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Invasion of mosquito salivary glands by malaria parasites: Prerequisites and defense strategies

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Abstract

The interplay between vector and pathogen is essential for vector-borne disease transmission. Dissecting the molecular basis of refractoriness of some vectors may pave the way to novel disease control mechanisms. A pathogen often needs to overcome several physical barriers, such as the peritrophic matrix, midgut epithelium and salivary glands. Additionally, the arthropod vector elicites immune responses that can severely limit transmission success. One important step in the transmission of most vector-borne diseases is the entry of the disease agent into the salivary glands of its arthropod vector. The salivary glands of blood-feeding arthropods produce a complex mixture of molecules that facilitate blood feeding by inhibition of the host haemostasis, inflammation and immune reactions. Pathogen entry into salivary glands is a receptor-mediated process, which requires molecules on the surface of the pathogen and salivary gland. In most cases, the nature of these molecules remains unknown. Recent advances in our understanding of malaria parasite entry into mosquito salivary glands strongly suggests that specific carbohydrate molecules on the salivary gland surface function as docking receptors for malaria parasites.

Keywords

Malaria; *Plasmodium*; Vector-borne diseases; Parasite-vector interactions

1. Introduction

Vector-borne diseases continue to be a major public health threat throughout the world. Usually transmitted by arthropods, their causative agents include helminths and protozoa, as well as microbial pathogens and viruses. Few vaccines are available and disease control and prevention in the majority of cases relies on vector control and, to a lesser extent, on drug treatment of the infected human population. Since the 1970s, vector-borne diseases have been on the rise. The underlying causes are complex and include political, sociological and biological factors, such as emergence of drug-resistant strains of pathogens, as well as insecticide-resistant vector populations. Other approaches to disease control are envisioned. These include the use of transmission-blocking vaccines against the pathogen (most recently

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reviewed by Coutinho-Abreu and Ramalho-Ortigao, 2010) or vector (Trager, 1939), and the use of refractory vectors for population replacement. The development of these strategies requires an intimate knowledge of vector biology and pathogen-vector interactions in order to identify potential molecular targets. In this article, we summarize our current understanding of salivary gland invasion, arguably the most important step for transmission in the majority of vector-borne diseases, using malaria as the best-studied example.

2. Salivation is the most common transmission strategy employed by vector-borne disease agents

Pathogens always enter their vector species with the blood meal. However, their transmission to the vertebrate host is more varied. While this transfer usually occurs during blood feeding on the vertebrate host, different exit strategies are used. Table 1 summarizes the different mechanisms of horizontal transmission, including salivation, active and passive escape through the cuticle, posterior exit with arthropod feces (stercoration), regurgitation, and in rare cases, ingestion of the vector. Host entry by salivation is the most commonly utilized and efficient transmission method, e.g. all known arthropod-borne viruses are transmitted this way. The pathogen is directly injected into the host during probing or blood feeding. This method cannot be employed by pathogens that remain in the gut or the hemocoel of the vector as it requires entry into the salivary glands. Therefore, invasion of salivary glands can be considered as one of the most important events required for vector-borne disease transmission.

The molecular make-up of salivary glands of a variety of hematophagous arthropods, including of several mosquito species (Valenzuela et al., 2002, 2003; Calvo et al., 2004, 2007, 2010; Ribeiro et al., 2004b; Arca et al., 2005, 2007), sandflies (Ribeiro et al., 2000), blackflies (Andersen et al., 2009), triatome bugs (Ribeiro et al., 2004a), fleas (Andersen et al., 2007), and hard and soft tick species (Ribeiro et al., 1991; Santos et al., 2004; Francischetti et al., 2005; Mans et al., 2008), have been analyzed in detail. As a consequence, we have detailed understanding of the saliva components, which contribute to blood feeding in different ways, including regulation of blood haemostasis by vasodilators, inhibitors of blood clotting and platelet aggregation. In contrast, the salivary gland surface molecules that are required for pathogen entry are mostly unknown. Additionally, the cell biological events occurring during this invasion process have only been described for a handful of vector-borne disease pathogens. The following sections discuss the currently best-understood system of salivary gland invasion: the cross-talk of the malaria parasite, *Plasmodium* spp. with its mosquito vector, *Anopheles* spp..

