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Differential macrophage polarisation during parasitic infections in common carp (*Cyprinus carpio* L.)

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Abstract

In many parasitic infections both classically activated macrophages (caMF) and alternatively activated macrophages (aaMF) play a pivotal role. To investigate if both types of macrophages also play an important role during parasitic infections in fish, we infected carp with either *Trypanoplasma borreli* or *Trypanosoma carassii* and determined the activation state of the head kidney leukocytes (HKL). Nitrite production was used as read-out for caMF and arginase activity as read-out for aaMF. Basal nitrite production and arginase activity of HKL were moderately different between the two infections. Differences were observed, however, after ex vivo re-stimulation of HKL. Re-stimulation with LPS and *T. borreli* lysates increased nitrite production by HKL of *T. borreli*-infected fish. Re-stimulation with cAMP increased arginase activity in HKL of *T. carassii*-infected fish. Our results indicate that *T. borreli*-infected carp are more prone to increase nitrite production by caMF while *T. carassii*-infected fish are more prone to increase arginase activity by aaMF.

Keywords: Macrophage; Alternative activation; Classical activation; iNOS; Arginase; Gene expression; Re-stimulation

1. Introduction

Macrophages are one of the most pleiotropic cell types within the immune system. Depending on the (cytokine) environment macrophages can differentiate into a continuum of different activation states with classical activation and alternative activation representing two extremes [1–6]. Classically activated macrophages (caMF) play an important role in type-I immune responses against intracellular pathogens by the production of reactive oxygen species (ROS) and nitric oxide (NO), and have been well characterized also in teleost fish [7–11]. Alternatively activated macrophages (aaMF) play an important role in type-II immune responses against extracellular pathogens by showing increased phagocytic activity and enhanced gene expression of MHC class II genes [1–6]. Recently, we described the

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existence of aaMF in carp (*Cyprinus carpio* L.) [12]. In this study, we identified the genes for both arginase 1 and 2 and we used inducible nitric oxide synthase (iNOS) activity and arginase activity as measures for the classical and alternative activation of head kidney-derived macrophages, respectively. We demonstrated a classical activation of macrophages after LPS stimulation and an alternative activation of macrophages after cAMP stimulation. To continue this initial in vitro study we now examined the role of caMF and aaMF in vivo during infection with two related parasites.

During a parasitic infection, macrophages are among the first host immune cells to encounter the infecting parasites and their derived products. The subsequent activation state of these macrophages plays a major role in the further development of the immune response and infection. In many parasitic infections the early phase is characterized by the presence of caMF and the production of the antimicrobial compound NO. Often, the later phase is characterised by the presence of aaMF and related arginase activity (reviewed by Noel et al. [6]). Alternatively, in some parasitic infections one type of macrophage prevails, such as during infections with the helminth *Brugia malayi* where macrophages are preferentially alternatively activated [6], or during *Leishmania major* infections where parasites are cleared by caMF [13].

So far, it remains unclear whether the induction of aaMF is beneficial to host or parasite. On one hand, the induction of aaMF downregulates the initial activation of caMF characterised by high iNOS activity, subsequent NO production and accompanying inflammation, thereby minimising immune-related pathology. On the other hand, the induction of aaMF can be beneficial for parasite development, enabling chronic infections instead of parasite clearance. For example, intracellular (*Leishmania*) as well as extracellular (*Trypanosoma brucei*) parasites can benefit from the induction of arginase in aaMF [14,15]. Arginase activity depletes cells of L-arginine, while L-arginine is also the substrate of iNOS. In addition, arginase activity results in the production of L-ornithine which is a precursor of the polyamines involved in DNA and trypanothione synthesis. Parasite trypanothione and its related enzymes are involved in the defence against damage by oxidants [16].

Trypanoplasma borreli and *Trypanosoma carassii* (Syn. *T. danilewskyi*, [17]), the parasite species used in the present study, are extracellular kinetoplastid protozoan parasites that are transmitted by blood-sucking leeches. The common carp is one of their natural hosts and while *T. borreli* and *T. carassii* infections are widespread in nature they cause mortality only in intensive aquaculture [18]. Experimental infections with *T. borreli* result in mortalities varying between 0 and 100%, depending on the carp strain used [19,20]. Experimental infections with *T. carassii* cause mortalities varying between 60 and 100% in goldfish [18]. The high but practically unnoticed prevalence in nature suggests a balanced evolution of these parasites and their fish hosts. In the laboratory, parasite dose and route can be tightly controlled by injection, which allows us to study the host-parasite balance in more detail [21].

Previous studies showed different responses of carp against the two parasite species with regard to nitrite production [11,22]. *T. borreli* was shown to induce a high amount of nitrite production by head kidney leukocytes, both in vitro and in vivo. In contrast, *T. carassii* did not induce nitrite production by head kidney leukocytes in vitro nor did serum nitrite levels increase in *T. carassii*-infected carp [11]. Additionally, in general, *T. borreli* infections are more severe than infections of carp with *T. carassii*. The observation that the induction of caMF with accompanying nitrite production is different between both infections, suggests that the presence of aaMF with accompanying arginase activity, could also be different between both infections. To test this hypothesis we infected carp with either *T. borreli* or *T. carassii* and followed the activation state of their head kidney leukocytes ex vivo.

2. Materials and methods

2.1. Animals

Common carp (*Cyprinus carpio* L.) were reared in the central fish facility 'De Haar-Vissen' at 23 °C in recirculating UV-treated tap water and fed pelleted dry food (Trouvit, Nutreco, France) daily. R3 × R8 heterozygous carp are the offspring of a cross between fish of Hungarian origin (R8 strain) and of Polish origin (R3 strain) [23]. Carp were 9 months old at the start of the experiment with an average weight of 120 g. All studies were performed with approval from the animal experimental committee of Wageningen University.

2.2. Parasites

Trypanoplasma borreli was cloned and characterised by Steinhagen et al. [24]. *Trypanosoma carassii* was cloned and characterised by Overath et al. [25]. Both parasites were maintained by syringe passage through carp, with