Developmental and Comparative Immunology 34 (2010) 387–395

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Review

Mosquito immune defenses against *Plasmodium* infection

Chris M. Cirimotich, Yuemei Dong, Lindsey S. Garver, Shuzhen Sim, George Dimopoulos *

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., Baltimore, MD 21205, USA

ARTICLE INFO

Article history:
Received 2 November 2009
Received in revised form 7 December 2009
Accepted 8 December 2009
Available online 23 December 2009

Keywords:
Malaria
Plasmodium
Mosquito
Anopheles
Innate immunity

ABSTRACT

The causative agent of malaria, *Plasmodium*, has to undergo complex developmental transitions and survive attacks from the mosquito's innate immune system to achieve transmission from one host to another through the vector. Here we discuss recent findings on the role of the mosquito's innate immune signaling pathways in preventing infection by the *Plasmodium* parasite, the identification and mechanistic description of novel anti-parasite molecules, the role that natural bacteria harbored in the mosquito midgut might play in this immune defense and the crucial parasite and vector molecules that mediate midgut infection.

Contents

1. Introduction

Within the mosquito vector, malaria parasites must go through a series of complex developmental transitions before transmission to a human host occurs. After being ingested by a mosquito, male and female gametocytes fuse within the midgut lumen and will over a period of approximately 18 hours develop into a motile ookinete that will migrate to the midgut epithelium and invade a single epithelial cell. The ookinete must travel to the basal lamina before the infected cell is extruded from the epithelial layer. Once it arrives at the basal lamina, the parasite differentiates into an oocyst and then further develops over a period of about 10 days into thousands of sporozoites that are released into the mosquito hemolymph. Sporozoites migrate to and invade the salivary glands and can be transmitted when the mosquito takes another blood meal. A major bottleneck for *Plasmodium* development takes place during the ookinete invasion of the midgut epithelium. The

* Corresponding author. Tel.: +1 443 287 0128.
E-mail address: gdimopou@jhsph.edu (G. Dimopoulos).

0145-305X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2009.12.005

majority of the parasite loss can be attributed to lumenal and epithelial immune responses mounted by the mosquito.

Insect (and especially dipteran) innate immunity has generally been resolved using the fruit fly *Drosophila melanogaster* as a model and bacteria or fungi as the challenging microbe. For a more thorough coverage of *Drosophila* immunity, the reader is referred to a review by Lemaitre and Hoffmann [1]. Cellular and humoral factors are major players in the response to microbial challenge, especially within the hemolymph (blood) of the insect. Hemocytes, the insect blood cells, are constantly circulating and can either engulf (by phagocytosis) or surround (encapsulation) a foreign invader as a defense mechanism. Humoral responses to pathogens involve melanization and antimicrobial effector molecules. During melanization, a serine protease cascade activates pro-phenoloxidases that, through a second catalytic cascade, generate the melanin and free radicals that are involved in killing microbes. Production of antimicrobial effector molecules are regulated by intracellular immune signaling pathways that are activated by pattern recognition receptors (PRRs) upon interaction with pathogen associated molecular patterns (PAMPs).

The intracellular immune signaling pathways have been extensively studied in *Drosophila*, with most information having been obtained by injection of bacteria or fungus directly into the fly hemolymph. Toll pathway activation occurs through pathogen detection by soluble peptidoglycan recognition proteins (PGRPs) that stimulate a serine protease cascade, culminating in the proteolytic activation of the extracellular ligand, Spätzle. Activation of a second pathway, the immune deficiency (IMD) pathway, occurs when a pathogen is detected by a membrane-bound class of PGRPs. From either pathway, extracellular signals initiate a series of intracellular reactions that lead to an increased expression of select immune-related genes, including antimicrobial peptides (Fig. 1).

Here we describe recent findings concerning the role of immune signaling pathways in preventing infection of the mosquito vector by the malaria parasite, the identification and mechanistic description of novel anti-parasite molecules, the role that natural bacteria harbored in the mosquito midgut might play in this overall immune response and the crucial parasite and vector molecules that mediate midgut infection. The role of pattern recognition receptors in activating anti-*Plasmodium* defense will be discussed in the different sections.