3. The cell biology of malaria parasite invasion of mosquito epithelia

The *Plasmodium* parasite undergoes a complex life cycle encompassing heterophasic generational changes, and obligatorily fulfills its sexual life cycle in the mosquito. Male and female gametocytes, taken up with the blood meal, undergo gametogenesis within the lumen of the mosquito midgut. Fertilization takes place within approximately 2 h after a blood meal and the resulting zygote undergoes meiosis and develops into the motile ookinete. Approximately 1 day after the infectious blood meal, the ookinete traverses the peritrophic matrix and subsequently the midgut epithelium itself. The ookinete then rounds up and forms the oocyst, the stage in which sporogony occurs. Approximately 2 weeks after the blood meal sporozoites are released into the hemocoel. They then reach the salivary glands and again traverse an epithelium, in this case to penetrate into the salivary gland lumen, where they mix with the saliva and are injected into the next vertebrate host (recently reviewed by Baton and Ranford-Cartwright, 2005b).

3.1. Midgut invasion

The cellular processes that occur during ookinete invasion and traversal of the midgut epithelium (Fig. 1A) have been under intense investigation and various aspects have recently been reviewed extensively (Baton and Ranford-Cartwright, 2005a,b;Kumar and Barillas-Mury, 2005;Vinetz, 2005;Vlachou et al., 2006;Whitten et al., 2006). The ookinete presumably migrates actively in the blood bolus before encountering its first barrier, the peritrophic matrix (Sieber et al., 1991). Some parasite species require the secretion of chitinase to cross this barrier in order to reach the microvilli of the midgut epithelium (Huber et al., 1991;Tsai et al., 2001). Midgut cell entry is thought to be mediated by a yet to be identified specific receptor-ligand interaction and occurs into the apical-lateral membrane where three epithelial cells converge (Baton and Ranford-Cartwright, 2004). Ookinete invasion is an active process that requires gliding motility, a type of movement typical for all invasive apicomplexan parasites (Keeley and Soldati, 2004). The invasion induces tyrosine nitration in the invaded midgut cells, which involves nitric oxide synthase (NOS) up-regulation followed by increased peroxidase activity (Kumar and Barillas-Mury, 2005). Such a defense reaction generating toxic chemicals is potentially harmful to the host, often leading to apoptosis. Indeed, ookinete invasion of midgut epithelia induces apoptosis of the invaded cells, which are expelled from the epithelium by actin-based restitution mechanisms (Time-bomb model; Han et al., 2000;Gupta et al., 2005). A single ookinete often serially invades several cells, which all become apoptotic and are excluded from the epithelium. The parasite exits the midgut epithelium at its basal side and is at that stage covered by lamellipodia that form a “hood” around the parasite (Vlachou et al., 2006). The accumulation of actin around the parasite at the time of egress has been also noted (Vernick et al., 1995;Whitten et al., 2006). The interaction of the extracellular ookinete with the basal lamina is believed to induce transformation to the oocyst stage (Weathersby, 1952). The passage of an individual ookinete is thought to take no more than 30 min. However, ookinete invasion is asynchronous and continuous up to 36 h after a blood meal. Eventually, the remaining parasites present in the midgut lumen are excreted with the digested blood meal.

3.2. Reaching the salivary gland

Once oocysts are established, mitotic divisions occur and ultimately sporozoites are formed. Upon egress from mature oocysts, sporozoites travel via the hemolymph to the salivary glands, where they invade salivary gland secretory cells. Sporozoites can be found throughout the mosquito hemocoel (Garnham, 1966; Golenda et al., 1990) and are passively carried through the hemolymph to the salivary glands (Akaki and Dvorak, 2005; Hillyer et al., 2007). Less than 20% of the sporozoites entering the hemocoel ultimately reside within the salivary gland and the remaining 80% of parasites are eliminated from the hemolymph (Hillyer et al., 2007) and within the salivary gland (Pinto et al., 2008) by an unknown mechanism.

