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*Entry*

# Extracellular Vesicles and Immunomodulation in Mosquitoes and Ticks

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**Definition:** Extracellular vesicles are small blebs that are secreted by cells, which are lipid-rich and contain proteomic and genomic material (including small RNAs, mRNA, and plasmid DNA). These materials are delivered into recipient cells leading to a phenotypic change. Recent studies have demonstrated the secretion of extracellular vesicles by mosquito and tick cells, as well as tick salivary glands. Further, these studies suggest vesicles play a role in the transmission of vector-borne pathogens, including viruses and bacteria, and are involved in the manipulation of wound healing and immune responses. Both of these processes are key in the host response to hematophagous arthropods' feeding. The role of mosquito and tick EVs in the modulation of immune responses and pathogen transmission is discussed in this entry.

**Keywords:** hematophagy; immune modulation; pathogen transmission; extracellular vesicles; arthropods



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## 1. Introduction

Arthropods are important vectors of pathogens that can affect humans, wildlife, and domestic animals [1]. The World Health Organization (WHO) has estimated that diseases caused by vector-borne pathogens result in more than 700,000 human deaths yearly [2]. In the US, damages to livestock production due to arthropod feeding account for approximately USD 100 billion in losses annually [3]. Mosquitoes and ticks are particularly impactful from a public and animal health perspective and are the focus of this entry.

Mosquitoes can transmit flaviviruses, protozoans, bacteria, and nematodes. With over 3000 different species, mosquitoes are a worldwide public health concern [4–6]. An anthropophilic adult female mosquito will need multiple bloodmeals to enhance their fitness, obtain the energy to search for a mate, and to initiate vitellogenesis [4,7–9]. Hard ticks, on the other hand, are obligatory blood feeders that can feed on a host for days to weeks at a time [10,11]. Ticks are considered second to mosquitoes in their public health relevance. It is estimated that tick bites are responsible for the transmission of over 100,000 cases of tick-borne diseases in humans throughout the world [12,13]. A recent study showed that 140,281 insured patients were diagnosed with Lyme disease in the US alone from 2010 to 2018, with incidences as high as 87.9/100,000 enrollees [14] and the Center for Disease Control and Prevention (CDC) now estimates that 476,000 Americans are affected by this disease [15]. Given that most of the work on vector-derived extracellular vesicles (EVs) has been carried out in these two vector species, the authors will limit this entry to what is known about mosquito- and tick-derived EVs.

Due to their need for a bloodmeal, either for survival or reproduction, arthropods have evolved intricate mechanisms that allow them to counteract immune and inflammatory responses by their host. For example, during feeding, arthropods can release EVs via their saliva [16,17]. EVs are double-layer vesicles that are secreted by all cells and are essential for cell-to-cell communication [18–22]. Physiological changes in the cell can

lead to an increase in vesicle secretion or cause changes in the cargo packed within the extracellular vesicles secreted by these cells. For example, pathogen-infected cells secrete EVs that carry infectious cargo, such as viral RNA. These vesicles can enhance pathogen transmission and replication [23–25]. EVs originating from infected vector cells can serve as the source of infection for host cells in vitro [24,26–28]. In other cases, EVs produced by virus-infected cells can inhibit pathogen transmission [29,30]. Nevertheless, the relevance of these phenomena during in vivo pathogen transmission is undetermined. This entry focuses on the function of EVs during arthropod feeding and their potential contribution in pathogen transmission.

## 2. Hematophagy in Arthropods

Blood feeding behavior appeared in arthropods on six independent occasions throughout evolution [31]. In the case of insects, hematophagy emerged on several separate occasions driven by two potential scenarios: (1) The association of insects within the nest of different vertebrate animals due to their attraction to the protected environment provided, which increased the contact of insects with the vertebrate host. The constant interaction with vertebrate animals may have led to behavioral and structural adaptations that allowed these insects to access a higher nutritional substrate, such as blood. (2) Ancestral insect lineages containing piercing mouthparts may have accidentally probed vertebrate animals [32]. In the case of ticks, fluid feeding is characteristic of the Parasitiformes, the major lineage that ticks belong to. Thus, it is probable that blood feeding arose as a result of this adaptation to feed on fluids [33]. For arthropods to successfully feed on blood, they had to acquire several molecular adaptations that allow them to overcome host immune and wound healing responses, permit the detoxification of harmful molecules found within blood, allow them to stop blood coagulation, and supplement nutritional factors that are missing in blood. Understanding the adaptation of arthropods to hematophagy is important due to the underlying connections with pathogen transmission [34]. As discussed in the introduction, both mosquitos and ticks secrete salivary factors that dampen immune responses, reduce coagulation, increase blood flow, alter the skin microbiome, and delay wound healing. Herein, the authors will focus on the effect that certain salivary factors have at the bite site.

