Early Dengue Virus Infection in Human Skin: A Cycle of Inflammation and Infectivity

Matthew O. Ivory[1,2], James C. Birchall[2], Vincent Piguet[1]*

1) Department of Dermatology and Academic Wound Healing, Institute of Infection and Immunity, Cardiff University and University Hospital of Wales, Heath Park, Cardiff, CF14 4XN, United Kingdom
2) School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3NB, United Kingdom

* To whom correspondence should be addressed: Prof. Vincent Piguet, Department of Dermatology and Academic Wound Healing, Institute of Infection and Immunity, Cardiff University and University Hospital of Wales, Heath Park, Cardiff, CF14 4XN, United Kingdom; Tel: +44 (0) 29 20 744721; Fax: +44 (0) 29 20 744312; E-mail: piguetv@cardiff.ac.uk

Abstract (60 Words)

Early events of dengue virus (DENV) infection remain poorly understood. In this issue, Schaeffer and colleagues utilise *ex vivo* human skin cells to investigate viral infection. They show that skin-resident immune cells are infected by DENV and their infectability is increased in the inflammatory conditions - particularly where IL-4 is released - that accompany mosquito bites that transmit the virus.

Body Text (1187 words)

Dengue virus (DENV) is a single-strand positive RNA virus and is the most common arthropod-transmitted virus (arbovirus), being transmitted to humans exclusively via *Aedes* mosquitos. Viral infection presents most commonly with fever, muscle/joint pains and headache that are often accompanied by a characteristic rash. More serious infection can progress to haemorrhagic dengue fever or dengue shock syndrome which can lead to death via haemorrhage and hypovolemic shock respectively. Neither vaccines nor specific antiviral treatments are available and the WHO estimates that almost half of the world's population are currently at risk. The potential severity of symptoms, significant at-risk population and lack of effective treatments have led to dengue disease being named as one of Bill and Melinda Gates Foundation's neglected tropical diseases requiring increased research to improve global health. In the search for new treatment/prevention strategies, improved understanding of the viral pathogenesis is essential. The study by Schaeffer *et al.* (2014) in this issue utilises an *ex vivo* human skin cell model to further the understanding of DENV infection.

As DENV is transmitted via mosquito bites, skin is the primary site of infection. Human skin contains a variety of immunological cells including Langerhans cells (LCs) in the epidermis and subsets of dermal dendritic cells (DCs) along with macrophages and T-cells in the dermis. These cells will be amongst the first to encounter the virus following transmission. These heterogeneous cell populations present a challenge to studying immune responses to the virus in skin. Both LCs and DCs have previously been shown to be infected by DENV (Cerny *et al*., 2014). In their study, Schaeffer *et al*. (2014) investigated the interactions between DENV and dermal DCs and macrophages in normal conditions and in a model of inflammatory conditions. Dermal DCs, unlike LCs, are a heterogeneous population. Three subsets are commonly referred to in the literature - $CD14^+$, $CD1c^+$ and $CD141^+$ DCs (Chu *et al*., 2012 and Haniffa *et al*., 2012). Each subset possesses individual properties in terms of viral infectability, antigen processing and immune response stimulation. Schaeffer *et al*. (2014) focus on $CD14^+$ and $CD1c^+$ DCs alongside dermal macrophages to elucidate the infectability of immune cells following viral entry to the skin.

C-type lectins such as Langerin (CD207), DC-SIGN (CD209) and mannose receptor (MR/CD206) enable immunological cells to uptake antigen from the extracellular environment (Engering *et al*., 2002 and de Witte *et al.,* 2007). This uptake method is exploited by a number of viruses such as HIV-1 and DENV to gain entry into the cell, which they can subsequently infect (Arrighi *et al*., 2004). The role of DC-SIGN in DENV pathogenesis has been confirmed through the study of patients with certain DC-SIGN polymorphisms possessing a higher risk of developing dengue haemorrhagic fever following viral infection (Sakuntabhai *et al*., 2005).

