How myeloid cells contribute to the pathogenesis of prominent emerging zoonotic diseases

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Abstract

Up to 75% of emerging human diseases are zoonoses, spread from animals to humans. Although bacteria, fungi and parasites can be causative agents, the majority of zoonotic infections are caused by viral pathogens. During the past 20 years many factors have converged to cause a dramatic resurgence or emergence of zoonotic diseases. Some of these factors include demographics, social changes, urban sprawl, changes in agricultural practices and global climate changes. In the period between 2014–2017 zoonotic viruses including ebola virus (EBOV), chikungunya virus (CHIKV), dengue virus (DENV) and zika virus (ZIKV), caused prominent outbreaks resulting in significant public health and economic burdens, especially in developing areas where these diseases are most prevalent. When a viral pathogen invades a new human host, it is the innate immune system that serves as the first line of defence. Myeloid cells are especially important to help fight viral infections, including those of zoonotic origins. However, viruses such as EBOV, CHIKV, DENV and ZIKV have evolved mechanisms that allow circumvention of the host’s innate immune response, avoiding eradication and leading to severe clinical disease. Herein, the importance of myeloid cells in host defence is discussed and the mechanisms by which these viruses exploit myeloid cells are highlighted. The insights provided in this review will be invaluable for future studies looking to identify potential therapeutic targets towards the treatment of these emerging diseases.

INTRODUCTION TO THE MYELOID CELL LINEAGE

Myeloid cells are comprised of granulocytes and monocytes, and are derived from a common bone marrow progenitor cell. The commitment to either the granulocyte or monocyte lineage is initiated by exposure to a distinct panel of transcription factors in combination with signalling by colony stimulating factors that leads to terminal differentiation. Myeloid cells are part of the innate immune system, and act as the first line of defence to invading pathogens. Following pathogen invasion, myeloid cells are recruited in rapid response to the tissues via chemokine receptors, where myeloid cell phagocytosis of pathogens, along with the release of cytokines from tissue resident immune cells, results in myeloid cell maturation and activation.

The various cells that make up the myeloid lineage are described in Table 1. This review will discuss the role of myeloid cells in both immunity and pathogenesis of emerging viral zoonotic diseases of current public health concern.

EBOLA VIRUS

Background

With increasing media attention and an ongoing epidemic in West Africa that has seen 28,646 total cases and over 11,323 deaths, ebola is arguably the most well-known emerging viral disease of 2014–2016 [1]. The Ebolavirus is a member of the Filoviridae family, and causes the disease commonly referred to as ebola virus disease (EVD). The Filoviridae family contains three genera Ebolavirus, Marburgvirus and the recently proposed ‘Cuevavirus’ [2]. Within the Ebolavirus genus, there are five species; Zaire
The outcome of which is devastating, with an 90% maternal mortality. Furthermore, transmission to the baby may occur during delivery and via breast milk [1]. Vertical transmission also occurs for EVD with 111 reported cases, the outcome of which is devastating, with an 90% maternal mortality. Furthermore, transmission to the baby may occur in utero, during delivery and via breast milk [1].

### Clinical features

The incubation period for EVD is between 3–13 days with a mean range of 7–10 days. Disease onset is non-specific in nature, being characterised by fever (39–40°C), chills, fatigue, headache, myalgia, nausea, vomiting and diarrhoea. Unless the patient is in an outbreak area, EVD is not usually suspected until signs of a non-pruritic, erythematous and maculopapular rash or mucosal haemorrhage appear. Patients are leukopenic, with high granulocyte (particularly neutrophils) levels that elevate to leukocytosis as disease progresses. Thrombocytopenia, increased serum levels of alanine and aspartate aminotransferase (markers of liver function), coagulopathy and increased blood urea nitrogen together with creatinine are common features of EVD. The degree to which liver enzymes are dysregulated is an indication of disease severity, with liver damage along with rhabdomyolysis correlating with severe to fatal cases [4, 5]. In fatal cases, death occurs between 6–16 days after the onset of symptoms (usually in the second week) due to hypotensive shock and multi-organ failure, including hepatic damage and renal failure. The general clinical findings of EVD are summarised by Kortepeter et al. and are consistent with the clinical findings reported in the current outbreak [6].

### Pathogenesis

Current recommendations state that if a person is suspected to have died from Ebola virus infection, no autopsy should be performed. However, a number of autopsies have been carried out primarily by the Centre of Disease Control, that have provided invaluable insight into the pathogenesis of human disease [7]. The histopathological features of EVD show widespread necrosis in several organs, including liver, spleen, kidney, gonads, gastrointestinal tract and endocardium [8].

