Virology 464-465 (2014) 26–32

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Role of skin immune cells on the host susceptibility to mosquito-borne viruses

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ARTICLE INFO

Article history:
Received 9 April 2014
Returned to author for revisions
15 May 2014
Accepted 17 June 2014
Available online 18 July 2014

Keywords:
Arboviruses
Mosquito saliva
Skin
Innate immunity
Dengue
Chikungunya
West Nile virus
Rift Valley Fever

ABSTRACT

Due to climate change and the propagation of competent arthropods worldwide, arboviruses have become pathogens of major medical importance. Early transmission to vertebrates is initiated by skin puncture and deposition of virus together with arthropod saliva in the epidermis and dermis. Saliva components have the capacity to modulate skin cell responses by enhancing and/or counteracting initial replication and establishment of systemic viral infection. Here, we review the nature of the cells targeted by arboviruses at the skin level and discuss the type of cellular responses elicited by these pathogens in light of the immunomodulatory properties of arthropod vector-derived salivary factors injected at the inoculation site. Understanding cutaneous arbovirus–host interactions may provide new clues for the design of future therapeutics.

Contents

Introduction

Arthropod-borne viruses, known as arboviruses, share the common property to be transmitted among vertebrate hosts by blood-feeding mosquitoes or ticks. Among them, mosquito-borne dengue (DENV), West Nile (WNV), Chikungunya (CHIKV) and Rift

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http://dx.doi.org/10.1016/j.virol.2014.06.023
0042-6822/

Valley Fever (RVFV) viruses represent major public health problems in regions with high invertebrate vector densities and over the last decades have become a global menace, not only in the tropics, but also threatening temperate areas colonized by the appropriate strains of competent mosquitoes.

The transmission cycle of mosquito-borne viruses is initiated when pathogen-containing fluids are ingested by the vector from an infected vertebrate during a blood meal. Once the virus crosses the midgut barrier and has replicated in the mosquito body, it reaches the salivary glands, leading to the presence of high infectious titers in the saliva of infected arthropods (Luplertlop et al., 2011; Salazar et al., 2007; Vazeille et al., 2010; Ziegler et al., 2011). During a subsequent blood meal, the proboscis of the infected mosquito probes the vertebrate host's skin, resulting in the extravascular delivery of most of salivary glands content in both the epidermis and dermis where resident and migratory cells will encounter the pathogen Heath and Carbone, 2013. During transmission, arboviruses contained in salivary glands are intimately associated with mosquito saliva. The simultaneous delivery of mosquito cofactors clearly potentiates the capacity of arboviruses to replicate at the anatomical site of the mosquito bite (Le Coupanec et al., 2013; Schneider et al., 2010; Styer et al., 2011; Surasombatpattana et al., 2014, 2012; Thangamani et al., 2010), leading to an enhanced viremia in the vertebrate host (McCracken et al., 2014) and to an acute viral pathogenicity (Le Coupanec et al., 2013; Schneider et al., 2010).

Interaction of arboviruses with mammalian skin: convict the guilty cell

The nature of skin cells first encountered during virus transmission is likely to have a significant impact on the establishment of a systemic infection and continuation of the transmission cycle between the vertebrate host and the arthropod vector. Depending on its capacity to replicate in resident or instead in migratory cells in this organ will have a real impact both on the propagation in the new host and on the pathogenesis of viral infection. This is especially of importance for understanding the occurrence of skin alterations detected in most arboviruses-induced symptoms. Questioning the tropism of these pathogens at the skin level and elucidating the nature of skin cells that first encounter viral pathogens following inoculation therefore remain key issues.

