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Original article

Cost of immune priming within generations: trade-off between infection and reproduction

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Abstract

Immune priming is a new paradigm in innate immunity. However, most studies have focused on the benefits of priming (enhanced survival and parasite clearance after a second challenge), while little attention has been paid to the costs. In this study, both factors were investigated in *Anopheles albimanus* primed against *Plasmodium berghei*. As previously observed in other invertebrates, compared to un-primed mosquitoes, those primed better controlled a challenge from the same parasite, and had a higher survival rate. Although there was no difference in the number of oviposited eggs between primed and control females, hatching rate was lower in primed than in control mosquitoes and it was more likely for control females to produce eggs than for primed females. Furthermore, a trade-off between parasite elimination and egg production was observed among primed mosquitoes, as primed females that successfully fought the infection were unable to produce eggs, but primed females that produced eggs were similarly infected as control un-primed ones. These results concord with recent mathematical models suggesting that reproduction affects immune priming outcomes, and may explain why in some species and under some conditions it seems that immune priming is not occurring.

Keywords: Cost of immune priming; *Anopheles*; *Plasmodium*; Trade-offs; Immune memory

1. Introduction

The selective pressure that parasites and pathogens impose on their host has favored the evolution and maintenance of the immune response [1,2]. However, evolutionary biologists suggest that the immune response is costly in terms of physiology, development and reproduction, which implies a trade-off between immune response and other characteristics such as survival and reproduction [3–5]. Evidence that supports this trade-off can be found in *Drosophila melanogaster*. Under conditions of scarce food supply, mutant flies with no investment in maintaining the immune system survived longer than wild type flies after an immune challenge [6]. Additionally, immune challenged damselfly males (*Calopteryx virgo*) were less successful in mating and had a lower survival rate than control or sham-manipulated males [7]. Thus, evolutionary costs could explain the immune response variations found within and among species [2–4].

In invertebrates, new and fascinating findings have revealed that parasites and pathogens have favored the evolution of innate immune priming, which is an enhanced protection resulting from a past experience with the same pathogen species or strain [8–10]. This improved protection can be found in individuals and populations [11–13], can persist across different life stages [14], and may even favor an increase in life span [10,15]. These observations suggest that immune priming is beneficial to the host because it is effective

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to suppress reinfections. However, considering that immunity responses seem to be traded-off against other costly life-history traits [16], we could expect that the ability to better fight a reinfection could also bear costs for the organisms. If the immune priming is indeed costly, it would be expected to be traded-off against other costly fitness traits, like reproduction [11–13]. That is, females that invest in immune priming would have a lower investment in reproduction [11]. A good model for testing this supposition is the *Anopheles–Plasmodium* system. Mosquitoes become infected by *Plasmodium* parasites through the ingestion of an infected blood meal from a vertebrate host. Gametocytes transform into gametes in the mosquito, and after fecundation the resulting ookinetes invade the insect midgut and transform into oocysts, where sporozoites (the parasite stage capable of infecting vertebrates) develop [17]. This parasite invasion triggers the innate immune response of mosquitoes [18]. However, given that parasite development coincides with vitellogenesis, mosquitoes confront a trade-off between reproduction and parasite elimination [19,20].

It has been suggested that immune priming in mosquitoes represents a very important field for the development of new strategies to better control vector-borne diseases like malaria [21]. Accordingly, a recent study with the *Anopheles gambiae–Plasmodium berghei* system showed that primed mosquitoes (mosquitoes confronted once with a sub lethal dose of the parasite and then challenged with a lethal dose) developed fewer parasites than control mosquitoes (mosquitoes confronted only once with a lethal dose) and that hemocyte differentiation is the potential mechanism [21]. However, the evolutionary scenario (i.e. life history trade-offs) that may limit the immune priming outcome to fight-off malaria parasites has not been tested. In the present study, the benefits of increased parasite elimination and mosquito survival were documented after immune priming. However, a trade-off between parasite elimination and egg production was also observed in primed mosquitoes. That is, immune priming of *Anopheles albimanus* and a later challenge with *P. berghei* resulted in a reproductive cost. Finally, although there were no differences in egg numbers, a lower hatching rate occurred in primed females compared to the control group.

2. Materials and methods

2.1. Parasites

A. albimanus females were first infected by feeding on BALB/c mice carrying gametocytes of *P. berghei* (ANKA 2.34 strain, kindly provided by RE Sinden, UK). Mouse gametocytaemia was assessed in Giemsa-stained tail blood smears. A genetically modified *P. berghei* sub strain that expresses GFP protein [Green Fluorescent Protein [22]] was used for challenge infections. Ookinetes were produced from GFP-*P. berghei* by culturing gametocyte-infected mouse blood [23] and offered to mosquitoes in artificial membrane feeders [24]. Mouse gametocytaemia was also assessed in Giemsa-stained tail blood smears before ookinete cultures. Only mice with about 5–6% parasitemia were included [see Ref. [20]].

2.2. Mosquitoes

The *A. albimanus* white stripe pupal phenotype was used [25]. Mosquitoes were bred under a photoperiod cycle of 12 h light: 12 h dark, at 28 °C and 70–80% relative humidity, and were fed *ad libitum* with cotton pads soaked in 0.8% fructose + PABA.

2.3. Priming experiments

Five days post-emergence, female mosquitoes were fed with either non-infected mouse blood (control group) or *P. berghei* 2.34 gametocyte-infected blood (primed group).

The analysis of Giemsa-stained blood meal bolus in a sample ($n = 15$) of mosquitoes, 24 h after gametocyte feeding revealed the presence of ookinetes in approximately 80% of the sample that was maintained between 20 and 21 °C. However, by changing fed mosquitoes from this low to high temperature (27–28 °C), the parasite infection was interrupted [20]. These mosquitoes were maintained during 13 days at 27–28 °C, and the analysis of their midguts ($n = 25$) stained with 1% mercurochrome (291774Y, BDH-Merck, Poole, UK) in PBS (Eyles 1950) under an optical microscope (OLYMPUS), indicated that oocysts did not further develop in these insects. Additionally, in another replicate of this experiment ($n = 25$), were mosquitoes were kept at high temperature (27–28 °C) since infected-blood feeding did not developed oocysts. Hence, this method using a change from low to high temperature interrupted the parasite development in mosquitoes. In this manner, we make it sure that a second (challenge) infection (with ookinetes) did not overlap with the priming (aborted infection with gametocytes) in the mosquitoes' midgut.

The control group received a similar treatment. On the day 4 after feeding, all females were provided with a substrate for oviposition (wet filter paper on wet cotton wool) and were kept on a 12:12 h light/dark cycle at 27–28 °C. Seven days after the first blood feeding, both control and primed mosquito were challenged with GFP- expressing *P. berghei* ookinetes in membrane artificial feeders. Five days after the challenge, the mosquito midguts were analyzed under a UV light microscope to determine the numbers of infected mosquitoes (prevalence) and the number of GFP oocysts per mosquito (intensity of infection; fluorescent developing oocysts are visible). This experiment is termed the short-term assay for testing the effects of priming, and it lasted a total of 17 days from emergence (representing about half the life span of the mosquitoes in normal conditions, which in our laboratory ranges from 30 to 35 days) before counting parasites in the mosquito midgut under the UV light microscope [see also [20]].

In a second experiment, primed and control mosquito groups were prepared as above; the first blood meal was with uninfected blood (control) or blood infected with *P. berghei* 2.34. Females were provided with a substrate for oviposition