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In vitro effects of prostaglandin E_2 on leucocytes from sticklebacks (*Gasterosteus aculeatus*) infected and not infected with the cestode *Schistocephalus solidus*

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ABSTRACT

Many helminth parasites have evolved strategies to evade the immune response of their hosts, which includes immunomodulation. Prostaglandin E_2 (PGE_2) is one of the best-described immunomodulators in mammalian helminth parasite infections. We hypothesized that also in teleost fish anti-helminthic immune responses are regulated via PGE_2. We used a model system consisting of the tapeworm *Schistocephalus solidus* and its host, the three-spined stickleback (*Gasterosteus aculeatus*), to investigate *in vitro* effects of PGE_2 on head kidney leucocytes (HKL) derived from sticklebacks that were experimentally infected with *S. solidus*. PGE_2 was tested alone or in combination with either *S. solidus* antigens or bacterial lipopolysaccharides (LPS). After *in vitro* culture, cell viability and changes in leucocyte subpopulations (granulocytes to lymphocytes ratios) were monitored by flow cytometry and HKL were tested for their capacity to produce reactive oxygen species (ROS) with a chemiluminescence assay. In short term (2 h) HKL cultures PGE_2 did not change the total numbers of live HKL, but the production of ROS decreased significantly with high (0.1 μmol L^{-1}) PGE_2 concentrations. In long-term (96 h) cultures high PGE_2 concentrations induced a sharp decrease of leucocytes viability, while low (0.1 pmol L^{-1}) and intermediate (0.1 nmol L^{-1}) concentrations of PGE_2 caused elevated leucocyte viability compared to controls. This coincided with reduced ROS production in cultures with high PGE_2 and elevated ROS production in cultures with low PGE_2. Granulocyte to lymphocyte ratios increased with high PGE_2 concentrations alone and in combination with *S. solidus* antigens and LPS, most prominently with HKL from *S. solidus* infected sticklebacks. The present study supports the hypothesis that PGE_2 might be an immunomodulator in tapeworm–fish parasite–host interactions.

1. Introduction

Coevolution between hosts and their parasites is predicted to be dynamic and rapid, mainly because fitness costs caused by parasites are high, parasites are ubiquitous, and they often evolve rapidly. The majority of parasites have evolved strategies to evade the immune response of their hosts [1]. The capacity of helminth parasites to modulate the immune system underpins their longevity in mammalian hosts [2]. There is consequently intense interest in understanding the molecular basis of helminth immunomodulation [3,4]. Most published work on immune evasion mechanisms deals with mammalian hosts, whereas interactions of parasites with fish hosts are to date not sufficiently investigated [5]. It was shown that cestodes can regulate anti-helmintic immune response of fish via excretory/secretory products [6,7]. However, the potential function of immunomodulating molecules produced by fish parasites is to date not well understood.

Possible candidates for substances that are derived by cestodes for host immunoregulation are prostaglandins (PGs). In mammalian parasite infections, PGs are known as a group of immunomodulating molecules, among those PGE_2, which was detected in parasitic Protozoa [8], trematodes [9], nematodes [10] and cestodes [11]. PGs appear to play a role in penetration, immune suppression, inflammation or modulation of host haemostasis, enabling parasite invasion and establishment [12].

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PGE_2 is a modulator of immunity affecting key cellular elements. In mammalian parasite infections, PGE_2 is known to modulate inflammatory responses and the subsequent activation of the adaptive immune system [13,14]. PGE_2 is responsible for the inhibition of IL-12 and TNF-α and enhances the production of IL-4 and IL-10 that suppresses T-helper cell 1 (Th1) responses [9,15–19]. PGE_2 inhibits the secretion of IFN-γ and IL-2 that exhibit a T-helper cell 2 (Th2)-promoting activity [20]. Thus, using PGE_2, parasites switch host immune response from Th1 to Th2 responses. A Th1 host immune response is protective against parasite invasions, and suppression of this response is advantageous for parasite survival. One of our previous studies demonstrated the presence of PGE_2 in the fish-infecting cestode parasite *Diphyllobothrium dendriticum*. Large cells located in the cortical parenchyma and subtegument of *D. dendriticum* displayed an intense PGE_2 stain. These cells belong to the type of frontal glands, which exists at all stages of Diphyllobothriidae ontogeny [21].

As in other vertebrates, the innate immune system of fish provides the first line of immune defence against parasites [22]. The increase of the numbers of phagocytic cells in response to fish parasites has frequently been reported, as well as the increase of functions associated with phagocytosis, i.e. production of reactive oxygen species (ROS), as mechanisms for the elimination of parasites [23,24]. Mobilization and activation of granulocytes was considered a significant part of the immune response of cyprinids [6,25–27] and rainbow trout [28] against helminth parasites. It is generally well established, that lymphocytes are activated in the defence of fish against parasites [5]. Similar patterns may be expected in at least some teleost species [29]. Infection of European eels with *Anguillicola crassus* already 8 days post infection leads to an activation of the cellular innate immunity, resulting in an increased migration activity of neutrophilic granulocytes compared to uninfected controls [30]. However, anti-*A. crassus* antibodies were first observed 8 weeks post infection, and appeared to be independent of both the number of infective larvae administered and the frequency of administration [31]. Therefore, both innate and adaptive immune responses are mounted by fish to control parasite infections, however innate immunity seems to play a more important role in anthelmintic host immune response.