### *2.1. Immune Modulation*

Both mosquitos and ticks can diminish innate and adaptive immune responses. In the case of the innate immune response, salivary components target immune cell migration, the secretion of cytokines and chemokines, and the complement system. The complement system comprises three complexes of plasma proteins and receptors that are part of the innate immune system and assist in skin homeostasis [35]. The complement system is initiated through the activation of the alternative, the lectin, or the classical pathway. Some of the key components of the complement system are the proteins C3 and C5, which form part of the alternative and lectin pathways. The classical pathway is initiated by the interaction of C1 and an antibody bound to its specific antigen. Some mosquitos, such as *Anopheles (Nyssorhynchus) aquasalis*, can inhibit the alternative pathway by blocking C3b deposition [36]. Similarly, saliva from *Rhipicephalus (Boophilus) microplus* ticks can hinder the activation of the classical and alternative pathways by affecting the cleavage of C4 by C1b and preventing the action of the C3 convertase [37], suggesting that the control of the complement system is a conserved mechanism within hematophagous arthropods.

For cell migration, mosquito and tick saliva have dissimilar effects. In mosquitos, salivary gland extracts (SGE) from *Aedes aegypti*, the vector of dengue, increased the permeability of endothelial cell monolayers in vitro and the leakage of the vasculature of mice ear in vivo [38]. This augmented vascular leakage may explain the enhanced migration of neutrophils, monocytes, and dendritic cells to the skin during inoculation of dengue virus (DENV) and the Semliki Forest virus (SFV), a close relative of the chikungunya virus [38,39]. In both cases, either the mosquito bite or the inoculation of SGE boosted the

severity of the viral infection in the skin. Although an increase in innate immune cells at the bite site is also observed during tick feeding [16,40], tick saliva decreases the migration of dendritic cells in vivo [41]. This effect on dendritic cell migration may be the result of the decreased expression of adhesion proteins in subcutaneous tissue, as shown in vitro [42], the reduced expression of chemokine receptors CCR5 and CCR7 and the secretion of TNF$\alpha$ and IL12p40. Tick saliva also inhibited the differentiation of dendritic cells [43,44]. These effects on differentiation and cytokine expression could be due to the induction of IL-10 secretion and the stimulation of the TLR2 receptor [45]. Thus, arthropods share some common mechanism to dampen innate immune responses. However, depending on the vector species, evident differences exist in how they affect signaling and cellular immunity. These differences are probably the result of the unique selective pressures that mosquitos and ticks face. The interaction of salivary compounds and immune modulation has been previously described in detail in [46,47].

### *2.2. Antihemostatic and Wound Healing*

Tissue repair, or wound healing response, takes place following an injury or abrasion of the tissue. This process involves multiple stages starting from hemostasis, inflammation, tissue proliferation, and remodeling [48]. Hemostasis includes the constriction of the blood vessels to diminish blood lost, coagulation, and platelet aggregation. Blood sucking arthropods have developed or acquired salivary molecules that restrict the hemostatic process. One interesting example is the gain of a vertebrate vasodilator by soft ticks of the genus *Ornithodoros* through horizontal gene transfer (HGT) [49]. Three isoforms of an adrenomedullin (ADM)-like protein are found within the genome of *Ornithodoros moubata*, *O. parkeri*, and *O. coriaceus*. Interestingly, the gene encoding this protein is missing from other soft ticks and invertebrates. The closest homologs are present in amphibians and fish, suggesting that this gene was acquired by an ancestor *Ornithodoros* probably during feeding on ancient reptiles. Comparatively, SGE from two hard ticks, *Rhipicephalus appendiculatus* and *Dermacentor reticulatus*, contain compounds that induce both vasodilation and vasoconstriction, depending on the feeding time. During the rapid feeding phase, male ticks that fed for 6 days showed vasodilatory properties, whereas females and males that fed during the early and late stages showed vasoconstricting properties [50]. Thus, this indicates that the vasoacitve properties of tick saliva may change depending on sex and feeding phase. For mosquitoes, only vasodilatory compounds have been reported. *A. aegypti* saliva contains proteomic compounds (susceptible to trypsin digestion) with vasodilatory properties [51,52]. These proteins were identified as tachykinins, which have also been identified in *Aedes triseriatus* [53]. Another vasodilator found within the saliva of mosquitoes is catechol oxidase [53]. Other antihemostatic properties of hematophagous arthropods are discussed in the review by D. Champagne [54].