Given the proposed role of DC-SIGN in DENV infection, Schaeffer et al (2014) initially determined the effect of skin inflammation on the number of $DC\text{-}SIGN^+$ cells, using bullous pemphigoid, hypereosinophilic syndrome, mastocytosis and scabies infection affected skin as

models for mosquito-bitten skin based on shared properties of basophil recruitment and eosinophilia. It was found that all of the inflammatory conditions studied produced increased the numbers of DC-SIGN$^+$ cells in skin, when compared against non-diseased tissue, leading the authors to suggest that the inflammation arising from a mosquito bite might lead to the recruitment of infectable cells to the site.

Schaeffer *et al.* (2014) obtained skin DCs (along with other migratory cells) using a well-established migratory cell protocol (Chu *et al*., 2012). Cells were infected in an unsorted state, then the infection levels determined post-infection via flow cytometry. They found that all of the studied skin DC subsets could be infected by DENV, with infectability being highest in CD14$^+$ dermal DCs and lowest in LCs, with higher DC-SIGN expression in CD14$^+$ cells suggested as a potential reason for this difference. The low infectability of LCs found by Schaeffer *et al.* (2014) contradicts previously reported results that found LCs were the most susceptible skin immune cell to DENV infection (Cerny *et al*., 2014).

Transmission of arboviruses is accompanied by trauma to the local site as well as delivery of insect salivary substances, which can elicit an immune response in isolation. It is speculated that the Th2-type immune response that occurs following insect bite leads to an increase in infection and clinical symptoms from DENV (Cox *et al*., 2012). Schaeffer et al (2014) focussed on interleukin-4 (IL-4), which is released from basophils, mast cells and T-cells during the immune response to foreign antigen, to determine whether inflammation from the mosquito bite promotes viral infection of skin immune cells. Exposing the DCs to IL-4 led to a marked increase in CD14$^+$ DC infection with the virus with little effect on other DCs/LCs, suggesting CD14$^+$ cell infection may become prominent following mosquito bite inflammation. Incubation of dermal DCs with IL-4 also enhanced their T-cell stimulatory ability as evidenced by proliferation of CFSE-labelled naïve T-cells following co-culture. Their presence in the skin and expression of the C-type lectin MR make macrophages a potential DENV-infectable cell population. Dermal macrophage infection was studied following enzymatic digestion of skin and cell sorting. This allowed them to obtain a population of CD14$^+$ macrophages - the authors speculate that CD14$^+$ DCs may have developed a macrophage-like phenotype in culture. It was found that IL-4 with or without GM-CSF also increased DENV infection. Infection led to release of TNF-α, most markedly in macrophages with higher infection levels. This led the authors to speculate that DENV infection of DCs would lead to potent T-cell antiviral responses whereas macrophage infection would most likely lead to the inflammatory symptoms associated with DENV infection.

Schaeffer *et al.* (2014) reason that since CD14$^+$ DCs are most readily infected, especially under inflammatory conditions, and since they tend to produce humoral immune responses, these cells are likely to be responsible for the increased severity of DENV disease on subsequent exposure to a different serotype of the virus. This occurs due to cross-reactive antibodies that can bind to other DENV serotypes without fully neutralising them, instead facilitating their entry into immune cells and leading to more severe infection (Endy *et al*., 2004). This could represent an important target for further study as moderating this response might be able to lower the risk of serious DENV disease developing. The release of TNF-α by macrophages in the dermis, especially when exposed to IL-4, is also likely to be important due to the cytokine's proposed central role in the development of dengue fever. It is also likely that the local inflammation brought about by TNF-α release would lead to the release of IL-4, creating a cycle of increasing infectability of the local immune cells.

Schaeffer *et al*'s (2014) study therefore builds upon previous animal studies that have shown an increase in arbovirus infection due to the immune response to mosquito salivary substances and provides novel data utilising primary human skin cells, which will much more closely resemble the *in*

vivo infection situation. Understanding how DENV can take advantage of inflammatory conditions for its propagation may highlight novel opportunities to block this virus when it tries to invade the host via skin immune cells.

Clinical Implications

- *Aedes* mosquito bites lead to local inflammation, which results in release of IL-4. This cytokine increases the infectability of both dermal dendritic cells and dermal macrophages.
- $CD14^+$ DCs are the most readily infected cells and are likely to be important in driving humoral immune responses against DENV.
- Dermal macrophages release TNF-α upon DENV infection, driving local inflammation and further increasing the infectability of skin immune cells.

Conflict of Interest

The authors state no conflict of interest.

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