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Insect parents improve the anti-parasitic and anti-bacterial defence of their offspring by priming the expression of immune-relevant genes

Ute Trauer-Kizilelma, Monika Hilker*

Institute of Biology, Dahlem Centre of Plant Sciences, Freie Universität Berlin, Haderslebener Str. 9, 12163 Berlin, Germany

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ABSTRACT

Insect parents that experienced an immune challenge are known to prepare (prime) the immune activity of their offspring for improved defence. This phenomenon has intensively been studied by analysing especially immunity-related proteins. However, it is unknown how transgenerational immune priming affects transcript levels of immune-relevant genes of the offspring upon an actual threat. Here, we investigated how an immune challenge of *Manduca sexta* parents affects the expression of immune-related genes in their eggs that are attacked by parasitoids. Furthermore, we addressed the question whether the transgenerational immune priming of expression of genes in the eggs is still traceable in adult offspring. Our study revealed that a parental immune challenge did not affect the expression of immune-related genes in unparasitised eggs. However, immune-related genes in parasitised eggs of immune-challenged parents were upregulated to a higher level than those in parasitised eggs of unchallenged parents. Hence, this transgenerational immune priming of the eggs was detected only "on demand", i.e. upon parasitoid attack. The priming effects were also traceable in adult female progeny of immune-challenged parents which showed higher transcript levels of several immune-related genes in their ovaries than non-primed progeny. Some of the primed genes showed enhanced expression even when the progeny was left unchallenged, whereas other genes were upregulated to a greater extent in primed female progeny than non-primed ones only when the progeny itself was immune-challenged. Thus, the detection of transgenerational immune priming strongly depends on the analysed genes and the presence or absence of an actual threat for the offspring. We suggest that *M. sexta* eggs laid by immune-challenged parents "afford" to upregulate the transcription of immunity-related genes only upon attack, because they have the chance to be endowed by parentally directly transferred protective proteins.

1. Introduction

Insects defend themselves against pathogens and parasitoids by an efficient immune system. They obtain protection from infection not only by their innate immunity, but also the experience of a microbial infection or parasitic attack can improve the insect's immune response to a subsequent exposure to pathogens or parasitoids. Such immune priming by a first immune challenge can be traced within a generation and even across generations. Parents that experienced an immune challenge are able to prepare their offspring for enhanced defence against impending infections (Schmid-Hempel, 2005).

Parental immune priming of insect eggs can in principal be provided by direct maternal transfer of immune-active or immune response eliciting compounds. Indeed, immune priming of eggs has been shown to be associated with a transmission of immune response inducing bacteria or bacterial fragments from the mother to the eggs (Freitak et al., 2014). Transgenerational immune priming that exclusively relies on the transgenerational transmission of immune-effective compounds would "dilute" in elder offspring stages. However, transgenerational immune priming does not only efficiently affect the immune activity of insect eggs (Freitak et al., 2009a, 2014; Hernández-Martínez et al., 2010; Moreau et al., 2012; Sadd and Schmid-Hempel, 2007; Trauer-Kizilelma and Hilker, 2015; Zanchi et al., 2012), but also of offspring larvae (Freitak et al., 2009b; López et al., 2014; Moret, 2006; Rahman et al., 2004; Shi et al., 2014; Tidbury et al., 2011; Trauer and Hilker, 2013) and even of F1-adults (Sadd et al., 2005; Roth et al., 2010; Zanchi et al.,

* Corresponding author.
E-mail address: monika.hilker@fu-berlin.de (M. Hilker).

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2011; Eggert et al., 2014). Therefore, insects as well as other organisms that show phenotypic, not DNA-sequence based, transgenerational transfer of immune traits rely on mechanisms affecting the transcript levels of immune-related genes (Eggert et al., 2014; Freitak et al., 2009a, 2009b, 2014). These mechanisms may involve e.g. epigenetic marks of immune-related genes, transfer of (inactive) transcription factors promoting the expression of immune-related genes or transmission of small, non-coding RNAs interfering with transcripts of genes involved in the regulatory immune network (Fitzgerald and Caffrey, 2014; Freitak et al., 2012; Gómez-Díaz et al., 2012; Mukherjee et al., 2015; Poulin and Thomas, 2008; Richards, 2006; Sha, 2008).

Most of the studies on transgenerational immune priming in insects focused on the analyses of immune-relevant proteins and their activities. A few studies also described the effect of a parental immune challenge on the expression of immune-relevant genes in the unchallenged juvenile insect offspring stages and showed elevated transcript levels of immune-related genes in eggs and larvae (Eggert et al., 2014; Freitak et al., 2009a, 2009b, 2014).

However, so far it is unknown whether increased transcript levels of immune-related genes of eggs laid by immune-challenged parents are still traceable in the adult progeny. Furthermore, no knowledge is available on how transgenerational immune priming affects transcript levels of immune-relevant genes of the offspring upon an actual threat. Here, we addressed these gaps of knowledge by studying the expression of immune-relevant genes in the egg and adult stage of the offspring of immune-challenged parents.