3.3. Salivary gland invasion

Morphological studies of the passage of *Plasmodium* sporozoites through the salivary gland of their mosquito vectors have been performed by electron microscopy using avian and rodent malaria models (Sterling et al., 1973; Meis et al., 1992; Pimenta et al., 1994; Ando et al., 1999). Similar to midgut invasion, the sporozoite takes an intracellular route through the salivary gland epithelium to reach its final destination in the mosquito, the salivary gland duct. However, the cell-biological events that occur during sporozoite invasion of salivary gland epithelial cells (summarized in Fig. 1B) differ substantially from those occurring during midgut invasion. The sporozoite approaches the salivary gland epithelium from the basal side and first has to recognize, attach to and subsequently penetrate the basal lamina in order to reach the epithelial cell. Invasion itself, as in midgut epithelia, is thought to be

mediated via receptor-ligand interactions and involves parasite gliding motility (Sultan et al., 1997) and the possible formation of moving junctions (Pimenta et al., 1994). During cell invasion, the sporozoite is initially surrounded by a second membrane (Pimenta et al., 1994), which has been interpreted as a transient parasitophorous vacuole (PV) (Rodriguez and Hernandez-Hernandez Fde, 2004). The origin of this membrane is unclear. The sporozoite subsequently escapes the vacuole by an unknown mechanism, and exits the host cell into the secretory cavity. The majority of sporozoites remain in these cavities and only a few enter the salivary duct (Frischknecht et al., 2004). The invasion process has little visible effect on the invaded host cell. No apoptosis or actin rearrangements of parasite-invaded salivary gland epithelial cells have been described. In contrast to midgut invasion, NOS is either not transcriptionally altered or is down-regulated in *Plasmodium berghei*-infected salivary glands (Rosinski-Chupin et al., 2007; Dimopoulos et al., 1998), and the enzyme is not detectable by immunofluorescence analysis (Pinto et al., 2008).

4. Parasite molecules required for *Plasmodium* spp. salivary gland invasion

The molecular mechanisms responsible for the interaction between the malaria parasites and molecules on the surface of mosquito salivary glands are complex and not well understood. Apicomplexan parasites are named after their apical complex, an anterior structure formed by three organelles: the rhoptries, dense granules and micronemes. While molecules released from the rhoptries participate in PV formation (Lingelbach and Joiner, 1998), molecules from dense granules complete the establishment of parasites in their host cell, and the content of micronemes is required for host cell invasion. Micronemes are vesicles containing molecules that after secretion are maintained on the surface of the parasite. These molecules participate in adhesion to target cells, gliding motility and the invasion process (Donahue et al., 2000).

To date, no data formally show rhoptry, dense granule or microneme secretions to be required for sporozoite invasion of the mosquito salivary gland. However, several micronemal proteins have been shown to be important in salivary gland invasion: the circumsporozoite protein (CSP), the apical membrane antigen/erythrocyte binding-like protein (MAEBL), the thrombospondin-related anonymous protein (TRAP), and the up-regulated-in-oocysts sporozoites protein 3 (UOS3) (Mikolajczak et al., 2008), also called S6/TREP (Combe et al., 2009; Steinbuechel and Matuschewski, 2009). CSP is a parasite surface molecule essential for sporozoite development within oocysts and invasion of salivary glands (Menard et al., 1997, Sidjanski, 1997). An 18 amino acid N-terminal peptide fragment of CSP that includes Region I has been shown to specifically bind to salivary glands (Sidjanski et al., 1997; Myung et al., 2004). Together with CSP, the malaria sporozoite utilizes a second protein, MAEBL, to facilitate attachment to salivary glands (Kariu et al., 2002). MAEBL displays adhesive features involved in attachment of target organs. Mutant parasites developed normally within the mosquito vector but were unable to invade salivary glands or hepatocytes. While MAEBL expression is necessary for salivary gland invasion, conflicting data exist on its role in sporozoite motility (Kariu et al., 2002), (F. Frischknecht, K. Michel, A.K. Mueller, unpublished data). Midgut and salivary gland sporozoites express two dominant alternatively spliced *MAEBL* mRNAs, encoding a transmembrane and a secreted MAEBL isoform (Singh et al., 2004). Using knockout and allelic replacement experiments, Saenz et al., (2008) recently showed that the MAEBL transmembrane isoform is essential for salivary gland invasion by human malaria parasites.