Following the inflammatory process, the tissue proliferation and remodeling process involves the restructuring of the extracellular matrix (ECM) of keratinocytes, fibroblast, epithelium, and endothelium [48]. The growth and restructuring of the tissue require the production and secretion of growth factors and the rearrangement of the cytoskeleton. Tick saliva contains growth factor binding proteins that interact with transforming growth factor beta (TGF-β1), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF-2), and hepatocyte growth factor (HGF), affecting cell proliferation and altering actin polymerization during in vitro assays [55]. Another group of tick salivary proteins that act by decreasing angiogenesis are serine protease inhibitors (Serpins). For a complete review of tick salivary serpins, please refer to [56]. Proteins and factors that act on the wound healing response during mosquito feeding have not been experimentally identified.

### *2.3. Control of Commensal Bacteria*

Commensal microbes colonize the skin of humans and animals early in life. These microbes form part of the skin microbiome, which interacts with immune and skin cells to prime and shape the immune system [57]. Studies indicate that ticks can alter the

skin microbiome by potentially killing *Staphylococcus epidermidis* through the degradation of peptidoglycan by the salivary protein $Dae^{Is}$ [58]. This protein is secreted during the bloodmeal to control the presence of bacteria that may be damaging to the tick midgut's integrity. Thus, this provides a fitness advantage to the tick.

## 3. Extracellular Vesicles in Mosquitoes

Mosquitoes are top vectors of many vector-borne pathogens that cause several human diseases, such as malaria, dengue, West Nile, and Zika [5,59]. They feed by piercing through the skin of their host using needle-like mouthparts (Figure 1A). Mosquito C6/36 cells secrete EVs in vitro [26,28]. Although the exact function of these vesicles during feeding has not been determined, recent studies indicate that mosquito vesicles carry viral cargo, which facilitates spread and potentially transmission. EVs that originated from Zika virus (ZIKV) or DENV-infected cells promote viral spread by carrying viral RNA to receiving naïve cells [26,28,60–62]. Along with the viral RNA, these vesicles also contain altered host-derived cargo that could enhance pathogen transmission and replication. For example, EVs from DENV3-infected monocyte-derived dendritic cells (Mo-DCs) contained mRNAs encoding the transcripts for Interferon-Induced Transmembrane Protein 1 (IFITM1), Interferon-Induced Protein with Tetratricopeptide Repeats 1 (IFIT1), and DExD/H-Box Helicase 58 (DDX58) involved in DENV infection [26,28]. These vesicles also transported microRNA-4327, which is used as a marker for severe dengue, and the tetraspanins (CD9, CD81, CD63) and Tsp29fb [61]. The modified vesicles may be involved in the translocation of DENV from DENV-infected C6/36 [26–28,61–63]. Nevertheless, EVs also inhibit viral replication. For example, EVs secreted by DENV-infected C6/36 cells restricted viral membrane fusion, inhibiting viral replication, and spread [28,30]. Thus, in the context of dengue infection, EVs can facilitate or dampen viral spread. Whether these conflicting effects are due to different vesicle populations being secreted by specific cells remains to be determined.