The most characteristic histopathological finding of fatal human EVD is found in the liver, with extensive hepatocyte necrosis in conjunction with varying degrees of inflammation [9]. In EBOV, mononuclear inflammatory cell infiltrates are found at the portal field, accompanied by extensive karyorrhexis [8]. Hepatocytes may show intracellular virus inclusions, mainly located in the perportal zones and adjacent to areas of necrosis [10]. Both Kupffer cell...
hyperplasia and small-droplet steatosis are also frequently observed [8, 9].

The spleen, which is a critical component of the mononuclear phagocyte system containing half of the body’s monocytes [11], displays extensive lymphoid depletion, associated with necrotic debris and apoptosis [8]. Viral antigen can be detected throughout the organ, particularly in monocytes, macrophages, dendritic cells (DCs) and fibroblasts [8]. Additionally, viral antigen is present in the extracellular space in close proximity to necrotic cells. Similar findings are seen in lymph nodes throughout the body.

Due to the haemorrhagic nature of EVD, fatal cases commonly show pulmonary congestion, focal intra-alveolar oedema and haemorrhage. Similar to the spleen, viral antigens are found predominantly in alveolar macrophages, but also in endothelial cells, fibroblasts and other interstitial cells. Further evidence of viral replication within alveolar macrophages has been shown by in situ hybridisation [9].

As mentioned earlier, the appearance of both exanthema and subsequent mucosal haemorrhage are indicative of EVD [12]. The histopathological changes in the skin tissue vary, and consist of varying degrees of necrosis, endothelial cell oedema, dermal oedema and haemorrhage [9, 12]. Epidermal DCs, endothelial cells and connective tissue fibroblasts all show viral inclusions and evidence of viral antigens [9, 12].

Descriptions of histopathological findings in other organs of the body are limited. It has been reported that the gastrointestinal tract shows mild focal mononuclear infiltrates in the lamina propria, with viral antigen detected in the infiltrating mononuclear cells [9]. Kidneys frequently show evidence of acute necrosis, with endothelial and interstitial viral antigen staining correlating to studies that have detected virus in urine by electron microscopy (EM) and PCR [13]. To date, no significant myocardial damage has been observed, however viral antigen has been detected in endocardial and endothelial cells, as well as in the extracellular space. Although no significant inflammation was observed in the testicular tissue from the single human EBOV case examined, experimental infection in NHPs displayed testicular inflammation and haemorrhage. EM analysis identified viral particles in interstitial cells, endothelium and monocytes [14]. Finally, no apparent morphological changes have been observed in the bone marrow. However, abundant inclusions in mononuclear cells, as well as extracellular antigens, have been detected. The finding that megakaryocyte numbers remain within the normal limits, and have not been identified as a site of viral antigen, supports the conclusion that EVD thrombocytopenia is not due to a reduction in platelet production [9].

The absence of extensive human data can be attributed to the fact that autopsies provided data only on the pathogenesis at the time of death and not during the course of infection. Taken together with research into potential antivirals and vaccines, prompted the development of several animal models of the disease. Both NHP and rodent (such as mouse, guinea pig and hamster) models have been developed to further elucidate the pathogenesis and treatment strategies for EVD.

NHP models have been used for several decades to better understand the physiopathology of *Ebolavirus* infection. EBOV infection and clinical disease in NHPs closely resembles that observed in humans, including the development of haemorrhagic manifestations, coagulation abnormalities, thrombocytopenia, lymphopenia and macular rash [15]. Species that have been employed to study EVD include African green monkeys (*Chlorocebus aethiops*) [16–18], cynomolgus macaques (*Macaca fascicularis*) [18–24], rhesus macaques (*Macaca mulatta*) [25–32] and hamadryad baboons (*Papio hamadryas*) [33–36]. However, macaques and baboons have been shown to best mimic human EVD.

Although NHPs provide the most biologically relevant data, being closely related to humans and naturally susceptible to *Ebolavirus* infection, they can be expensive and their use poses a number of ethical issues. The use of rodent models have aided in filling in the gaps of knowledge regarding disease pathogenesis. Recently, the development of a Syrian hamster (*Mesocricetus auratus*) model of EVD has shown promise, with clinical disease closely mimicking that seen in NHP models including the development of coagulopathy, which is absent in mouse and guinea pig models [37]. For a detailed overview on the pathogenesis of EVD in animal models, please refer to the following review [15].