The human skin: a physical and immunological barrier

The skin is a complex organ that exerts multiple vital protective functions against environmental aggressions. This crucial role is rendered possible thanks to an elaborate structure, associating multiple cell types organized in three layers: the outermost epidermis, the dermis and the deepest hypodermis (Fig. 1). Keratinocytes contribute to the integrity and the infrastructure of the outer layer in the skin and represent the major cell population in the epidermis. While the outermost cornified skin layer results from differentiation of keratinocytes into corneocytes, the deeper epidermis is a living cell layer of cells generated by tight junctions between adjacent keratinocytes. This population has a key innate role in the detection or pathogens and defense facilitated by the expression of many pattern recognition receptors, including Toll-like receptors (TLR) (TLR-1, TLR-2, TLR-3, TLR-4, TLR-5, TLR-6 and TLR-9) and Nod-like receptors (NLR), and by the capacity of keratinocytes to produce antimicrobial peptides (LL-37, β-defensins, RNase 7 and S100 family members), chemokine and cytokines (CXCL9, CXCL10, CXCL11, CXCL20, TNF-α, IL-1α and β, IL-6, IL-10, IL-18 and IL-33) critical for local recruitment of immunocompetent cells. Besides keratinocytes low proportions of Merkel cells and melanocytes also form part of the resident cells in the epidermis. This skin layer also hosts Langerhans cells, a resident dendritic cells population situated above the basal layer of proliferating keratinocytes that can sample and capture antigens within the cornified epidermis (Kubo et al., 2009). These cells subsequently undergo maturation while they migrate to local draining lymph nodes, where their antigen-presenting properties allow activation of effector T cells and initiation of an immune response (Macatonia et al., 1987; Silberberg-Sinakin et al., 1976). Among other immune cells, dendritic epidermal T-cells, a subset of T cell receptor (TCR) γδ-expressing cells with migratory properties, are detected in mice epidermis whereas they represent a minor subset in the human skin (MacLeod et al., 2013). In opposition to the epidermis, the dermis, the deepest skin layer, is enriched in elastin and collagen fibers and furthermore consists of an extracellular matrix produced by fibroblasts. It is highly vascularized and interspersed with draining lymphatics traversing the deeper layers to access the lymph nodes. The dermis contains immunologically relevant cells, including mast cells, macrophages, neutrophils, innate lymphoid cells and both TCRαβ and TCR γδ T cells (for review see Heath and Carbone (2013). In addition, $CD11b^+$ DCs and $CD103^+$ DCs represent the two subsets identified in mice that correspond to $CD1c^+$ $CD14^+$ DCs and $CD141^+$ DCs, respectively, in humans. Below the dermis, the subcutis layer consists of adipocytes surrounded by fibroblasts, nerves and blood vessels. Accordingly the skin barrier is equipped with a vast range of resident and migratory immunocompetent cells capable to direct and drive an efficient immune response aimed to control early replication of invading pathogens.

Facts and queries on mosquito-borne viruses in the skin

Mosquito-borne viruses have evolved to bypass the physical skin barrier by hitch-hiking on blood-sucking arthropod vectors. As keratinocytes are the most abundant cell population in the epidermis, acquiring the capacity to replicate in these resident cells represents an attractive strategy for host colonization. In recent years, their capacity to support replication of a variety of mosquito-borne viruses was questioned. First, epidermal keratinocytes were identified as the initial target for WNV infection both *in vivo* and *in vitro* (Lim et al., 2011). More recently, we have reported that *ex vivo* cultured primary human epidermal keratinocytes can also support DENV replication (Surasombatpattana et al., 2011). Consistent with this observation, basal keratinocytes were reported positive for DENV antigens in the epidermis of experimentally inoculated skin explants (Limon-Flores et al., 2005). In these cells, infection significantly enhances expression of TLR3, RIG-I, MDA5 and PKR, resulting in IFN-β, IFN-γ, β-defensin and RNase 7 release most likely accounting for the initiation of an antiviral innate immune response (Surasombatpattana et al., 2011). Based on these observations we explored the contribution of keratinocytes in early CHIKV infection. When studied in *ex vivo* infection models, we observed that primary human keratinocytes, despite supporting fusion with viral envelope glycoproteins, are non-permissive for viral replication, regardless of their differentiation stage. These cells display no evidence of cytopathogenicity, the hallmark of CHIKV replication (LB, personal communication). This intriguing result contrasts with the presence of high copy numbers of CHIKV genomes in vesiculobullous skin lesions of infected patients attesting for CHIKV replication in another cutaneous cell type and with observation of necrotic keratinocytes that may result from an indirect effect of CHIKV infection (Pakran et al., 2011). Nevertheless, results from animal models of CHIKV infection revealed the transient presence of viral antigens in the skin of experimentally infected macaques (Labadie et al., 2010) or adult mice within one week of infection (Couderc et al., 2008). However, instead of keratinocytes, histological detection of CHIKV antigens revealed the presence of virally-infected cells at the level of the deep dermis, and at lesser extent in basal layers, suggesting a role