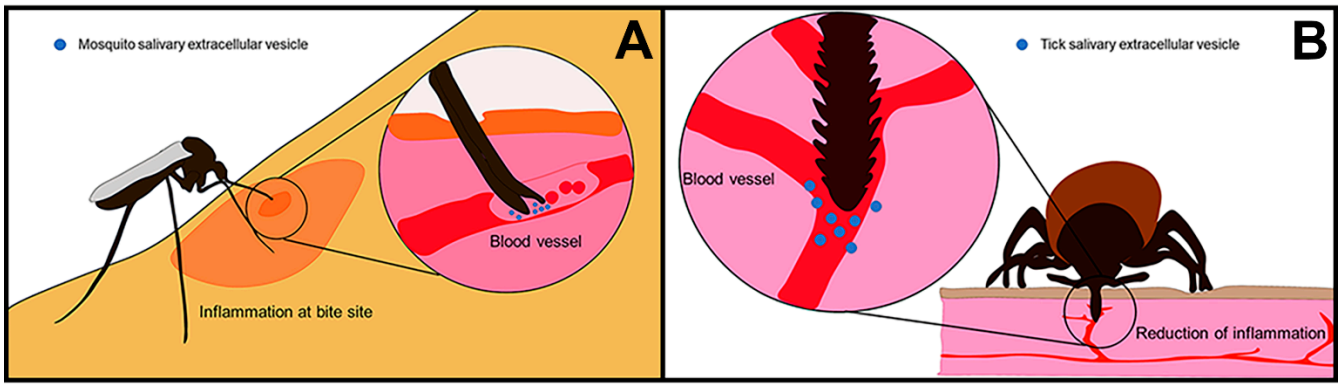


**Figure 1.** Extracellular vesicle secretion during arthropod feeding on vertebrate host. (**A**) Mosquitoes pierce the skin of their host using modified mouthparts. Their labella, labrum, and labium form needle-like structures that can pierce directly to the blood vessels where they feed. Once inside the blood vessels, they release saliva-containing EVs, which potentially interact with blood cells. (**B**) Ticks, on the other hand, use scissor-like structures called chelicerae to cut through the skin. They introduce their hypostome, which harbors several hook-like structures that allow the ticks to anchor themselves in the skin while they feed. Another mechanism that allows ticks to anchor firmly to the host skin is the secretion of cement proteins. These cement proteins polymerize and interact with other proteins to form a cone-like structure that presents antimicrobial properties, facilitates tick attachment, seals the feeding lesion, and assists in pathogen transmission. Through the introduction of their hypostome, they damage blood vessels and cells in the dermis and epidermis. They secrete their saliva, containing EVs, into the pool of blood that forms at the bite site.

During Zika virus infection, EVs can serve as the inoculum of infection [61]. EVs secreted by infected C6/36 cells carrying viral E protein (ZIKV E-protein) are infectious for human peripheral blood monocytes (THP-1) cells and endothelial vascular (HMEC-1) cells [26,62]. Similarly, EVs secreted by ZIKV-infected C6/36 mosquito cells can activate a pro-inflammatory response and induce an immunophenotype in human monocyte cells, comparable to that observed during ZIKV infection [62]. When compared to mock cells, the stimulation of healthy endothelial vascular cells with ZIKV C6/36 EVs also favored pro-coagulant state and pro-inflammatory responses [26,62]. Despite the observed involvement of mosquito derived vesicles in the spread of both DENV and ZIKV in vitro, whether mosquito salivary EVs contain viral particles remains to be confirmed. Similarly, how mosquito EVs may affect host immune responses at the bite site needs to be explored.

## 4. Extracellular Vesicles in Ticks

Ticks are hematophagous arthropods that, during feeding, secrete salivary substances with anticoagulatory, anti-inflammatory, and vasodilatory functions [64]. Ticks cause significant damage to the skin of humans and animals (Figure 1B). Thus, these substances function to circumvent the host's immunological response and indirectly facilitate the transmission of pathogens [65], including protozoan, bacterial, and viral agents. Several studies have demonstrated the secretion of EVs within the saliva of *Ixodes scapularis*, *Amblyomma maculatum*, and *Haemaphysalis longicornis* ticks [16,17,66].

EVs transfer intracellular information such as proteins and miRNAs between cells or tissues and mediate changes in cellular activity and pathways in the recipient cell [67,68]. The proteomic analysis of tick salivary EVs has identified several known effector proteins with immunomodulatory properties, including lipocalins, serine protease inhibitors, and cement [16,66], and several host proteins including histones, metabolic proteins, and immunoglobulins [66]. Various known exosomal markers, such as heat shock protein 70 (HSP70), the tetraspanin CD63, ALG-2-Interacting Protein X (ALIX), and Tumor Susceptibility 101 (TSG101), have been detected in these exosomes through proteomics and Western blot analysis [16,17,66].

Tick salivary EVs have also been shown to contain miRNAs, including novel miRNAS and previously described small RNAs [69]. Several miRNAs showed an upregulation during feeding, indicating a potential role in the tick's response to host immunity. Salivary exosomes carrying HSP70 and miRNAs potentially aid in fibrinogen degradation and modulate host gene expression at the vector–host interface [70]. The vesicle's cargo may also regulate key homeostatic responses in the host [71]. In fact, Zhou et al., 2020 [17] made the first report implicating tick salivary gland derived exosomes in the modulation of the wound healing responses at the tick bite site [17]. Later, independent studies demonstrated that tick EVs had a localized action on skin immunity by facilitating feeding [16]. EVs may allow ticks to bypass the immune barriers present in the skin successfully to complete their bloodmeal.

Due to their involvement in the manipulation of host immune responses, EVs are suspected to aid in the transmission and establishment of tick-borne pathogens. Oliva Chavez et al. [16] showed that the inoculation of the intracellular bacterium *Anaplasma phagocytophilum* along with tick salivary EVs increased the frequency of bacterial infection in the skin of naïve mice. Further, in 2018, Zhou et al. [28] demonstrated that cell lines derived from *I. scapularis* ticks are capable of secreting EVs. Interestingly, the vesicles from Langat virus-infected tick cells were shown to carry viral proteins and RNA and aid in the viral spread to invertebrate and vertebrate host cells. Nevertheless, what exact function EVs play during pathogen transmission remains to be defined.

