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Effects of ingested vertebrate-derived factors on insect immune responses

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Abstract

During the process of blood feeding insect vectors are exposed to an array of vertebrate-derived blood factors ranging from byproducts of blood meal digestion to naturally occurring products in the blood including growth hormones, cytokines and factors derived from blood-borne pathogens themselves. In this review, we examine the ability of these ingested vertebrate blood factors to alter the innate pathogen defenses of insect vectors. The ability of these factors to modify the immune responses of insect vectors offers new intriguing targets for blocking or reducing transmission of human disease-causing pathogens.

Keywords

mosquito; sand fly; tsetse fly; Reduviidae; *Plasmodium*; Leishmania; insulin; insulin-like growth factor 1 (IGF1); transforming growth factor-beta (TGF-β); complement; chitinase

Introduction

Insect vectors of human disease-causing pathogens are exposed to a unique range of vertebrate blood factors that can persist through the process of blood digestion and directly impact their immune system. This review provides a summary of the various effects of vertebrate-derived blood factors on insect immune responses. Blood feeding behavior has evolved independently several times during insect evolution and, as a result, the feeding stage and rate and frequency of feeding vary greatly among hematophagous insect vector species. This review will focus on the best-studied of these insects: mosquitoes, sand flies, and kissing bugs. Only female adult mosquito and sand fly species feed on blood while non-holometabolous kissing bugs require blood at every life stage. For most hematophagous

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insects a blood meal is necessary for the successful completion of a reproductive or gonotrophic cycle; however, there are species that are capable of autogenous reproduction.

Hemoglobin

A hematophagous insect can ingest up to 10 times its body weight in vertebrate blood, which is primarily composed of hemoglobin (Hb) [1••]. The degradation of Hb during the digestive process releases heme and can yield antimicrobial peptides that are bioactive in both humans and insects [2]. These Hb-derived peptides are an important part of both vertebrate and insect innate immune responses and adversely affect the growth of parasites, fungi, and bacteria. The presence of these antimicrobial peptides in the midguts of hematophagous insects can inhibit the growth of invading organisms. For example, Hb peptides with activity against *Trypanosoma cruzi* (the causative agent of Chagas disease) have been isolated from the midguts of the kissing bugs *Triatoma infestans* [3] and *Rhodnius prolixus* [4]. The fact that these antimicrobial Hb peptides exists in both humans and insects [5], implies that this physiology is both ancient and highly conserved. The release of heme during Hb digestion can also catalyze the synthesis of reactive oxygen species (ROS), which can directly lyse blood stages of *Trypanosoma* and *Plasmodium* (the causative agent of malaria) parasites [6, 7]. In mosquitoes, blood digestion generates elevated levels of ROS that are further enhanced in the presence of malaria parasites [8•]. In response to these damaging levels of ROS, hematophagous insects have evolved an array of heme-inactivating mechanisms [1••]. However, these responses are not immediately saturating and ROS are likely to be present throughout the process of blood digestion.

In addition, low concentrations of ROS can regulate the innate immune responses of a variety of organisms. For example, in mosquitoes the control of dengue virus in *Wolbachia*-infected *Aedes aegypti* is mediated by ROS-dependent activation of the Toll pathway [9]. In contrast, ROS induced by the insulin/insulin-like growth factor signaling (IIS) pathway in *Anopheles stephensi* favors malaria parasite development [10]. Given the conserved nature of ROS physiology, other insect vectors are likely have these signaling responses as well.

Pathogen-derived factors

Pathogen-derived factors present in the vertebrate blood meal also have the potential to alter mammalian and insect biology. Examples of such pathogen-derived factors are the glycosylphosphatidylinositols (GPIs) and GPI-anchored proteins. *Plasmodium, Leishmania* (the causative agent of leishmaniasis), and *Trypansoma* GPIs anchor proteins to parasite cell surfaces and are also secreted [11••]. The GPIs of all three parasite genera can modulate the production of pro-inflammatory cytokines in infected mammals [11••]. In addition, parasite-derived GPIs can modulate the innate immune responses of insect vectors. For example, *Plasmodium falciparum* GPIs can induce anti-microbial peptide secretion [12•] and *NOS* expression [13] in *Anopheles* mosquitoes. The GPI-anchored cell surface lipophosphoglycans (LPGs) of *Leishmania* [14•] and *Trypansoma* [15] parasites are critical for their survival and infectivity in their respective insect vectors.