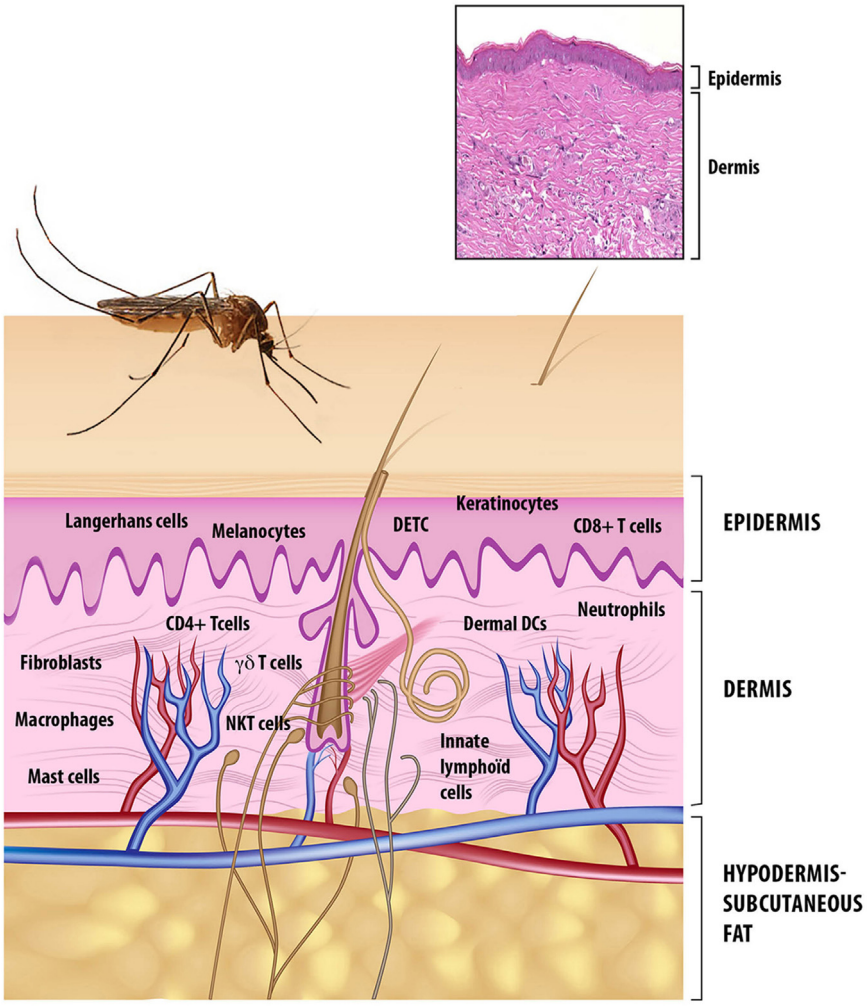

Fig. 1. Skin immune sentinels. Human infections with arboviruses occur during blood feeding by infected mosquitoes. During blood meal, mosquito's mouthpieces are introduced into the skin and released viral particles with saliva which are in contact with many cells types. The epidermis is composed of the outermost layers of cells in the skin. Specialized cells in the epidermis include keratinocytes, melanocytes, and Langerhans cells. In addition, rare T cells, mainly CD8+ cytotoxic T cells and dendritic epidermal T-cells can be found in the epidermis. The dermis is anatomically composed of many immune cells, including dermal dendritic cells (DCs), and T cell subsets, including CD4+ T cells, γδ T cells and natural killer T (NKT) cells. Moreover, fibroblasts, macrophages, and mast cells are also present. This layer of the skin is richly supplied with blood vessels and collagen fibers. The dermis also contains sensory nerve endings sweat glands, oil glands, and hair follicles. Below the dermis is the subcutaneous layer, a layer of tissue composed of adipose tissue.