**Mechanism of disease**

In addition to analysis of fatal human cases and the use of *in vivo* animal models, *in vitro* models of infection have significantly contributed to the greater understanding of disease mechanisms. By combining the studies to date, an overall picture of pathogenic mechanisms can be determined. *Ebolavirus* is known to invade its host primarily through the breach of the body’s mucosal surfaces or through abrasions in the epithelium. Once inside the host, the virus primarily targets macrophages and DCs. After initial replication, the virus spreads into surrounding tissues infecting fibroblasts, epithelial and endothelial cells. As macrophages and DCs are capable of migration via lymphatics to draining lymph nodes following exposure to antigen, it is thought that the infection of monocytes, macrophages and DCs are responsible for spreading the virus from the primary site of infection to the lymph nodes, then to the liver and spleen via blood [38, 39]. *Ebolavirus* subsequently infects secondary tissue macrophages, including Kupffer cells, tissue DCs and fibroblastic reticular cells [37, 38]. The infection of these myeloid cells has been shown to impair their critical function in antiviral defence, resulting in high levels of viraemia. During the infection process, macrophages and DCs respond by initiating inflammation through the release of pro-inflammatory soluble factors and vasoactive mediators. This results in an influx of innate immune cells to the site of infection, which only acts to aid virus dissemination in the host.
The targeted replication of *Ebolavirus* in macrophages and DCs significantly contributes to extensive tissue damage in EVD. Infection of macrophages has been shown to contribute to the development of fever, lymphopenia, hypotension and shock in fatal cases. Upon viral invasion, macrophages and DCs release a range of soluble mediators, including IL-1, IL-6, IL-8, IL-15, IL-16, MIP-1 α/β, monocyte chemotactic protein-1 (MCP-1), macrophage-colony stimulating factor, macrophage migration inhibitory factor (MIF), IFN gamma-induced protein-10 (IP-10), eotaxin and nitric oxide (NO) [27, 40–46]. Most notably there is a marked increase in tumour necrosis factor-α (TNF-α), which induces both fever and lymphocyte apoptosis (shown as lymphopenia in patients) as well as an inhibition of IFN, a critical component of the host’s antiviral defence [42, 47–50]. Overall, the release of these mediators attract additional immune cells, including monocytes and neutrophils, to the site. They also facilitate their migration from the bloodstream into the tissues by causing vasodilation, increased endothelial permeability and the expression of endothelial cell surface adhesion molecules [51–54].

In addition to playing a significant role in tissue damage and shock, macrophages have been shown to play a critical role in the development of coagulation abnormalities. During EVD, defects in coagulation can present as petechiae, ecchymoses, mucosal haemorrhage, congestion, and uncontrolled bleeding at injection sites. Experimental infection of rhesus macaques has shown increased levels of tissue factor (TF), a key initiator of the coagulation protease cascades, associated with lymphoid macrophages [55]. Further research on primary human monocytes/macrophages demonstrated that increased levels of cell surface TF were produced in response to EBOV infection, whereas endothelial cells showed no such increase [55]. *Ex vivo* studies on *Ebolavirus* infection on human peripheral blood mononuclear cells (PBMCs) has shown that 60–70% of macrophages undergo apoptosis by day 8 post-infection and the cells are phenotypically characterised by active caspase 3, annexin-V and B-cell lymphoma 2 (Bcl2)low [56]. The role of *Ebolavirus* in inducing macrophage apoptosis is hypothesised to further impair the host’s antiviral immune response and the development of an acquired antibody production, thereby contributing to high fatality rates [57].

As mentioned earlier, DCs have been shown to be a target of infection in *vivo* both in human cases and in animal models [9, 38]. Further studies have shown that DCs support productive virus replication in *vitro* [40, 58, 59]. Infection of DCs derived from human monocytes has been shown to impair IFN-α/β production, affect pro-inflammatory cytokine expression, and inhibit activation of naive T cells [40, 58, 60]. Interestingly, it has been shown that *Ebolavirus* infection prevents DC maturation through a VP35-dependent mechanism [60, 61]. The suppression of DC function by the EBOV VP35 protein has been found to act by general antagonism of RIG-like receptor signalling and can hinder both RIG-I- and MDA5-mediated induction of IFN-α/β responses [61], without inhibiting toll-like receptor (TLR)-mediated expression of pro-inflammatory cytokines. These data suggest VP35 inhibition of DC function is likely to be a significant factor contributing to the suppression of innate immune function [60–62]. It has been well-established that the activation of DC pattern recognition receptors and the subsequent maturation of DCs is critical in linking the innate and adaptive immune responses [63], and therefore it is speculated that *Ebolavirus* suppression of DC maturation contributes to the extreme viraemia and viral growth observed in EVD (see Fig. 1).