The third micronemal protein, TRAP, also displays complex interactions with salivary glands (Sultan et al., 1997; Kappe et al., 1999). TRAP is a member of a family comprising at least six type I transmembrane invasins, which also include circumsporozoite and TRAP-related protein (CTRP; Dessens et al., 1999), merozoite TRAP (MTRAP, Baum et al.,

2006), *Plasmodium* thrombospondin-related apical merozoite protein (PTRAMP; Thompson et al., 2004), TRAP-related protein (TREP, originally named UOS3/S6), and TRAP-like protein (TLP). In contrast to CSP and MAEBL, TRAP is not required for initial attachment to salivary glands but for the gliding movement of sporozoites, by functioning as a bridge between the outer membrane surface and the intracellular actin-myosin motor complex through binding to the actin-bridging molecule aldolase. TRAP is also needed for the invasion process of salivary glands and hepatocytes (Sultan et al., 1997; Kappe et al., 1999; Matuschewski et al., 2002a). These two functions can be genetically isolated and are mediated by distinct domains of the protein (Kappe et al., 1999; Matuschewski et al., 2002a). The extracellular portion of TRAP needs to be shed from the parasite surface for gliding motility and host cell invasion to occur. This process is potentially mediated by the rhomboid protease ROM4, which in cell-based assays cleaves TRAP within its transmembrane domain and causes the release of its extracellular portion (Baker et al., 2006). The function of the cytoplasmic domain of TRAP during sporozoite invasion of mosquito salivary glands and hepatocytes can be partially complemented by the cytoplasmic domain of at least one other member of the type I transmembrane invasins, TLP (Heiss et al., 2008). However, knockout-TLP *P. berghei* parasites produce normal numbers of salivary gland sporozoites, indicating that TLP might not be involved or only plays a non-redundant role for sporozoite invasion of salivary glands (Heiss et al., 2008). In contrast, TLP is required for cell/tissue traversal in the vertebrate host, as knockout parasites exhibit defects in hepatocyte cell traversal in vitro and in mouse infectivity in vivo (Moreira et al., 2008).

Whole genome approaches have been used to identify parasite genes required for infection of specific tissues (Matuschewski et al., 2002b; Kaiser et al., 2004; Mikolajczak et al., 2008; Tarun et al., 2008). Two of these studies identified a putative transmembrane protein, UOS3/S6, a member of the TRAP protein family, also called TREP (Combe et al., 2009). Unlike TRAP, UOS3/S6 lacks apparent adhesion motifs such as the Von Willebrand factor A-domain and only contains a degenerate thrombospondin type I motif in its extracelluar region. UOS3/S6 knockout sporozoites display reduced infectivity to salivary glands (Steinbuechel and Matuschewski, 2009). Using advanced microscopy techniques, UOS3/S6 has been shown to mediate attachment and detachment to substrate surfaces, and to interact with TRAP and TLP during gliding motility (Hegge et al., 2010).

In addition to the proteins described above, the sporozoite surface proteins Cysteine Repeat Modular Proteins (CRMPs) 1 and 2 are also required for salivary invasion (Thompson et al., 2007). Knockout of these two proteins does not seem to affect motility and sporozoites remain infectious to the vertebrate host, however no salivary gland sporozoites were observed.

5. Salivary gland surface molecules required for parasite binding

As described in Section 4, sporozoite attachment to salivary glands is facilitated by several different proteins on the sporozoite surface. The sporozoite encounters the salivary gland from the basal side. It initially attaches to the basal lamina of this epithelium and subsequently binds to the basolateral membrane of the epithelial cells. Initial attachment and presumably invasion are facilitated by the interaction of sporozoite surface molecules with molecules present on the salivary gland surface; so what do we know about its molecular composition?

Mosquito salivary glands bind a variety of lectins, indicative of sugar molecules on their surface, most likely present in the basal lamina (Molyneux et al., 1990; Mohamed and Ingram, 1993; Barreau et al., 1995). Indeed, salivary glands prepared from adult female

Anopheles stephensi mosquitoes contain chondroitin sulfate and heparan sulfate, members of the glycosaminoglycan family of heteropolysaccharides (Sinnis et al., 2007). These sugar moiteties are likely to participate in parasite entry as lectin binding to *Aedes aegypti* salivary glands blocks *Plasmodium gallinaceum* sporozoite invasion (Barreau et al., 1995). The parasite surface molecule CSP binds to mammalian heparan sulfate (Sinnis et al., 1994; Ying et al., 1997; Rathore et al., 2001), which is required for liver cell invasion (Frevert et al., 1993; Pinzon-Ortiz et al., 2001). The demonstration that CSP binds to mosquito heparan sulfates (Sinnis et al., 2007) suggests that this may be the salivary gland molecule to which CSP binds.

