evasion might be achieved either through molecular mimicry, when the parasite prevents its immune detection by mimicking host epitopes (Damian, 1964; Salzet et al., 2000; Zambrano-Villa et al., 2002), or through direct alteration of the host immune system that leads to immunosuppression (Duvaux-Miret et al., 1992; de Jong-Brink, 1995). These mechanisms may involve parasite excretory–secretory products that impair or inhibit both humoral and cellular effectors of the host immune system (Duvaux-Miret et al., 1992; Shelby et al., 2000; Humbert and Coustau, 2001; Labrosse et al., 2003; Guillou et al., 2007). Molecules such as lectins and mucins are of importance in host–parasite interactions and avoidance of host immune processes (Loukas and Maizels, 2000; Theodoropoulos et al., 2001). Nevertheless, immunosuppression may not only be beneficial for the parasite as it could potentially favour subsequent infections by other pathogens (Graham, 2008), which could then challenge the already established immunosuppressing parasite (Hurst et al., 2003; Wedekind and Little, 2004). This is particularly the case for endoparasites with complex life cycles. These parasites often use one or more invertebrate intermediate hosts in which they undergo successive growth events until they become infective to their definitive host, usually a vertebrate (Lafferty, 1999). These parasites need to ensure their own survival through immune evasion in the intermediate host until transmission to a suitable definitive host. However, a reduction of immunocompetence might increase the probability of host death by favouring super-infections by pathogens. Hence, parasites that rely on immune depression mechanisms have to balance the benefits and the costs of their

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immunosuppressive effect to ensure both their own survival and that of their intermediate host until transmission has occurred.

Rigaud and Moret (2003) found that natural infection by parasites with complex life cycles (the acanthocephalans *Pomphorhynchus laevis* and *Polymorphus minutus*) was associated with a reduced activity of the enzyme phenoloxidase (PO) in their intermediate host, the amphipod crustacean *Gammarus pulex*. This suggests that these parasites are potentially able to depress the activation of the prophenoloxidase (ProPO) system, an important component of the immune system in invertebrates (see below). In rivers of Burgundy (Eastern France), three species of acanthocephalan parasites, *P. laevis*, *Pomphorhynchus tereticollis* (see Perrot-Minnot, 2004), and *P. minutus*, currently infect *G. pulex* as an intermediate host before being transmitted via predation to their definitive host (fish and water birds, respectively Kennedy, 2006). Gammarids ingest parasite eggs released in the faeces of their definitive hosts. After hatching, the parasite larva (acanthor) passes through the gut wall and develops in the haemocoel until reaching a cystacanth stage. During their development, acanthocephalans are therefore exposed to the gammarid immune system. The immune system of crustaceans provides innate immunity, which relies on both cellular (Johansson et al., 2000) and humoral (Bachère et al., 1995) components. Pathogens entering the host haemocoel are usually phagocytosed or encapsulated by haemocytes. These reactions are accompanied by the proteolytic activation of the ProPO system (Cerenius and Söderhäll, 2004) that leads to the activation of the key enzyme, PO, which synthesises melanin and is also used to signal non-self, as well as kill and isolate internal parasites.

The extent to which acanthocephalans affect the gammarid immune system has not been well characterised. Rigaud and Moret (2003) found a correlation between infection and low PO activity, but there is a lack of knowledge of whether the differential immune activity between uninfected and parasitised animals is the consequence of parasite infection or the cause of differential infection (only animals with low PO activity could be infected). Moreover, whether or not the parasites alter several immune pathways and the general consequences of such alterations on host immunity to other pathogens are not known. In addition, there is now clear evidence showing that acanthocephalans are able to affect several phenotypic traits of *G. pulex* such as behaviour (Bethel and Holmes, 1973; Cézilly et al., 2000; Bauer et al., 2005; Lagrue et al., 2007), reproduction (Bollache et al., 2002) and physiology (Bentley and Hurd, 1996; Plaistow et al., 2001), to favour host exploitation and their own transmission. It is still unknown whether these multiple alterations in phenotype are related or independent. Pleiotropy, or a single change inducing a cascade of effects, could explain multiple changes and the exploration of this aspect of parasite manipulation has yet to be explored (Cézilly and Perrot-Minnot, 2005). In particular, the developing field of neuropsychoimmunology proposes that the immune and the nervous systems might be connected (Maier and Watkins, 1999; Adamo, 2002, 2006). There is now clear evidence that both systems share common molecular effectors such as neuromodulators (serotonin, octopamine and dopamine) (Adamo, 2002; Demas, 2004), which could be targeted by manipulative parasites. Indeed, recent work has demonstrated that the changes in behavioural responses of *G. pulex* to light when infected by acanthocephalans (infected animals are attracted by light instead of being repulsed) specifically involve the serotonergic system (Tain et al., 2006), which also affects immunity in other model systems (Baines et al., 1992; Mössner and Lesch, 1998). The confirmation or affirmation of potential links between immune and behavioural alterations by parasites would provide important insights about the mechanisms and therefore the evolution of parasitic manipulation.

