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Rhodnius prolixus: Identification of immune-related genes up-regulated in response to pathogens and parasites using suppressive subtractive hybridization

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Abstract

We report the identification of immune-related molecules from the fat body, and intestine of *Rhodnius prolixus*, an important vector of Chagas disease. Insects were challenged by introducing pathogens or *Trypanosoma cruzi*, the parasite that causes Chagas disease, into the hemocoel. RNA from intestines, or fat body were isolated 24 h after stimulation. We used suppressive subtractive hybridization to identify immune-related genes, generated three subtracted libraries, sequenced the clones and assembled the sequences. The functional annotation revealed expressed sequence tags (ESTs) generated in response to various stimuli in all tissues, and included pathogen recognition molecules, regulatory molecules, and effector molecules.

Keywords: *Rhodnius prolixus*; Parasitic infection; Innate immunity; Suppressive subtractive hybridization; Expressed sequence tags

1. Introduction

Rhodnius prolixus (family: Reduviidae) is an important vector of *Trypanosoma cruzi*, a protozoan parasite and etiological agent of American trypanosomiasis (Chagas disease) in Northern-South and Central America. Chagas disease affects an estimated 13 million people in the Americas causing significant morbidity; most acute infections are asymptomatic, yet 25–30% of these become chronic, leading to approximately 14,000 deaths annually [1]. Currently, there is neither a preventive vaccine nor an effective treatment to cure chronic Chagas disease as the drugs used, based on nitro heterocyclic compounds, have a very limited efficacy in the chronic stage and toxic side effects often lead to treatment cessation.

Transmission of *T. cruzi* is atypical and shares very little with other major insect-borne diseases in which the parasites invade the salivary glands and are injected into the vertebrate as it takes a blood meal. *T. cruzi*, resides in the intestine/rectum of triatome insects. As the insect engorges, the insect defecates and droplets containing the parasites are deposited on the host's skin and may enter via the bite site or a mucosal membrane. This transmission strategy is inefficient, and we have hypothesized previously that by remaining exclusively in the gut,

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T. cruzi is not exposed directly to the hemolymph which contains the most potent components of the insects' immune response [2]. The immune response of insects is innate, lacks the acquired component of vertebrates yet still is very efficient in eliminating pathogens using a combination of humoral and/or cellular defense responses.

The first step in the immune response requires the recognition of parasites as non-self. Insects recognize unique pathogen-associated molecular patterns (PAMPs) that are characteristic of microbial organisms [3] using host pattern recognition receptors (PRRs) [4]. The two major PRRs in insects are the peptidoglycan recognition proteins (PGRPs) and the Gram-negative bacteria-binding proteins (GNBPs) [5]. Once specific PRRs are activated by the appropriate PAMP, signaling cascades are initiated. Surface molecules present on Gram-negative bacteria are PAMPs recognized by the receptors in the IMD pathway which results in the nuclear translocation of Relish (an NF-κB-like transcription factor), and the induction of antimicrobial peptides (AMPs) such as Cecropin, Drosocin, defensin and Diptericin [6,7]. In *Drosophila melanogaster*, challenge with fungi and Gram-positive bacteria activates the Toll pathway, which results in the NF-κB-like transcription factor, *Dif*, being translocated to induce expression of Drosomycin. This activation process also triggers various other proteolytic cascades, including melanization and coagulation, in which serine proteases and serpins are involved [5] and cellular-mediated mechanisms including phagocytosis, nodulation, and encapsulation by hemocytes [8]. This insect immune system is very efficient and large numbers of bacteria can be removed within minutes of entry into the hemocoel [9]. In addition, the humoral response can contribute to the release of reactive intermediates of nitrogen or oxygen [10] all of which can contribute to the removal of parasites.

Insect innate immunity against larger parasites, has been studied mostly in mosquitoes given their importance as vectors of major human diseases [11]. Approximately 2 weeks after acquisition of an infected blood meal, *Plasmodium* sporozoites are released into the hemocoel and face both humoral and cellular immune responses. Despite massive parasite mortality, malaria parasites infect the salivary glands and subsequently are transmitted to the vertebrate host during a blood meal. Parasite mortality in mosquitoes is mediated by phagocytosis and the anti-plasmodial activity of AMPs has been shown in vitro [12,13]. The exact molecular mechanisms by which eukaryotic parasites are recognized and killed are not well characterized and are an active research area.

Studies on the molecular interactions between *T. cruzi* and triatome vectors are scarce compared with other insect/parasite combinations. Ultra-structural studies have revealed potential and probable ultra-structural interactions occurring in vivo between *T. cruzi* and the intestine of the vectors [14], but because different regions of the intestine vary in their nutritional potential and surface characteristics, we do not know how these differences affect local gene expression that may affect *T. cruzi* development. If the parasite is injected into the hemolymph of *R. prolixus*, lysozyme, prophenoloxidase (proPO), and agglutination are activated [15], and the parasite is killed and cannot be recovered [16]. However, *T. cruzi* normally does not enter the hemocoel. In vitro studies have demonstrated the susceptibility of *T. cruzi* to insect immue peptides [17,18], and in vivo studies have generated insects refractory to the parasite by engineering the bacterial gut symbionts to express a potent AMP in the midgut [19]. Studies on a closely related organism, *Trypanosoma rangeli,* which crosses the midgut epithelia and survives in the hemolymph, suggest that this parasite avoids the humoral immune system by infecting hemocytes and has the capacity to disable the proPO pathway that normally leads to melanization [20,21]. Subsequent studies [22] have demonstrated host immune responses in which lectins bind to carbohydrate moieties on the surface of *T. rangeli,* preventing their attachment to midgut and salivary glands. Identifying the specific pool of genes involved in host–parasite interactions could provide an insight into molecular mechanisms involved in parasite development and the specificity of these interactions.

The expression of these immune factors is pathogen specific; insects such as *D. melanogaster* discriminate between fungal and bacterial infections and use two main pathways, the Toll and the IMD pathways, to express specific molecules involved in their defense [23]. We have identified similar pathogen-specific responses in *R. prolixus* to bacteria and *T. cruzi* using suppressive subtractive hybridization (SSH). This technique selectively identifies differentially expressed genes in response to a particular stimulus rather than a general transcriptome analysis. We report here the generation