In addition to lectins, polyclonal antibodies raised against salivary gland extracts can also block sporozoite salivary gland invasion (Barreau et al., 1995; Brennan et al., 2000). These antibodies recognize epitopes in the basal lamina, suggesting that this specialized extracelluar matrix may have receptors that mediate sporozoite attachment (Barreau et al., 1995). Subsequently, using purified monoclonal antibodies that specifically bound to median and distal lateral salivary gland lobes, the regions preferentially invaded by sporozoites (Sterling et al., 1973), a new Salivary Gland Surface (SGS) protein family was identified (Korochkina et al., 2006). SGS1 is a large protein of more than 220 kDa that is present in the basal lamina of female salivary glands. It contains heparin-binding and tyrosin O-sulfation motifs, suggesting that this protein is highly glycosylated in vivo. How SGS1 contributes to parasite invasion is unclear. Based on its heparin-binding domain, it is possible that SGS proteins bind a soluble heparin-like glycosaminoglycan present in the haemolymph that in turn could bind to CSP (Korochkina et al., 2006).

Two additional proteins that are only expressed in the distal lobes of female salivary glands have been shown to serve as sporozoite receptors (Brennan et al., 2000; Korochkina et al., 2006). One of these proteins, called Saglin, is a secreted protein that contains several putative glycosylation sites (Okulate et al., 2007) and its expression is induced by blood feeding (Korochkina et al., 2006). Furthermore, Saglin can bind to the A domain of TRAP in vitro (Ghosh et al., 2009), the same domain that had previously been shown to be required for parasite invasion (Matuschewski et al., 2002a). It is likely that the binding of TRAP to Saglin is also required in vivo, as this interaction can be inhibited in vivo by a synthetic peptide, called SM1, which binds to Saglin and abolishes the parasite's ability to invade salivary glands (Ghosh et al., 2001).

Taken together, these studies indicate that malaria sporozoites utilize at least two receptor-ligand interactions to bind to the mosquito salivary glands. Given that at least five other sporozoite surface proteins with unknown binding partners are involved in the invasion process, the interaction between parasite and gland surface is likely to be more complex.

6. Epithelial defense mechanisms limit parasite success during salivary gland invasion

Once the sporozoite has attached to and migrated through the basal lamina, it actively invades the salivary gland epithelial cells. Under laboratory conditions, invasion of often hundreds to thousands of malaria sporozoites into a single salivary gland has seemingly little effect on the invaded cells (Pimenta et al., 1994; Pinto et al., 2008). In contrast, ookinete invasion of the mosquito midgut epithelium causes major cytoskeleton restructuring that ultimately leads to apoptosis and expulsion of the invaded cells from the epithelium (Han and Barillas-Mury, 2002; Baton and Ranford-Cartwright, 2004; Vlachou et al., 2004). In parallel, midgut and haemolymph-derived factors kill nearly 80% of the invading ookinete population (Blandin et al., 2004). These observations are reflected in the transcriptional changes observed in these two epithelia during parasite infection. While up to 7% of the

mosquito midgut transcriptome was altered (Abraham et al., 2004; Vlachou et al., 2005; Xu et al., 2005; Dong et al., 2006), less than 1% of salivary gland transcripts changed (Rosinski-Chupin et al., 2007). Among these were 37 immune-related genes, indicating that salivary glands mount an acute immune response against invading sporozoites. Interestingly, four genes showed similar regulation in both epithelia: a fatty acid synthase was downregulated by parasite invasion in both tissues, while a GTP-binding nuclear protein, a lysosomal thioreductase precursor and SRPN6, (which belongs to a mosquito-specific expansion group (Michel et al., 2005) that is ortholgous to *Drosophila Spn28D*, (Scherfer et al., 2008)) were up-regulated in both tissues. *SRPN6* knockdown in mosquito midguts and salivary glands leads to an increased number of parasites in the respective tissue (Abraham et al., 2005; Pinto et al., 2008), indicative of a common epithelial immune response to malaria parasite invasion.

Additionally, transcriptomes of naïve salivary glands of blood-feeding arthropods encode for anti-microbial peptides, lysozyme and pathogen pattern recognition polypeptides (Arca et al., 2005; Calvo et al., 2010). Their potential effect on pathogens and parasites within the salivary gland has not been characterized.