This study examined potential immune manipulation by acanthocephalans of the gammarid immune system, by addressing four questions. We first used experimental infections under controlled laboratory conditions (Franceschi et al., 2008) to confirm the immunodepressive effect of this parasite on *G. pulex*. Second, we examined whether acanthocephalans affect several compartments of the immune system, by studying changes in humoral (two activities of the ProPO system) and cellular immunity (haemocyte concentration) in *G. pulex* naturally parasitised with three acanthocephalan species: *P. laevis*, *P. tereticollis* and *P. minutus*. Third, we estimated the potential cost of the observed immune depression, by investigating the probability of infection by bacteria in gammarids according to their status of infection by acanthocephalans. Finally, we investigated relationships between these parasitic immune modifications and behavioural changes associated with the infection in both experimentally and naturally-parasitised gammarids.

2. Materials and methods

2.1. Sampling

Gammarus pulex were collected in the River Ouche at Dijon in October 2006 and 2007 to study, respectively, *P. laevis* and *P. tereticollis* infection and in the River Bèze at Noiron sur Bèze in June 2007 for *P. minutus* infection. Since three different samplings occurred, in all the following analyses we compared acanthocephalan infections with a set of uninfected gammarids sampled at the same time and at the same site. Prevalence of different acanthocephalan species was relatively low (usually between 1% and 2%, e.g. Lagrue et al., 2007), so infected animals were actively sought and consequently the samples did not reflect the natural prevalence of infection. Infected gammarids could easily be identified as the parasite appeared as orange–red dots through the cuticle of the host. Animals were maintained in the laboratory under standard conditions (15 ± 1 °C, light:dark cycle 12:12 h) in aerated tanks filled with de-chlorinated u.v.-treated tap water and fed ad libitum with elm leaves. At the end of the experiments, all individuals were measured (size of the fourth coxal plate) using a stereoscopic microscope (Nikon SMZ-10A) and a video analysis system (VTO 232, Linkam Scientific Instruments). They were then dissected and the parasite species identified following Perrot-Minnot (2004). Behavioural activity, level of immune defences and immunocompetence (resistance to a bacterial challenge) were estimated the day after sampling (see below).

2.2. Laboratory infection experiments

Controlled infections were made following Franceschi et al. (2008). Both gammarid host *G. pulex* and parasite *P. laevis* were collected in the River Ouche. In the laboratory, uninfected gammarids were acclimated to laboratory conditions for 15 days prior to the experiment. Only males were used. Parasites were taken from naturally-parasitised definitive hosts, the chub, *Leuciscus cephalus*. Fish were anaesthetized, killed and dissected. Adult parasites were immediately collected from the intestines. Eggs were obtained from female worms, placed in 400 μL of water and parasite tissues were preserved in ethanol for molecular species identification. Chub may be infected by several acanthocephalan species that could not be reliably identified morphologically, so we used a PCR-based method (see details in Franceschi et al., 2008) to identify *P. laevis*. Egg maturity was evaluated under a microscope (200× magnification) and nine clutches with more than 75% of mature eggs were kept for experimental infection with 972 parasites. Prior to parasite exposure, gammarids were deprived of food for