Also in teleosts, PGE_2 might be an important molecular agent to modulate host immunity during parasitosis. However, there is no information on the role of PGE_2 in cestode–fish interactions. Here, we hypothesize that fish anti-helminthic immune response is regulated via PGE_2. Deduced from studies in mammals, PGE_2 might suppress inflammation at the site of contact between cestode and fish, by inhibition of the production of pro-inflammatory cytokines [9,13–20]. Therefore, firstly it is important to study the influence of PGE_2 on innate immunity parameters such as respiratory burst and numbers of granulocytes. On the other hand, PGE_2 can potentially regulate Th1/Th2 immune responses in fish as in mammals. Consequently, it is also necessary to investigate acquired immunity parameters such as numbers of lymphocytes.

The tapeworm *Schistocephalus solidus* and its host, the three-spined stickleback (*Gasterosteus aculeatus*), is a well-suited model for research of the influence of cestodes on fish immunity. Techniques for culturing the parasite *in vitro* and generating infective stages that can be used to experimentally infect sticklebacks have been developed, and the system is increasingly used as a laboratory model for investigating aspects of host–parasite interactions [32–35]. Since the teleost head kidney is a site of antigen presentation, leucocyte activation, proliferation and maturation, and consequently interacts closely with immunological activity in the periphery, information derived from head kidney leucocyte studies can be regarded as representative for immune activity in the periphery, even if specific interactions at the site of infection might remain concealed [22,35].

In the present study, to the best of our knowledge for the first time, we investigate the influence of PGE_2 on basic characteristics of the three-spined stickleback cellular immune response. Since PGE_2 is an easily decomposed substance, we preferred the *in vitro* investigation of PGE_2 effects on stickleback leucocytes, to exclude considerable loss of reactant. In addition, for better understanding of immunomodulating mechanisms, we tested PGE_2 in stickleback leucocytes also stimulated with antigens and used co-stimulation with *S. solidus* antigens and bacterial lipopolysaccharides (LPS). Furthermore, we tested leucocytes from *S. solidus* infected sticklebacks to analyse potential interactions of the infection with the *in vitro* PGE_2 concentration. After *in vitro* exposure to PGE_2 and antigens, head kidney leucocytes (HKL) were analysed by flow cytometry for cell viability and the frequency of leucocyte subsets (granulocyte to lymphocyte ratio) and by a chemiluminescence assay for the production of reactive oxygen species (ROS).

2. Material and methods

2.1. Propagation of G. aculeatus and S. solidus

Both sticklebacks and tapeworms were laboratory-raised offspring of individuals originating from the brook Ibbenbürner Aa (52°17′31.76″N, 7°36′46.49″E) in the area of Münster (Germany). Sticklebacks were raised and kept in groups of 10–20 fish in 14 L tanks connected to a water filtration/recirculation system at 18 °C and a day:night cycle of 16:8 h. During the first weeks after hatching, fish were fed daily with *Artemia* sp. naupliae, and later with frozen mosquito larvae and occasionally frozen cladocerans *ad libitum*. Sticklebacks were maintained and treated in accordance with the local animal welfare authorities and the EU Directive 2010/63/EU for animal experiments.

Mature *S. solidus* plerocercoids were removed from the fish body-cavity aseptically and allowed to reproduce sexually in an *in vitro* system modified from Smyth [36], as described in Wedekind et al. [37]. Eggs released by the worms were washed and stored in tap water at 4 °C. After 21 d incubation at 20 °C, hatching of coracidia was stimulated by light [38].

2.2. Infection experiments

As first intermediate hosts, laboratory-bred copepods (*Macrocyclops albidus*) were incubated individually with 3 *S. solidus* coracidia each, and screened for intensity of infection after 2 weeks. Subsequently, individual sticklebacks (randomly taken form a pool of 8 families) were exposed to three infected/uninfected copepods containing together a total of three procercoids in tanks with 500 mL of water for 48 h. In order to exclude infection failure, ingestion of the intermediate hosts was confirmed by subsequent filtration of the water and screening for remaining copepods. Exposed but not infected sticklebacks were detected during fish dissection. In total, 16 sticklebacks were exposed to infected copepods, which resulted in 12 infected and 4 exposed but not infected sticklebacks. From the same pool, 10 sticklebacks were provided with three copepods that had not been exposed to the tapeworm, to produce sham-exposed fish as controls. Leucocytes were collected from the sticklebacks 64 d after exposure to *S. solidus*. For short-term leucocyte cultures 6 control and 4 infected sticklebacks were used. For long-term cultures 4 control, 8 infected and 4 exposed but not infected fish were used.