of fibroblasts located in the basal skin layer (Couderc et al., 2008) as well as endothelial cells in capillaries that are described as permissive *in vitro* in the efficiency of CHIKV infection in host vertebrate (Sourisseau et al., 2007). Nevertheless, as observed for DENV, CHIKV challenge induces a strong innate immune response in keratinocytes (DM, unpublished data). In this respect, it was recently revealed that the simultaneous knock-down of IRF3 and IRF7 genes leads to accumulation of CHIKV antigens in keratinocytes of experimentally infected mice, favoring the development of focal skin necrosis with ballooning degeneration, pale cytoplasm and karyorrhetic nuclei five days after infection while absent in wild-type animals (Rudd et al., 2012). However, the capacity of immune signaling elicited in human keratinocytes to control CHIKV replication and its associated cell death has not been clearly demonstrated as yet. A large variety of mosquito-borne viruses including WNV, VEEV and DENV viruses actively replicate in migratory LCs (Byrne et al., 2001; Gardner et al., 2008; Welte et al., 2009; Wu et al., 2000) suggesting that this capacity may represent an attractive strategy for propagation in vertebrates. Regarding DENV, this property was more specifically assigned to precursor DC-SIGN+ CD14+ interstitial cells that reside beneath the epidermis of skin and mucosal tissue which were proposed as preferential targets (Kwan et al., 2005). Interestingly, intradermal inoculation of WNV results in migration of infected LCs from the initial inoculation site to draining lymph nodes (Byrne et al., 2001). Moreover, during migration, LCs are maturated into activated lymphoid dendritic cell with antigen presenting capacities, expressing major histocompatibility class I and II antigens, CD54 and CD80 (Johnston et al., 1996). Similarly, LCs, dermal/interstitial DCs, and monocytes-derived DCs were proposed as initial replicating cells in DENV-inoculated cadaveric skins (Marovich et al., 2001; Wu et al., 2000) as well as in skin biopsies from volunteers inoculated with live-attenuated dengue vaccines

Interaction of mosquitoes with mammalian skin: mosquito saliva is the ideal accomplice for corrupting cell responses

Mosquito's saliva and Blood meal

Arboviruses are transmitted to the host or the vector during a blood meal taken by an adult female mosquito to provide the necessary resources for egg development. During blood meal, mosquito's mouthpieces are introduced into the skin. The process

of blood-feeding can be divided into two steps. The first is the probing phase, during which the arthropod seeks a blood vessel. It is during this period that saliva is released below the skin, to counteract physiological responses to the arthropod, such as hemostasis and inflammation. Once a blood vessel has been found, the engorgement step begins and continues until complete repletion of the arthropod is achieved (Video S1).

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.virol.2014.06.023.

The mosquito vector secretes anti-hemostatic, angiogenic, anti-inflammatory and vasodilatory molecules within its saliva to maintain blood flow during feeding (Patramool et al., 2012; Ribeiro and Francischetti, 2003; Schneider and Higgs, 2008). Recent proteomics studies have clearly shown that these activities can be ascribed to the presence of a vast variety of molecules, as evidenced for *Aedes* and *Anopheles* saliva (Choumet et al., 2007; Fontaine et al., 2011; Patramool et al., 2011; Sor-Suwan et al., 2013; Wasinpiyamongkol et al., 2010). However, despite the recent advance in our knowledge of these molecules and our understanding of their role in blood feeding, more than half of them remain without clearly defined functions (Schneider and Higgs, 2008). The vasodilatory sialokinin (Champagne and Ribeiro, 1994) and D7 proteins (Calvo et al., 2007) present in *Aedes aegypti* saliva are among the best characterized salivary proteins. *A. aegypti* also secretes an apyrase that inhibits ADP-dependent platelet aggregation and inhibits or scavenges platelet-aggregating factors (Champagne and Ribeiro, 1994). This platelet activation paves the way to secondary hemostasis by exposing the surface of activated platelets to coagulation proteins. A 48-kDa factor Xa inhibitor belonging to the serpin family of serine protease inhibitors in the saliva of *A. aegypti* has been found in the *Aedes* saliva (Stark and James, 1998). Finally, the adenosine deaminase enzymes detected in *A. aegypti* which appears to suppress pain perception may help blood feeding by degrading adenosine (Ribeiro et al., 2001).

