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Molecular cloning and expression of a Toll receptor in the giant tiger shrimp, *Penaeus monodon*

Joop A.J. Arts [a], Ferry H.J. Cornelissen [b], Tony Cijsouw [c], Trudi Hermsen [a], Huub F.J. Savelkoul [a,*], René J.M. Stet [d]

[a] *Cell Biology and Immunology Group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands*
[b] *Erasmus MC, PO Box 1738, 3000 DR Rotterdam, The Netherlands*
[c] *Academisch Medisch Centrum, PO Box 22660, 1100 DD Amsterdam, The Netherlands*
[d] *University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen AB24 2TZ, Scotland*

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Abstract

Invertebrates rely completely for their protection against pathogens on the innate immune system. This non-self-recognition is activated by microbial cell wall components with unique conserved molecular patterns. Pathogen-associated molecular patterns (PAMPs) are recognised by pattern recognition receptors (PRRs). Toll and its mammalian homologs Toll-like receptors are cell-surface receptors acting as PRRs and involved in the signalling pathway implicated in their immune response. Here we describe a novel partial Toll receptor gene cloned from a gill library of the giant tiger shrimp, *Penaeus monodon*, using primers based on the highly conserved Toll/IL-1R (TIR) domain. The deduced amino acid sequence of the *P. monodon* Toll (PmToll) shows 59% similarity to a Toll-related protein of *Apis mellifera*. Analysis of the LRRs of shrimp Toll contained no obvious PAMP-binding insertions. Phylogenetic analysis with the insect Toll family shows clustering with Toll1 and Toll5 gene products, and it is less related to Toll3 and Toll4. Furthermore, RT-qPCR shows that PmToll is constitutively expressed in gut, gill and hepatopancreas. Challenge with white spot syndrome virus (WSSV) shows equal levels of expression in these organs. A role in the defence mechanism is discussed. In conclusion, shrimp possess at least one Toll receptor that might be involved in immune defence.

Keywords: *Penaeus monodon*; Toll; WSSV; Innate immunity; Phylogeny

Abbreviations: PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors; PGRPs, peptidoglycan recognition proteins; GNBPs, Gram-negative binding proteins; LRR, leucine-rich repeat; TM, transmembrane domain; TIR, Toll/interleukin-1 receptor; LRR-CT, leucine-rich repeat C-terminal; WSSV, white spot syndrome virus; RT-qPCR, Real-time quantitative polymerase chain reaction; EF2, elongation factor 2.

* Corresponding author. Tel.: +31 317 483509; fax: +31 317 483955.
E-mail addresses: joop.arts@wur.nl (J.A.J. Arts), f.cornelissen@erasmusmc.nl (F.H.J. Cornelissen), t.cijsouw@amc.uva.nl (T. Cijsouw), trudi.hermsen@wur.nl (T. Hermsen), huub.savelkoul@wur.nl (H.F.J. Savelkoul), r.stet@abdn.ac.uk (R.J.M. Stet).

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1. Introduction

The innate immune system is of crucial importance in host defence against pathogens of invertebrates. The non-self-recognising immune response cascade is triggered by receptors that recognise pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides, peptidoglycans and mannans, through pattern recognition receptors (PRRs). Upon recognition these receptors activate signal transduction pathways leading to the translocation of transcription factors to the nucleus and eventually control the expression of immune response genes. Depending on the pathway used the response will either be the activation of haemocytes (cellular response) or the production of antimicrobial peptides (humoral response) [1]. The clearest example of the cellular response is the strong phagocytic activity and encapsulation of pathogens by the haemocytes using the prophenoloxidase (proPO) system. Granular haemocytes also produce a variety of defence molecules like clotting factors, proteinase inhibitors, lectins, and antimicrobial peptides. The activity spectra of these antimicrobial peptides are directed either against fungi (Drosomycins and Metchnikowin), Gram-positive (Defensin) or Gram-negative (Attacins, Cecropins, Drosocin, and Diptericins) bacteria [2,3].

The recognition of infectious non-self by pattern recognition receptors in *Drosophila* occurs through two major signal pathways. Firstly, the Imd signal transduction is activated by the interaction of a putative transmembrane peptidoglycan recognition protein receptor with peptidoglycan or LPS as ligand [4–6] and is primarily involved in defence against Gram-negative bacteria. Secondly, the Toll pathway is initiated by cleavage of Spätzle. Extracellular recognition proteins such as peptidoglycan recognition proteins (PGRPs) and Gram-negative binding proteins (GNBPs) activate an extracellular serine protease, which cleaves the Spätzle protein. Binding of the proteolytic cleaved ligand Spätzle to the cell-surface receptor Toll leads to the activation of the intracellular signalling domain [7,8].

The characteristics of Toll-like receptor proteins are extracellular amino-terminal leucine-rich repeat (LRR) areas, one transmembrane domain (TM), and an intracellular carboxyl terminal Toll/interleukin-1 receptor domain (TIR), occasionally supplemented with an extracellular leucine-rich repeat C-terminal (LRR-CT). The extracellular part with its LRRs is involved in the binding of proteins like Spätzle. The intracellular part of this receptor, TIR domain, interacts with homologs of NF-κB regulators, such as MyD88 and Traf2 [9,10]. MyD88 is an intracellular protein consisting of a TIR domain and a death domain. Traf2 is a protein that contains a TRAF domain and is homologous to a human TRAF protein. Two other proteins Tube and Pelle are involved in the next signal transduction step by activation of an unknown kinase through the kinase domain of Pelle. Eventually, this results in the phosphorylation of Cactus leading to its degradation and subsequent release of Dif, which is complexed with Cactus. Dif has a DNA-binding REL homology domain, which upon translocation to the nucleus acts as a transcription factor of a multitude of immune response genes [11]. In *Drosophila*, the Toll pathway is important for anti-fungal, some anti-Gram-positive bacterial and anti-viral responses by the synthesis of antimicrobial peptides [12–14].

The immune system of crustaceans, including *Penaeus monodon* has been investigated in detail only for some response systems, such as the prophenoloxidase (proPO) activating system [15,16], clotting [17–19] and phagocytosis [20].

Currently many research efforts are devoted to elucidate the immune response to white spot syndrome virus (WSSV). This virus has become a pandemic within a relative short time span, and is particularly virulent in *P. monodon*. Given the relatively close relationship between insects and crustaceans [21] it is anticipated that the major immune signalling components and effectors can be identified using the information from insects. However, this only applies for anti-bacterial and anti-fungal responses, but not for the immune response to pathogenic viruses.

Here the identification of a Toll homolog in the black tiger shrimp *P. monodon* is reported, that is possibly involved in the defence against pathogens.

2. Materials and methods

2.1. Shrimp culture

Healthy *P. monodon* shrimp were imported as postlarvae from Malaysia and maintained in a recirculation system (pH 7.8–8.0, salinity of ±20 ppt, 0.3 mg/l NH_4^+, 0.1 mg/l NO_2^-, 200 mg/l NO_3^- at 28 °C) at the facility ''De Haar vissen'' of the Wageningen University. Each shipment was tested for the presence of WSSV, Monodon baculovirus (MBV), yellow head virus (YHV), Taura syndrome virus (TSV) and infectious hypodermal and haematopoietic