First, we investigated how transgenerational immune priming affects the transcript levels of immune-related genes in insect eggs that are actually exposed to a parasitic attack. We used *Manduca sexta* as a model since previous studies showed (a) that immune-related genes in eggs of this species are inducible upon attack by the parasitoid *Trichogramma evanescens* (Abdel-latief and Hilker, 2008) as well as by bacterial attack (Gorman et al., 2004), and (b) that the antimicrobial and phenoloxidase activities of eggs show a stronger increase in response to parasitism when eggs are laid by parents that have been immune-challenged by a treatment with peptidoglycan (PGN) than by unchallenged control parents. The improved immune activity of *M. sexta* eggs laid by immune-challenged parents results in a more efficient suppression of the development of egg parasitoids inside the host eggs, while hatching rates of host neonates are not affected (Trauer-Kizilelma and Hilker, 2015). In our current study we challenged the parental generation by injection of the bacterial cell wall component PGN because insect immune responses to PGN and parasitoids show some parallels. PGN treatment triggers increased phenoloxidase (PO) and antibacterial activity (reviewed by Yu et al., 2002), i.e. immune activities which also increase in response to parasitic attack that may be associated with bacterial invasion (Carton et al., 2008; Godfray, 1994; Strand and Pech, 1995). Furthermore, the formation of oxidation products that are toxic to both bacteria and parasitic wasps is catalysed by PO (Zhao et al., 2011). Hence, an immune challenge by PGN injection may affect parental and offspring immune parameters that are relevant in response to both bacterial infection and parasitic attacks.

Furthermore, we investigated the effect of transgenerational immune priming on the expression of immune-related genes in *M. sexta* F1-adults that have themselves also experienced an immune challenge by PGN injection. We addressed the question whether immune priming effects on the eggs laid by immune-challenged parents are traceable in the F1-adults developing from these eggs. We focussed on gene expression analysis in the ovaries of the female progeny rather than in the typically immune-related tissues (fat body or haemocytes) to account for possible tissue-specific expression of the genes which is expected to be more similar in eggs and ovaries than in eggs and fat body or haemocytes (e.g. Bao et al., 2013; Wu et al., 2010). Detection of transgenerational immune priming of gene expression in the ovaries of F1-females would indicate a priming effect even on a further generation that develops from the progeny produced by the F1-females.

We analysed the transcript levels of genes involved in recognition of invaders and in killing them *via* the so-called humoral immune defence mediated by antimicrobial peptides (AMPs) and the so-called cellular immune response characterised by melanisation of the invader, phagocytosis and/or encapsulation (Gillespie et al., 1997; Kanost et al., 2004; Lavine and Strand, 2002; Lemaitre and Hoffmann, 2007). *PGRP-1* is a gene encoding a peptidoglycan recognition protein that can sense invading bacteria (Jiang, 2008; Ragan et al., 2009). This haemolymph protein activates immune signal transduction pathways and proteolytic cascades that generate AMPs and is involved in the proteolytic activation of prophenoloxidase (pro-PO) (Sumathipala and Jiang, 2010). The gene *dorsal* encodes a transcription factor that promotes the expression of AMPs (Park and Lee, 2012), as, for example, gloverin. For the cellular immune defence, the aggregation and spreading of plasmatocytes is mediated by the cytokine plasmatocyte-spreading peptide (PSP), which is generated from its precursor protein pro-PSP. The gene encoding the biologically inactive precursor pro-PSP is expressed in insect fat body cells in response to bacterial infections; the precursor protein is released into the haemolymph and finally cleaved by a protease to the mature protein PSP (Eleftherianos et al., 2009). Melanisation of invading bodies requires the pro-PO cascade (González-Santoyo and Córdoba-Aguilar, 2012) which involves prophenoloxidase activating proteinases (PAPs) that cleave inactive pro-PO to generate active PO (An and Kanost, 2010; Jiang et al., 2010). The role of insect lipoproteins, called lipophorins, and of their subunits, the apolipohorins (encoded in the inactive form by pro-ApoLp), in insect immune defence is discussed by e.g. Schmidt et al. (2010) and Zdybicka-Barabas and Cytrynska (2013). There is increasing evidence that apolipohorins, especially apoliphorin III, are multifunctional immune-relevant proteins taking on tasks ranging from non-self-recognition to mediation of humoral and cellular immune responses (Schmidt et al., 2010; Zdybicka-Barabas and Cytrynska, 2013). Expression of apolipohorin-encoding genes in the ovaries has been shown in several insect species (e.g. Kim et al., 1998; Liu et al., 2015; Telang et al., 2013). Hence, transgenerationally primed expression of genes encoding apolipophorins in the ovaries of female progeny may contribute to immune priming of the eggs developing inside the ovaries and thus, contribute to immune priming of a further generation.

2. Material and methods

2.1. Insects

Manduca sexta were reared on artificial diet in our laboratory at 24 °C, 70% r.h., and a 16:8 h L:D cycle as described by Trauer and Hilker (2013). Adults were kept in flight cages (50 × 50 × 50 cm). Four females were kept together with four males per cage. The moths were fed with a 10% honey solution.

Adults of the egg parasitoid *T. evanescens* were obtained from parasitised eggs of the moth *Sitotroga cerealella* that were purchased from a local company (AMW Nützlinge, Pfungstadt, Germany). An original stock population of *T. evanescens* was purchased from AMW Nützlinge. Our laboratory colony was kept at 20 °C, 70% r.h. and 18:6 h L:D cycle.

2.2. Parental priming treatment

For the priming treatment of the parental (F0) generation, both