Mosquito's saliva and the immune system

The capacity of mosquito saliva to generate an immune response has recently been an area of active research (Table 1). According to these studies *Anopheles stephensi* and *Anopheles gambiae* saliva display an intense chemotactic activity based on vascular permeabilization and activation of dermal mast cells degranulation (Choumet et al., 2012; Demeure et al., 2005). They were shown to result in the recruitment of DCs to the feeding site and of neutrophils to the draining lymph node (Demeure et al., 2005; Owhashi et al., 2001). Following their recruitment, these cells play important roles in the early signaling that activates and orchestrates the immune response. The saliva-induced release of MIP-2 may also contribute to these processes (Depinay et al., 2006). In addition to histamine-releasing factors like TCTP found in *A. gambiae* saliva (Choumet et al., 2007; Rosinski-Chupin et al., 2007), some components of *Aedes, Culex* and *Anopheles* saliva are allergenic (Arca et al., 2007; Peng et al., 2007). Salivary compounds are also susceptible to deregulate immune functions at least locally. *A. aegypti* saliva drastically reduces proliferation of murine T and B lymphocytes (Bizzarro et al., 2013; Wanasen et al., 2004; Wasserman et al., 2004) and reduces TNF-α or IL-2 and IFN-γ secretion, respectively, in mast cells or splenocytes (Bissonnette et al., 1993), a result reproduced in murine spleen cells (Cross et al., 1994). A long lasting suppression of IFN-γ production, together with increased levels of IL-4 and IL-10, was also reported in murine splenocytes upon exposure to *A. aegypti* or *Culex pipiens* (Zeidner et al., 1999). This general dysregulation of immune functions characterizes a polarization from the Th1 type cytokine

Table 1
Effect of mosquito salivary gland extracts or mosquito feeding on cells of the immune system.

	Mast cells	Polynuclear cells	Dendritic cells	Splenocytes	Lymphocytes
Anopheles gambiae	**Degranulation** (Choumet et al., 2012; Demeure et al., 2005)	**Attraction** to the bite site (Choumet et al., 2012; Demeure et al., 2005) **Eosinophil** chemotactic factor (chitinase) (Owhashi et al., 2001)	**Recruitment** of DCs to the feeding site (Demeure et al., 2005; Owhashi et al., 2001)		
Aedes aegypti	**Inhibition** of TNFα release (Bissonnette et al., 1993) **Inhibition** of mast cell degranulation (Ribeiro et al., 2001)	**Recruitment** of eosinophils, neutrophils at the bite site (Karppinen et al., 1996)		**Suppression** of IL2 and INFγ production (Bissonnette et al., 1993; Zeidner et al., 1999) **Increased** levels of IL-4 and IL-10 Zeidner et al., 1999)	**Reduction** of T cell recruitment at the bite site (Schneider et al., 2010) **Induction** of apoptosis of CD4+ and CD8+ T cells, and B cells (Bizzarro et al., 2013) **Reduced proliferation** of murine T lymphocytes (Wasserman et al., 2004) **Differentiation** of Th2 effector CD4′ T cells (Boppana et al., 2009) **Secretion** of TH2-cytokine IL4 by CD4 T cells (Boppana et al., 2009) **Shift** of a Th1 to Th2 type response (Cross et al., 1994; Limesand et al., 2000; Schneider et al., 2004; Thangamani et al., 2010; Wanasen et al., 2004; Zeidner et al., 1999)
Culex pipiens	**Inhibition** of mast cell degranulation (Ribeiro et al., 2001)			**Suppression of** INF γ production (Zeidner et al., 1999) **Increased** levels of IL-4 and IL-10 (Zeidner et al., 1999)	
Armigeres subalbatus					**Induction** of apoptosis Fas ligand **Suppression** of proinflammatory cytokines without changing IL-10 levels (Liu et al., 2012)

