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Review

The effects of ingested mammalian blood factors on vector arthropod immunity and physiology

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Abstract

The blood feeding behavior of disease-transmitting arthropods creates a unique intersection between vertebrate and invertebrate physiology. Here, we review host blood-derived factors that persist through blood digestion to affect the lifespan, reproduction, and immune responses of some of the most common arthropod vectors of human disease.

Keywords: Tick; Mosquito; *Plasmodium*; Insulin; Transforming growth factor-beta (TGF-β); Macrophage migration inhibitory factor (MIF)

1. Introduction

The blood feeding behavior of disease-transmitting arthropods creates a unique intersection between vertebrate and invertebrate physiology. Human blood contains a myriad of nutrients, growth factors, cytokines/chemokines, pathogens, and pathogen-associated molecules that can interact with arthropod vectors. Many of these blood-derived factors persist during blood digestion to signal in the arthropod vector. This review summarizes the conserved signal transduction pathways that are activated by these vertebrate host-derived factors, and describes their downstream effects on lifespan, reproduction, and innate immune responses of the most common arthropod vectors of human disease agents. Expanding our understanding of these complex interactions can guide novel approaches for the control of a variety of vector-borne diseases.

2. Insulin and insulin-like growth factor-1

Normal circulating levels of insulin in the blood of healthy humans can range between 17 pM at fasting to 590 pM without fasting [1]. Infection with malaria parasites can induce a rise in blood insulin levels above that of a normal healthy adult by as much as 10- to 35-fold [1,2]. Given that the occurrence of type 2 diabetes is rising in malaria-endemic countries [3], compounded hyperinsulinemia in individuals affected by both diseases could become prevalent. In particular, a hallmark of type 2 diabetes is insulin resistance, which can often result in increased insulin secretion to compensate for the inability of the body to respond to insulin. If female mosquitoes feed on co-morbid hosts, they would ingest higher than average levels of human insulin in an infectious blood meal.

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The mosquito midgut is a critical site for *Plasmodium* development [4], and a tissue that is exquisitely responsive to ingested human insulin [5–7]. Recent experiments using radiolabeled human insulin spiked into artificial blood meals fed to *Anopheles stephensi* revealed that ingested, intact insulin persisted in the midgut for up to 24 h and in the head and thorax for up to 18 h post-blood feeding [8]. These data indicate that insulin persists long enough to signal in the mosquito midgut, and can cross the midgut epithelium to persist in the hemocoel to activate signaling in other tissues.

The insulin/insulin-like growth factor signaling (IIS) pathway is highly conserved and consists of two main signaling branches: a mitogen-activated protein kinase (MAPK)-dependent branch and a phosphatidylinositol 3-kinase (PI3K)/Akt-dependent branch. Both branches of the IIS pathway play integral roles in the regulation of growth, longevity, reproduction, and immunity in vertebrates and invertebrates [9]. Although these pathways can differ in their downstream physiological effects, most of the IIS proteins and their interactions appear to be conserved in mosquitoes [5–7,10–16] (Fig. 1). Orthologs of IIS proteins exist in ticks, lice, tsetse flies and sand flies (Table 1). Expression of endogenous insulin-like peptides (ILPs) has been confirmed in *Anopheles gambiae*, *A. stephensi*, *Aedes aegypti*, and *Culex pipiens*; partial ILP sequences have been identified in the Reduviidae 'kissing' bug *Rhodnius prolixus*, the human body louse *Pediculus humanus humanus*, and in the tick species *Ixodes scapularis* and *Dermacentor variabilis* [17]. Therefore, it is not surprising that exogenous insulin can activate the IIS pathway in arthropods. Bovine insulin can activate the neurosecretory cells of *D. variabilis*, and can regulate glycogen accumulation, via PI3K signaling, in cell lines derived from the hard tick *Rhipicephalus* (*Boophilus*) *microplus* [18,19].