7. Future perspectives

Our understanding of pathogen and parasite interactions with salivary glands of their arthropod vectors remains incomplete. Over the last 10 years substantial progress has been made in the identification of the molecular interactions between *Plasmodium* spp. sporozoites and *Anopheles* spp. salivary glands. Two receptor-ligand interactions have been characterized and several other sporozoite surface molecules are now known to be required for invasion. Additionally, an epithelial immune response common in midguts and salivary glands against invading ookinetes and sporozoites, respectively, has been identified. However, even in this arguably best understood parasite-vector system, several basic questions are unanswered. Firstly, no detailed morphological description of the sporozoite invasion process of mosquito salivary glands exists for any of the human malaria parasite species. Second, proteomic studies of sporozoite surface or micronemes have not been performed and the molecular make-up of the salivary gland basal lamina or the surface of the basolateral epithelial cell membrane is largely uncharacterized. A recent description of the micronemal proteome of ookinetes (Lal et al., 2009) identified more than 50 putatively secreted proteins of unknown function, of which at least some are most likely important for midgut epithelial cell invasion. A similar description of the micronemal proteome of midgut sporozoites would be highly desirable. However, preparation of enough material for these studies is severely hampered by the lack of culturing methods to produce sporozoites in vitro. Third, the mechanism of the SRPN6-dependent common epithelial immune response against ookinete and sporozoite invasion of mosquito epithelia is unknown. Its manipulation could simultaneously affect the two bottlenecks of parasite development in the mosquito, which makes it an ideal tool for creating refractory mosquitoes.

Overall, the study of *Plasmodium* parasite-mosquito salivary gland interactions is severely limited by the lack of experimental ex vivo or in vitro systems. The development of salivary gland epithelial cell lines or robust organ culturing systems as well as culturing techniques for the mosquito stages of malaria parasites should be research priorities and would greatly facilitate the description of the molecular parasite-vector interface.

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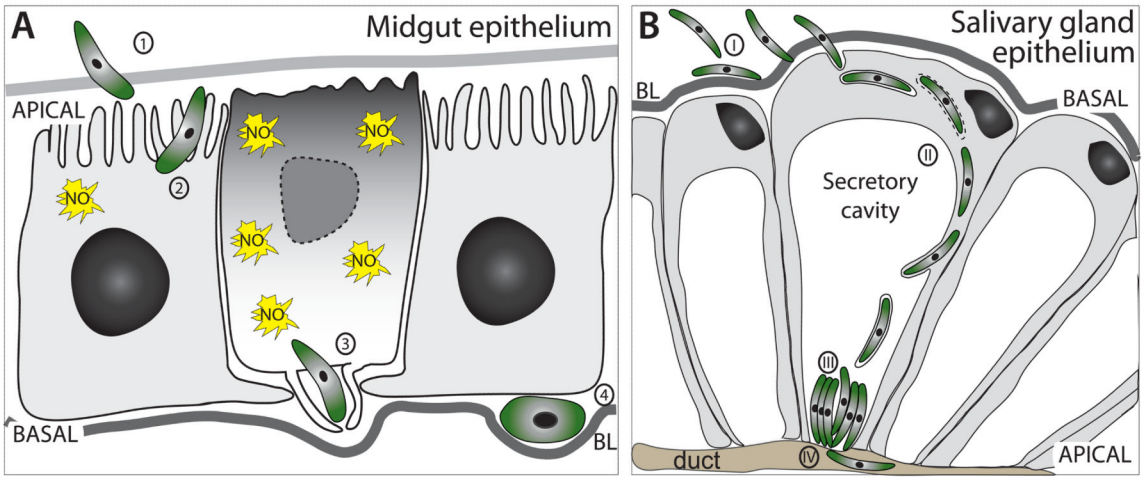

Fig. 1.

Comparison of malaria parasite entry into midgut and salivary gland epithelia. A) Ookinete invasion of midgut epithelia. The ookinete crosses the peritrophic matrix (1) and actively enters the epithelial cell 24–28 h after a bloodmeal, often sequentially invading several midgut cells (2). The ookinete egresses the epithelial cell at the basal site, which is accompanied by lamellipodia formation by the invaded cell as well as its neighbors (3). In the extracellular space of the basal labyrinth the ookinete starts to round up beneath the basal lamina (BL) and transforms into the oocyst (4). The invaded midgut cells undergo many severe physiological changes, among those nitric oxide (NO) production. Ultimately, the cells undergo apoptosis and are expelled from the epithelium. B) Salivary gland infection by sporozoites can be divided into several stages: (I) Sporozoite attachment, likely due to receptor-ligand interactions; (II) sporozoite invasion of epithelial cells with the formation of a transient vacuole; and (III) maturation phase, where sporozoites rest in the extracellular space of the secretory cavity, while (IV) few enter the salivary duct.

