Anopheles gambiae eicosanoids modulate Plasmodium berghei survival from oocyst to salivary gland invasion

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Eicosanoids affect the immunity of several pathogen/insect models, but their role on the Anopheles gambiae response to Plasmodium is still unknown. Plasmodium berghei-infected mosquitoes were injected with an eicosanoid biosynthesis inhibitor, indomethacin (IN), or a substrate, arachidonic acid (AA), at day 7 or day 12 post-infection (p.i.). Salivary gland invasion was evaluated by sporozoite counts at day 21 p.i. IN promoted infection upon sporozoite release from oocysts, but inhibited infection when sporozoites were still maturing within the oocysts, as observed by a reduction in the number of sporozoites reaching the salivary glands. AA treatment had the opposite effect. We show for the first time that An. gambiae can modulate parasite survival through eicosanoids by exerting an antagonistic or agonistic effect on the parasite, depending on its stage of development.

Key words: eicosanoid - Anopheles - Plasmodium
lymph. These effects are reversed by AA treatment, suggesting that eicosanoids modulate this response (Garcia et al. 2004a). It was also shown that in Anopheles albimanus, a primary vector for malaria vivax in Mexico, DEX inhibits the expression of mRNAs corresponding to specific antimicrobial peptides (AMPs), such as cecropin, gambicin and attacin (Garcia-Gil-de-Muñoz et al. 2008).

Bearing this information in mind, we hypothesised that eicosanoid biosynthesis is altered during mosquito infection and plays a role in the mosquito response to sporozoites in the haemolymph. To test this hypothesis, Plasmodium berghei-infected mosquitoes were injected with either an eicosanoid biosynthesis inhibitor (IN, Fluka, Sigma) or a substrate (AA, Fluka, Sigma). Both compounds were used to prepare solutions of 0.1, 1 and 10 mg/mL in Schneider medium (Sigma) with 6% ethanol. The toxicity of IN and AA to the mosquitoes was tested by injection of 69 nl of each solution into Anopheles gambiae Yaoundé female mosquitoes using a Nanojet II (Drummond Scientific). For each group, 20 mosquitoes were injected intrathoraxically with 69 nl of the respective solution and then compared with controls. Survival was determined by counting dead mosquitoes for seven days and the data were analysed using a Mantel-Cox Log-Rank test. There were no differences in mortality between the groups tested (Fig. 1).

To evaluate the influence of IN and AA on infection, three-five-day-old mosquitoes were allowed to feed on mice infected with P. berghei ANKA. At day 7 post-infection (p.i.), when the oocysts were still developing, the infected mosquitoes (~100 oocysts) were injected with Schneider medium plus 10% ethanol (control group), IN at 1 mg/mL (IN group) or AA at 1 mg/mL (AA group). Other P. berghei-infected mosquitoes were also injected with control solution, IN or AA at day 12 p.i., which corresponds to the time the majority of oocysts are developed and the sporozoites are starting to egress to the haemolymph. Three replicates were performed for each experiment. All mosquitoes were dissected at day 21 p.i., and salivary glands were placed in a glass homogeniser and crushed to release the sporozoites; the sample was mixed with 400 μL of phosphate buffered saline. The number of sporozoites in the suspension was counted using a haematocytometer. The data were evaluated for statistical significance by applying a ratio t test using the GraphPad software (Prism).

Treatment of the infected mosquitoes with the inhibitor (IN) resulted in a decreased number of sporozoites recovered from the salivary glands when inhibition was performed at day 7 p.i., whereas an increase was observed when the inhibitor was used at day 12 p.i. (Fig. 2A, B). The opposite trend was observed when the eicosanoid biosynthesis substrate (AA) was injected (Fig. 2C, D). The injection of AA at day 12 p.i. led to a significant reduction in salivary gland sporozoite number to half of that found in the control mosquitoes (Fig. 2D), confirming that eicosanoids are necessary to clear sporozoites from the mosquito haemolymph. Additionally, at day 7 p.i., a 1.7-fold increase was observed in the parasite number in the salivary glands of the AA-injected mosquitoes when compared to the controls (Fig. 2C), corroborating that eicosanoids are at some point required for parasite development. This may reflect a role for mosquito-synthesised eicosanoids in Plasmodium biology and development.

An increase in parasite development in oocysts might reflect a parasitic metabolic need for lipids. Although the parasite has its own eicosanoid biosynthetic pathways, which are different from those present in mammals, it is not capable of the de novo synthesis of fatty acids, requiring AA from its host (Holz 1977). Furthermore, interactions of oocysts with AA might alter the lipid composition in the oocyst membrane, thereby influenc-
ing recognition by the immune system. However, there is also some evidence that points to a role for eicosanoids in controlling phase changes and differentiation in protozoa (Novert et al. 2003). Nevertheless, our data do not allow confirmation of any of these hypotheses.

In vertebrates, eicosanoids mediate inflammation and immune responses, having roles in platelet aggregation, intensity and duration of pain and fever and blood pressure regulation. In insects, eicosanoid biosynthesis has also been linked to immune responses. Indeed, accumulating data suggest that eicosanoids have an important role in mediating responses to bacterial, fungal and parasite infections in six orders of insects [reviewed by Stanley and Kim (2014)].

The fact that AA injection boosted the mosquito haemolymph response to sporozoites suggests that eicosanoid biosynthesis in infected mosquitoes is not operating at a maximum level. One possible explanation would be an ability of the parasite to immunosuppress its mosquito host. The ability of the parasite to evade and/or suppress the host’s immune response has been extensively studied in vertebrate hosts. In the blood stages of the P. falciparum life cycle, haemozoin was shown to be responsible for the inhibition of human PGF2 gene expression, the reduced levels of which led to a subsequent increase in tumour necrosis factor alpha levels, accounting for anaemia (Keller et al. 2004, 2006). In its mosquito vector, Plasmodium was also shown to possess immune evasion and suppression behaviours. In late oocyst stages, parasites are believed to camouflage themselves by incorporating mosquito-derived proteins into their surface and, in early stages, become wrapped in the mosquito’s plasma membrane when traversing the midgut epithelium (Vlachou et al. 2004).

Some mosquito proteins, such as CTL4 and CTLMA2, were found to be protective for the parasite, possibly assisting in immune evasion. Furthermore, midgut stages of Plasmodium gallinaceum reduce the ability of Aedes aegypti to encapsulate Sephadex beads (Boete et al. 2004) and P. falciparum infection was found to repress the expression of nitric oxide synthase, a gene involved in local epithelial responses (Tahar et al. 2002).

An intriguing property of host immunosuppression by pathogens has been observed in two models of infection in insects, including both bacterial and parasite infections that compromise the respective host’s immunity by interfering with eicosanoid biosynthesis. The bacterium Xenorhabdus nematophila is able to inhibit Spodoptera exigua PLA2 activity, as the injection of AA into S. exigua larvae reduces host mortality by 50%; in contrast, treatment with inhibitors potentiates bacterial mortality (Park & Kim 2000, 2003). This inhibition accounts for a specific time frame of cellular immunity inhibition (Eom et al. 2014) and AMP production suppression (Hwang et al. 2013). Moreover, in a protozoan infection, T. rangeli in R. prolixus, AA treatment increases the microaggregation reaction and reduces parasite numbers in the insect’s haemolymph. T. rangeli was proposed to inhibit the release of AA in its host, though it is not known whether the parasite has a direct action on PLA2 (Garcia et al. 2004b). In A. gambiae, eicosanoids have a dual role on the outcome of Plasmodium infection, promoting parasite development in the oocyst stage or controlling infection when parasites are released to the haemolymph.

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