2. Immune signaling pathways and *Plasmodium* infection

Immune signaling pathways, which direct insect immune responses to a variety of pathogens, have recently been shown to regulate anti-*Plasmodium* immunity in mosquitoes. The three major immune signaling pathways (Toll, IMD, and Jak/Stat) that were originally described in *Drosophila* or mammals have been identified through orthology in *Anopheles gambiae* [2]. A schematic representation of the Toll and IMD pathways is provided in Fig. 1.

2.1. The Toll pathway

The classical Toll pathway is activated upon infection with Gram-positive (G+) bacteria and fungi. It has also been implicated in the defense against viruses in fruit flies [3] and mosquitoes [4] and against the rodent malaria parasite *P. berghei* in *Anopheles* mosquitoes [5] (see below). PAMP recognition by Toll pathway PRRs is well documented, but the underlying mechanism is currently unresolved.

While *Drosophila* has two different transcription factors that separate the expression of Toll-mediated immune and developmental gene expression (Dif and Dorsal, respectively), *Anopheles* mosquitoes appear to express only an ortholog of Dorsal, the NF-

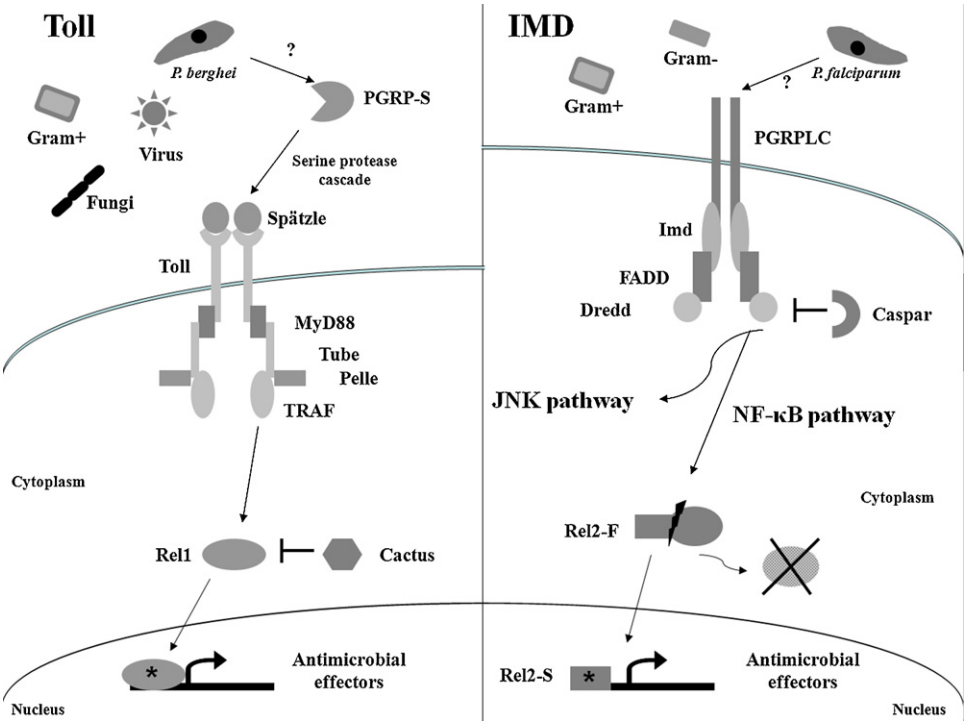

Fig. 1. Toll and IMD immune signaling pathways involved in anti-*Plasmodium* defense. Following recognition of a microbe, or unknown *Plasmodium* ligand, by soluble PGRP molecules, the Toll pathway is stimulated by binding of the ligand Spätzle with the Toll transmembrane receptor. This triggers a series of molecular events that culminate in the activation (*) and translocation of Rel1 into the nucleus, up-regulating transcription of immune genes that are responsible for microbial killing. The IMD pathway is stimulated when the transmembrane PGRP-LC receptor binds peptidoglycan or an unknown *Plasmodium* ligand that leads to the cleavage of Rel2-F and translocation of active Rel2-S (*) into the nucleus. A different set of anti-*Plasmodium* genes are up-regulated when the IMD pathway is stimulated. Branching of the IMD pathway is indicated, but the JNK pathway has not been extensively characterized in *Anopheles* mosquitoes.