production profile that promotes a pro-inflammatory response, capable to kill intracellular parasite, towards a Th2 type response profile that has a counteractive effect on the production of IFN-γ. Such effects have been clearly related to the concentration of saliva proteins delivered to the vertebrate, as lower concentrations of salivary gland extracts inhibited Th1 cytokine production and T cell proliferation, while higher concentrations suppressed the secretion of Th1, Th2, as well as pro-inflammatory, cytokines and decreased T cell viability (Schneider and Higgs, 2008). Accordingly, an immunosuppressed environment is created at the immediate feeding site, whereas decreasing saliva concentrations at more distal regions rather cause a dysregulation of the immune response. The shift of a Th1 to Th2 type lymphocyte response was confirmed by several studies and may persist in mice *in vivo* at seven days post *A. aegypti* feeding (Cross et al., 1994; Limesand et al., 2000; Schneider et al., 2004; Wanasen et al., 2004; Zeidner et al., 1999). Similar effects were reproduced when the mice were inoculated with *A. aegypti*-derived vasodilator sialokinin (Zeidner et al., 1999). Recently, SAAG-4 has been reported to be an important *A. aegypti* salivary protein that can program Th2 effector $CD4^+$ T cell differentiation in mice (Boppana et al., 2009). Based on these observations from experimental models, as well as on the capacity of mosquito saliva to create a cytokine-mediated polarization of the host immune response, the cellular and molecular biology of arbovirus infections should be considered in light of co-modulatory properties of mosquito saliva at concentrations that mimic the physiological reality.

Modulation of virus behavior by salivary components: committing the crime

There is mounting evidence that mosquito's saliva may be a critical factor in vector-borne disease transmission, either increasing the infectiousness of the pathogen it carries or/and attenuating the host immune response. The discovery of the immuno-modulatory properties of invertebrate saliva has prompted several research groups to study the involvement of salivary proteins from diverse vectors in the transmission and the establishment of the corresponding pathogens in their hosts (for review see Fontaine et al. (2011)).

Mosquito saliva components also have been proven to represent highly biologically active molecules susceptible to modulate early viral replication in addition of assisting mosquito blood feeding. Significantly higher WNV titers were observed in the serum of chickens infected by *C. pipiens* mosquito feeding, as compared to needle-inoculated animals (Styer et al., 2006). The recent observation of five- to ten-fold higher viremia and tissue titers in mice infected by WNV *via* the bite of a single infected *Culex tarsalis* mosquito correlates with faster neuro-invasion than observed in mice inoculated with WNV by needle (Styer et al., 2011). This aggravated disease course can be explained at the cellular level by the presence of saliva that is associated with enhanced early viral replication, especially in the skin and draining lymph nodes (Schneider et al., 2010). The results of the latter study corroborates the capacity of saliva to increase IL-10 production, to dysregulate antiviral signaling by antigen presenting cells and to elevate influx of WNV-susceptible cell types to the inoculation site probably, providing further insight into the role of mosquito cofactors in the acute pathogenesis of the infection (Schneider et al., 2010). Similar experiments in CHIKV-infected mice revealed that cutaneous immune responses elicited by bites from infected mosquitoes also significantly differ from those induced by needle inoculation (Thangamani et al., 2010). Indeed, needle transmission polarized host cutaneous cytokine response to a T_H1 profile with an upregulation of IFN-γ and IL-2 while CHIKV-infected mosquitoes generated a drop in IL-4 production, concomitant with decreased Th1 cytokine release and TLR3 expression. Similarly, co-inoculation of Sindbis virus (SINV) and *A. aegypti* SGE, resulted in higher IL-4 and IL-10 expression levels, as compared to those in mice injected with SINV alone (Schneider et al., 2004). DENV replication and the associated pathogenesis are strongly affected by factors contained in salivary glands of mosquito vectors as well. Indeed, levels of TNF-α, IL-4 and IL-10 are enhanced by *A. albopictus* saliva upon DENV inoculation in humanized mice (Cox et al., 2012). Mice skin probing prior to dermal DENV2 inoculation was found to be associated with a significant reduction in TLR7, RelA, IFN-γ and IP10 mRNA levels within 3 h of injection (McCracken et al., 2014). This modulation may reduce the recognition of viral material and therefore is likely to generate a more permissive environment for the establishment of infection with a possible repercussion on the pathogenesis of DENV infection. An increased reactivity against salivary components, including apyrase, was observed among the individuals displaying the more severe forms of dengue disease (Machain-Williams et al., 2012). The identification of salivary components accounting for such modulatory function deserves continued attention, as it could not only help to better understand the origin of severity, but also serve as targets for the control of DENV replication in mammalian hosts. In such an attempt, a functional proteomic analysis conducted by some of us demonstrated that a 34-kDa protein in salivary glands of infected *A. aegypti* mosquitoes enhances DENV replication in human keratinocytes by suppressing innate immune responses in the earliest stages of infection (Surasombatpattana et al., 2011). In the context of DENV infection, this molecule was found to decrease IRF3 and IRF7 mRNA expression, resulting in a reduced expression of IFN type 1 and IFN-γ transcripts, as well as mRNA encoding antimicrobial peptides, such as LL-37, RNAse 7 and S100A7 (Surasombatpattana et al., 2011). The 34 kDa protein is specific for the *Aedes* spp. and has been shown to be immunogenic in DENV-infected patients (Wasinpiyamongkol et al., 2010). Besides modulation of host immune responses, salivary components may also facilitate DENV propagation in other ways as reported very recently (Conway et al., 2014). The direct proteolysis of dermal extracellular matrix proteins by serine protease activity contained in *A. aegypti* saliva may increase DENV particles attachment to heparin sulfate proteoglycans and enhance interactions between virions and permissive cells, including LCs and macrophages. Such studies provide an urgent lead to characterize such immunomodulant saliva factors and advocate their characterization which will open a new insight into the design of performing strategies against arboviral infections.