Complement

An important component of the vertebrate innate immune response is the complement cascade which recognizes and induces the targeted lysis of invading organisms. Elements of both the classical and alternative complement cascades of humans can persist and alter pathogen development in insect hosts [16–18]. In mosquitoes, human complement can reduce malaria parasite development by either binding directly to zygotes and inhibiting their development into ookinetes [17] or by killing the parasites through complement-mediated lysis [18]. To evade complement-mediated killing in the mammalian host, malaria gametocytes have evolved the ability to bind complement regulator factor H. Factor H is a regulatory protein found in circulation that normally protects vertebrate host cells from complement activation and is therefore likely to present in a blood meal as well [19•].

Chitinase

Most blood feeding insects synthesize a peritrophic matrix (PM) composed of proteins and chitin around an ingested blood meal to protect their gut [20]. To establish an infection, and avoid digestion and expulsion by the insect midgut, pathogens must traverse the physical barrier of the PM. Chitinases are highly conserved enzymes that facilitate the breakdown of the PM in insects. The human ortholog chitotriosidase (CHIT) can similarly catalyze the hydrolysis of chitin [21]. During *P. falciparum* infection, plasma CHIT activity is elevated in humans [22] and mosquitoes fed blood supplemented with human CHIT exhibited a reduction in PM thickness [23•]. Leishmaniasis can also increase CHIT levels in human blood [24], which could similarly alter the PM of sand flies upon ingestion to impact the transmission of *Leishmania* parasites.

Insulin and insulin-like growth factor-1

The IIS pathway is highly conserved and regulates a variety of physiological functions in insects including immunity [25••]. IIS protein orthologs can be found in a broad range of insect species including the true bug *R. prolixus*, tsetse flies, sand flies, mosquitoes, and the human body louse *Pediculus humanus humanus* [26–32]. In addition to conservation of IIS architecture, mammalian insulin and invertebrate insulin-like peptides (ILPs) share a conserved structure that facilitates the binding of mammalian insulin to insect ILP receptors [33]. Indeed, exogenous insulin from vertebrate blood activates IIS in mosquitoes [26, 27] and tsetse flies [34]. In anopheline mosquitoes, physiological levels of insulin (170 pM) can significantly increase *P. falciparum* oocyst development [26–28], and control of malaria parasite infection requires at least three IIS proteins (ERK [35], Akt/PKB [36••, 37], PTEN [38]). In humans, IIS modifies innate immune responses through the regulation of NF-κB transcription factors [39]. Insects also possess NF-κB transcription factors (reviewed in [40]) and in mosquitoes IIS inhibits NF-κB-dependent immune responses [30•].

Although human insulin and insulin-like growth factor-1 (IGF-1) are structurally similar, they vary considerably in their effects in both humans and blood feeding insects [32]. Unlike insulin, ingested human IGF-1 increases resistance of *A. stephensi* to *P. falciparum* through the induction of midgut mitochondrial ROS and nitric oxide (NO) [32, 41•]. In humans, IGF binding proteins (IGFBPs) regulate the bioavailability of IGF-1 and can also independently

activate the IGF receptor [42]. In the fruit fly *Drosophila melanogaster* ILP-2 and ILP-5 signaling is regulated, in part, by interaction with IGFBP-like proteins [43]. IGFBP-like proteins have been described in *Ae. aegypti* [44] and in the moth species *Spodoptera frugiperda* [45], raising the possibility that insect vectors may also possess IGFBP-related proteins that could interact with ingested vertebrate growth factors to alter their downstream effects.

TGF-β1

Mammalian transforming growth factor (TGF)-β1 is a cytokine that is often present in peripheral blood during infection and is critical in regulating host immune responses [46]. In addition, TGF-β1 is also induced by infection with *Trypanosoma* and *Leishmania*, and these parasites may benefit directly from its subsequent downstream signaling effects [46]. Most mammalian cells produce TGF-β1 in its latent form and it is only after its activation that TGF-β1 exerts is cellular effects.