## 5. Conclusions and Prospects

Blood feeding or hematophagy has evolved independently in around ~15,000 species of arthropods [11,31,72]. To facilitate blood feeding, these arthropods have gained mechanisms to counteract blood coagulation [31], the presence of endosymbionts within their

microbiome for the production of B vitamins [73], the ability to prevent damage by heme molecules resulting from the digestion of hemoglobin [74], and to escape host immune responses [75,76]. As a side effect of the immunomodulation of host immune responses, arthropod saliva enhances the establishment and transmission of the pathogens they carry [77]. Recent studies in ticks have demonstrated that EVs secreted within tick saliva can regulate the secretion of chemokines and cytokines, as well as affect specific immune cell populations within the skin [16]. These vesicles can also affect wound healing responses [17]. Likewise, in vitro studies using both tick and mosquito cells lines have indicated that arthropod-derived vesicles may serve in the dissemination of viruses from infected vector cells onto mammalian host cells [28,62]. A graphical representation of the most common cargo found in EVs derived from mosquito-cells and tick salivary glands is depicted in Figure 2. This figure also shows some of the modifications in EV cargo that occur during viral infection of vector cells. Because of the implications in the infectious process of vector-borne pathogens, EVs could serve as an alternative for the design of vaccines and therapeutics to stop the transmission of these pathogens. Similarly, more studies should focus on defining the molecular and cellular mechanism by which EVs affect signaling and immune responses at the bite site. This information may lead to the discovery of novel mechanisms that arthropods have acquired to counteract host responses.

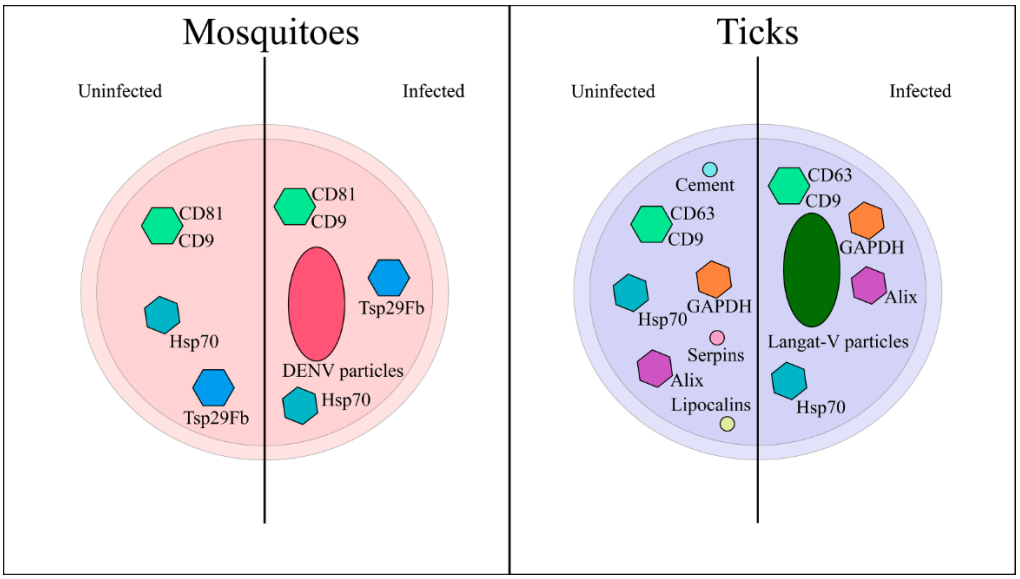


**Figure 2.** Extracellular vesicle cargo found within mosquito- and tick-derived vesicles. Mosquito-derived vesicles contain several well-known extracellular vesicle markers, such as tetraspanins (CD81 and CD9) as well as Heat Shock Protein 70 (Hsp70). These proteins are present within vesicles derived from infected and uninfected cells. EVs secreted by DENV-infected cells contain viral particles that can start an infection in naïve cells. Tick vesicles, on the other hand, contain EV markers (CD9, CD63, Alix, and Hsp79), metabolic proteins (GAPDH), and tick salivary effectors (Lipocalins, Cement, and Serpins). Whether these salivary effectors are affected by bacterial or viral infection is unknown. EV markers and viral particles are found within EVs secreted by virus-infected tick cells. In both systems, arthropod-derived vesicles appear to facilitate viral spread and pathogen transmission.

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