Table 1

Vector-borne pathogens/parasites and their mechanisms of transmission.

Infectious agent Parasites	Class	Vector	Transmission	Disease
Parasites				
Babesia microti	Apicomplexa	Tick	salivation	Babesiosis
Brugia malayi	Nematoda	Mosquito	active escape	Filariasis
Drancunculus medinensis	Nematoda	Copepod	ingestion	Dracunculiasis
Leishmania spp.	Euglenozoa	Sandfly	regurgitation	Leishmaniasis
Loa loa	Nematoda	Deer fly	active escape	Filariasis
Mansonella ozzardi	Nematoda	Blackfly	active escape	Mansonellosis
Mansonella perstans	Nematoda	Biting midge	active escape	Mansonellosis
Onchocerca volvulus	Nematoda	Blackfly	active escape	River blindness
Plasmodium spp.	Apicomplexa	Mosquito	salivation	Malaria
Trypanosoma brucei	Euglenozoa	Tsetse fly	salivation	Sleeping sickness
Trypanosoma cruzi	Euglenozoa	Triatome bug	stercorarian	Chagas disease
Wuchereria bancrofti	Nematoda	Mosquito	active escape	Filariasis
Viruses				
CCHF virus[c]	Bunyaviridae	Ixodid tick	salivation	Crimean Congo hemorrhagic fever
Chikungunya virus[c]	Togaviridae	Mosquito	salivation	Chikungunya fever
DEN virus	Flavirviridae	Mosquito	salivation	Dengue
JE virus[b]	Flavirviridae	Mosquito	salivation	Japanese Encephalitis
LCE virus[b]	Bunyaviridae	Mosquito	salivation	LaCrosse Encephalitis
Ross River virus	Togaviridae	Mosquito	salivation	Ross River fever
RSSE virus[c], CEE virus[c]	Flavirviridae	Ixodid tick	salivation	Tick-Borne Encephalitis
RVF virus[a]	Bunyaviridae	Mosquito	salivation	Rift Valley Fever
SLE virus	Flavirviridae	Mosquito	salivation	Saint Louis Encephalitis
VEE virus[b]	Togaviridae	Mosquito	salivation	Venezuelan Equine Encephalomyelitis
WNE virus[b]	Flavirviridae	Mosquito	salivation	West Nile Encephalitis
Yellow fever virus[c]	Flavirviridae	Mosquito	salivation	Yellow Fever
Bacteria				
Anaplasma phagocytophilum	Alphaproteobacteria	Hard Tick	salivation	Human Granulocytic Anaplasmosis
Bartonella bacilliformis	Alphaproteobacteria	Sand fly	salivation	Carrion's disease
Bartonella quintana	Alphaproteobacteria	Louse	stercorarian	Trench fever
Borrelia burgdorferi	Spirochaeta	Tick	salivation	Lyme Disease
Borrelia hermsii	Spirochaeta	Soft Tick	salivation	Tick-borne relapsing fever
Borrelia recurrentis	Spirochaeta	Louse	passive escape	Louse-borne relapsing fever
Ehrlichia chaffeensis	Alphaproteobacteria	Hard Tick	salivation	Human Monocytic Ehrlichiosis
Francisella tularensis[a]	Gammaproteobacteria	Hard Tick, Deer fly	salivation	Tularemia
Orientia tsutsugamushi	Alphaproteobacteria	Mite	salivation	Scrub typhus
Rickettsia prowazekii[b]	Alphaproteobacteria	Louse	stercorarian	Epidemic typhus
Rickettsia rickettsii[c]	Alphaproteobacteria	Hard Tick	salivation	Rocky Mountain spotted fever

Infectious agent Parasites	Class	Vector	Transmission	Disease
Rickettsia typhi[c]	Alphaproteobacteria	Flea	salivation	Murine typhus
Yersinia pestis[a]	Gammaproteobacteria	Flea	regurgitation	Plague

National Institute of Allergy and Infectious Diseases Biodefense Category A[a], B[b], C[c] priority pathogens (http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/research/pages/cata.aspx)