Considering an integrated model of virus saliva co-inoculation in future preventive and therapeutic strategies: the perfect picture

Clearly, mosquito saliva does not only impact human susceptibility to arboviruses, but also affect viral pathogenesis. By modulating the local immune responses and attracting a variety of susceptible cells at the inoculation site, mosquito saliva probably favors early infection and determines future host invasion. An intriguing aspect of arthropod–saliva interaction lies in the response of human skin to arbovirus infection. A complex interplay between skin injury, mosquito saliva and viral pathogens takes place in this tissue during early transmission. The resulting cytokine and chemokine secretion, antimicrobial molecule release, attraction of immunocompetent and/or susceptible cells for viral infection, as well as fluid extravasation, create a favorable environment for the establishment of viral infection. Accordingly, this step represents a key process both in the setup of the adaptive immune response and in host invasion. The finely tuned balance

created by the interplay among the virus, the host and salivary compounds will determine the outcome of infection and associated pathogenesis. According to this information and in light of recent comparisons performed using animal models of infection, it has become obvious that the impact of mosquito cofactors absolutely needs to be taken into account to provide the perfect picture of arboviruses skin infection. This aim will be achieved using appropriate integrated host-virus-mosquito model systems. However, one should be careful in transferring insight from the mouse model systems into translational research studies focusing on human pathology. Arbovirus infection in mammals is frequently associated with a variety of cutaneous symptoms some times of severe amplitude (maculopapular exanthema, roseola-like and morbilliform eruptions, vesiculobullous lesions, purpuric macules, *etc.*) (Bandyopadhyay and Ghosh, 2010; Del Giudice et al., 2005; Pakran et al., 2011; Riyaz et al., 2010). From the histological point of view, perivascular lymphocytic infiltrates are observed in biopsy lesions (Inamadar et al., 2008). The link between skin manifestations and the capacity of arboviruses to replicate in the skin remains to be elucidated and the contribution of mosquito saliva in these clinical manifestations is still uninvestigated. New efforts aiming at identifying viral tropism in the skin and early target cells infected in this organ, viral receptors and characterizing the molecules in saliva accounting for immune modulation and facilitation of viral replication may uncover the means to elaborate new currative or preventive therapeutics against arbovirus infection in humans.

Ethics statement

All studies on animals followed the guidelines on the ethical use of animals from the European Communities Council Directive of November 24, 1986 (86/609/EEC). All animal experiments were approved and conducted in accordance with the Institut Pasteur Biosafety Committee. Animals were housed in the Institut Pasteur animal facilities accredited by the French Ministry of Agriculture to perform experiments on live mice, in appliance of the French and European regulations on care and protection of the Laboratory Animals (accreditation number B 75 15-01 and B 75 15-07). The study protocols were approved by the Comité d'Ethique pour l'Expérimentation Animale of Pasteur Institute (CETEA) under the reference 0762.01.

Funding

This work was supported by grants from the French Research Agency “Agence National de la Recherche” (ANR-12-BSV3-0004-01).

Acknowledgments

The authors warmly thank Hans Yssel for critical discussion affiliation and Frederic Thomas for constant support as well as their colleagues for contributing to experimental work cited in this review.

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