Mosquitoes ingest human TGF-β1 primarily in a latent form that is rapidly activated by factors such as heme and NO that are released during the digestion of a blood meal [47•]. Levels of circulating latent TGF-β1 in healthy, uninfected humans can reach 5 ng/ml, therefore mosquitoes ingest a biologically relevant level of TGF-β1 [48]. Orthologous proteins from the TGF-β signaling pathway have been identified in a diversity of blood feeding insects [49], raising the possibility that ingested human TGF-β1 activates endogenous TGF-β1 signaling pathways in other insect vectors as well. One of the most potent effects of TGF-β1 is the regulation of NO production, which is used by both mammals and mosquitoes to kill *Plasmodium* parasites [50]. In mosquitoes, low levels of human TGF-β1 (≤ 200 pg/ml) ingested in an infectious blood meal induce a moderate increase in nitric oxide synthase (NOS) activity that inhibits malaria parasite development. In contrast, high concentrations of TGF-β1 (2,000 pg/ml) do not alter malaria parasite development, but instead induce negative feedback to regulate NO synthesis [35]. The dose dependent effects of TGF-β1 signaling observed in mosquitoes are consistent with findings from mammalian biology that highlight the ability of TGF-β1 to regulate NOS activity on multiple levels [51].

Other cytokines

Both vertebrates and invertebrates use cytokines and cytokine-like factors to regulate immunity and wounding healing. To date, no mammalian cytokines have been identified that signal in insect vectors. However, the strong conservation of signaling pathways between insects and their vertebrate hosts suggests that mammalian cytokines capable of altering the physiology of insect vectors exist. For example, human interferon-γ (IFNγ) signals through the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway. Binding of IFNγ by its membrane receptors leads to the activation of JAK, which phosphorylates the immune-regulatory transcription factor STAT1. JAK/STAT signaling is regulated in part by suppressor of cytokine signaling-1 (SOCS-1) [52]. Orthologs of STAT, JAK, and SOCS proteins exist in *Anopheles, Aedes* and *Culex* mosquitoes [53, 54] and activation of JAK/STAT signaling in *A. gambiae* can inhibit the

development *Plasmodium* parasites [55]. In addition, a cytokine with homology to mammalian interferon has been identified in West Nile virus (WNV)-infected *Culex quinquefasciatus* cell lines [56•]. This secreted peptide, termed Vago, restricts WNV infection in mosquito cells through the activation of the JAK/STAT signaling pathway [56•]. The use of cytokines by insect vector species to regulate their innate immune responses and the presence of a clear signaling architecture suggests that exogenous human cytokines may signal in insect vectors as well.

Conclusions

In this review we highlighted a variety of vertebrate blood-derived factors that modify the innate immune responses of insect vectors. The conservation of these signaling pathways, and the breadth of cross-talk identified, suggest that other connections remain to be discovered between mammalian hosts and blood feeding insects. Although in this review we discussed blood-derived factors and their impact on insect immunity individually, a single blood meal will most likely contain a multitude of these factors concurrently. Therefore, considerable work is still required to understand how these signaling pathways network with one another to understand their ultimate downstream affects on insect immunity and pathogen transmission.

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Abbreviations

CHIT	Chitotriosidase
GPIs	glycosylphosphatidylinositols
Hb	hemoglobin
IIS	insulin/insulin-like growth factor signaling
IGF-1	insulin-like growth factor-1
IGFBPs	IGF binding proteins
ILPs	insulin-like peptides
IFNγ	interferon-γ
JAK/STAT	Janus kinase/signal transducers and activators of transcription
LPGs	lipophosphoglycans
NO	nitric oxide
NOS	nitric oxide synthase
PM	peritrophic matrix
ROS	reactive oxygen species

SOCS-1	suppressor of cytokine signaling-1
(TGF)-β1	transforming growth factor
WNV	West Nile Virus