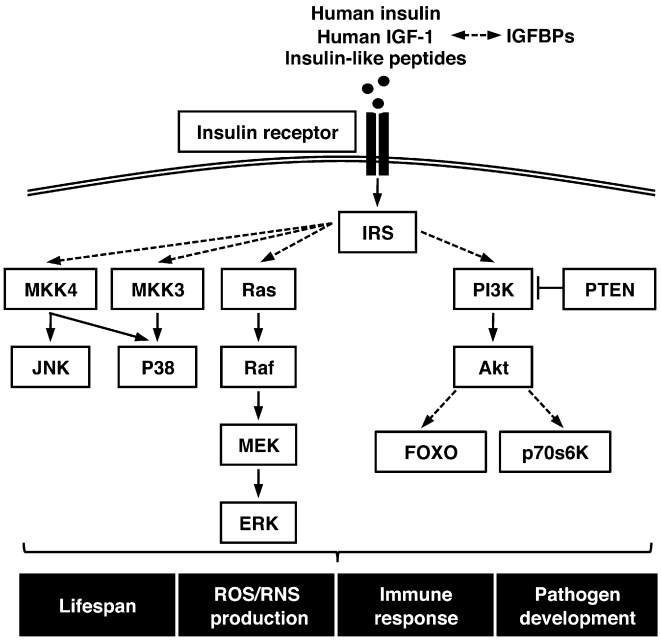

Fig. 1. Model of insulin/IGF-1 signaling pathway in vector arthropods. Solid lines indicate proven direct interactions between proteins, and dashed lines indicate indirect interactions that may involve other signaling proteins.

Among vector species, the mosquito response to ingested insulin has been the most well-studied. Beier et al. [5] were the first to demonstrate that ingestion of human insulin – at levels vastly exceeding those reported to occur in human blood – could significantly increase oocyst densities of *Plasmodium falciparum* in anopheline mosquitoes. We have since shown that physiological doses (170 pM) of human insulin in *A. stephensi* can activate both the PI3K/Akt and MAPK branches of the IIS pathway in mosquitoes [6,7,12,14,16] and that at least two IIS proteins – Akt/PKB and ERK – are critical for the control of malaria parasite infection [16,20]. Moreover, we have shown that the expression levels of *A. stephensi* ILPs change in response to human insulin and to *P. falciparum* infection, suggesting that ILP expression is finely tuned to IIS activation [11,12].

One of the best-known effects of IIS is the control of lifespan. The free radical hypothesis of aging posits that the accumulated damage caused by reactive oxygen species (ROS), such as superoxide and hydrogen peroxide (H_2O_2), potentiates aging [21]. Among invertebrate model organisms, the importance of oxidative stress in aging has been demonstrated in studies with *Drosophila melanogaster* and *Caenorhabditis elegans* [9]. We have shown that stimulation by human insulin increases H_2O_2 synthesis by mosquito cells, perhaps in part via a reduction in the activity of antioxidants such as the mitochondrial manganese superoxide dismutase (MnSOD) [7]. This increase in damaging ROS is most likely responsible for the decreased lifespan of insulin-fed *A. stephensi*, as provision of a cell-permeable SOD mimetic agent to insulin-fed mosquitoes returned survivorship to control levels [7]. The positive effects of insulin-induced ROS on *P. falciparum* development appear to be a consequence of ROS-dependent signaling in *Anopheles* mosquitoes, and not due to ROS-induced damage to the midgut epithelium [6]. These data suggest that early regulation of IIS-induced ROS primes an anti-inflammatory state in which parasite development is favored [6].

We have also shown that ingested human insulin and Akt over-expression lead to increased nitric oxide (NO) production [12,22], which likely contributes to increased midgut damage and decreased survivorship of mosquitoes. The induction of NO by insulin has also been observed in mammals [9], suggesting that this is a highly conserved response. Induction of NO production in *A. stephensi* midguts can limit malaria parasite development through the formation of inflammatory levels of toxic reactive nitrogen oxides [22–24]. In insulin-fed *A. stephensi* induction of nitric oxide synthase (NOS) expression – the enzyme that catalyzes the synthesis of NO – did not exceed control values until 36 h after feeding [12], a point at which *P. falciparum* ookinete invasion is largely completed [25]. In contrast, in transgenic Akt over-expressing mosquitoes NOS is induced very early after ingestion of an infected blood meal and inhibits parasite development in the midgut lumen [22]. Thus, differences in the kinetics of IIS can have distinct and dramatic impacts on malaria parasite development. Further, the beneficial effect of insulin on