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Highlights

- Ingested blood-derived factors can alter the immune response of insect vectors
- Factors released by hemoglobin digestion can limit the growth of pathogens
- Pathogen-derived factors can signal in the insect midgut to alter immunity
- Human chitotriosidase can alter the peritrophic matrix of insects
- Human insulin, IGF-1, and TGF-α1 signal in the insect midgut to alter immunity

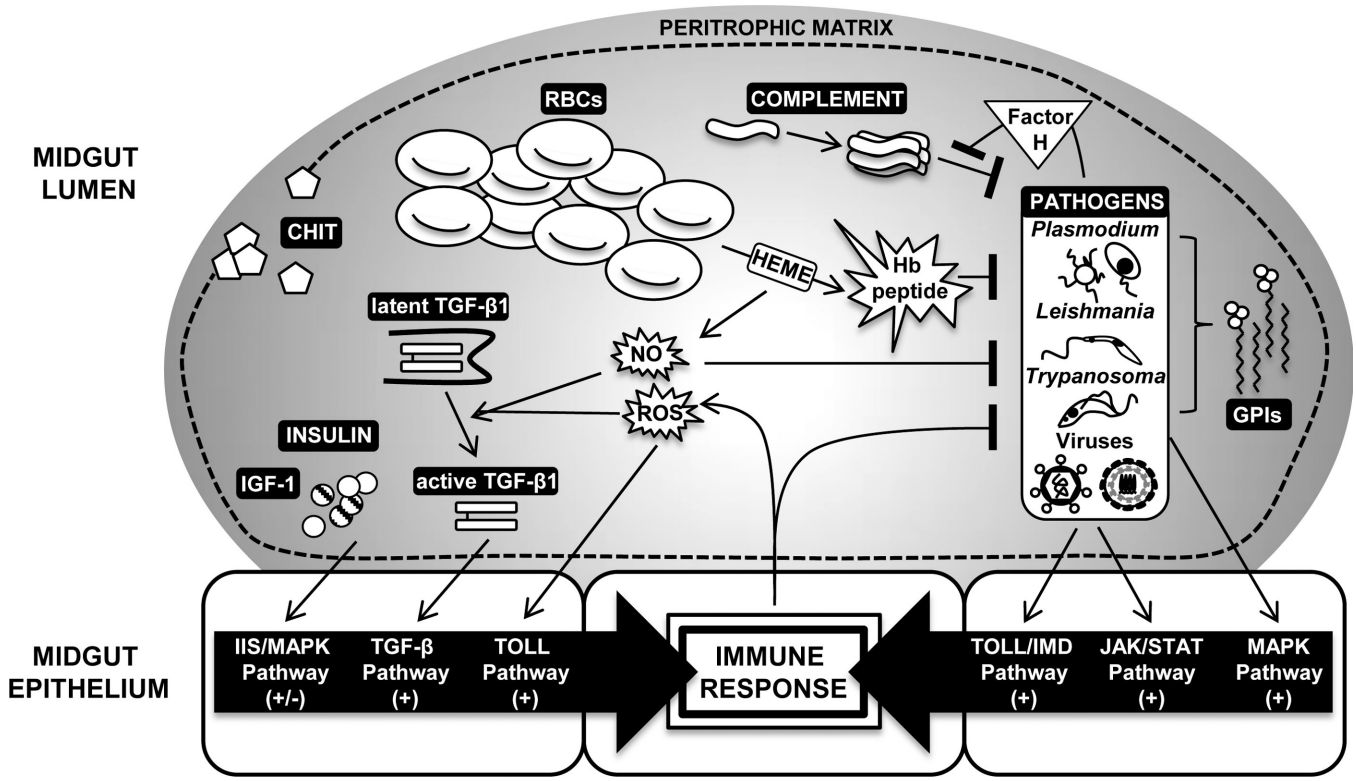

Figure 1.

The effects of ingested blood from an infected vertebrate host (large gray oval, top) on the insect midgut epithelium (large white squares, bottom). Dashed line indicates peritrophic matrix and black boxes indicate ingested factors/pathogens that are directly active or activated after reactions in the midgut lumen. Insect signaling pathways activated by these ingested factors and their downstream effects on insect immunity are indicated.