Recent studies have focused on the role of the *Ebolavirus* glycoprotein (GP) in immune modulation and disease pathogenesis. Surface GP has been shown to enhance viral transfer to non-infected cells via cell-to-cell contact [9, 38]. Several studies have found that following infection, GP is also released from infected cells in soluble forms. Large amounts of both non-structural surface GP and truncated surface GP have been detected in the blood of patients and in experimentally infected animals [64, 65]. In addition to acting as a decoy, by binding and neutralising virus-specific antibodies [64], soluble shed GP has also been shown to activate non-infected DCs and macrophages. This activation induces the release of high levels of cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-12p40, and IL-1-RA, IL-10), as well as increasing vascular permeability [66]. This activation occurs by GP binding to the surface of the macrophage/DC via TLR-4, and subsequently stimulating the TLR-4 signalling pathway (see Fig. 1). Therefore, it is dependent on both the structure of the GP and its glycosylation [66–68]. In addition, GP activation of macrophages and DCs is associated with an increase in the co-stimulatory molecules classification determinant (CD) 40, CD80, CD83 and CD86. Given that approximately 70% of transcribed GP is subsequently shed from the virus into the bloodstream [64], it is likely that soluble GP causes the induction and release of inflammatory cytokines from non-infected macrophages and DCs, contributing to the dysregulated host inflammatory responses and increased vascular permeability observed in fatal EVD cases. Another cell surface myeloid receptor important in the dysregulation of inflammation during EBOV is triggering receptors expressed in myeloid cells (TREM). A study by Mohamadzadeh *et al.* [69] demonstrated that direct activation of neutrophil TREM-1 by EBOV, causes DAP12 intracellular phosphorylation, resulting in TREM-1 exocytosis, calcium flux and secretion of proinflammatory cytokines [69].

**Summary and future directions**

Summation of several decades of research on the pathogenesis of EVD indicates that both an impaired and ineffective antiviral immune response results in high levels of virus and pro-inflammatory factors, which subsequently causes the development of haemorrhage and shock. The current hypothesis suggests that infection and activation of myeloid macrophages and DCs is fundamental to the development of ebola haemorrhagic fever. The release of pro-inflammatory cytokines, chemokines, NO and TF from these antigen-presenting...
cells causes impairment of the vascular and coagulation systems, which in turn leads to multi-organ failure, shock syndrome and high case fatality rates.

Based on detailed studies on the virulence of Ebola virus proteins in the impairment of macrophage and DC function, a number of therapeutic strategies are in the process of being developed or tested in animal models. Due to studies on the role of VP35 in preventing DC maturation, it is speculated that disruption of VP35 could increase both the innate and adaptive immune responses to EBOV infection, thereby facilitating viral clearance. Furthermore, the data obtained on the effect of shed GP on both macrophages and DCs provides a potential link between systemic inflammation and increased endothelial permeability and provides new insights into therapeutic strategies. In particular, due to the dependence of GP on TLR-4, it is possible that anti-TLR4 antibodies may reduce the inflammatory response caused by shed GP in a similar manner to what has been shown in the treatment of LPS-induced septic shock in mice [70]. Equally, it is likely that neutralising shed GP with specific anti-GP antibodies could also assist in alleviating systemic shock syndrome in EVD and significantly aid in reducing case fatality. Overall, the targeting of ebola virus to macrophages and DCs is a key mechanism aiding the virus to cause; high viraemia, lymphopenia, coagulation disorders including haemorrhage, widespread tissue necroses, overall impairment of the host immune response, systemic shock and multi-organ failure in EVD.

**CHIKUNGUNYA VIRUS**

**Background**

Chikungunya virus (CHIKV) is part of the *Togaviridae* family and a member of the *Alphavirus* genus. Alphaviruses are generally divided into Old World and New World
alphaviruses. Members of the Old World alphaviruses include CHIKV, Ross River virus, o’nyong-nyong virus, Barmah Forest virus, Mayaro virus and Semliki Forest virus [71]. Infections by these Old World viruses classically leads to debilitating pain, characterised by myalgia, polyarthralgia and swelling that can last for months to years [72]. CHIKV is normally maintained by a sylvatic cycle comprised of NHPs as reservoir hosts, including African green monkeys (Chlorocebus sabaensis), patas monkeys (Erythrocebus patas) and Guinea baboons (Papio papio). It is thought to infect humans via bridge vectors, which are mosquitoes that feed on both NHPs and humans including the forest dwelling Aedes (Ae) mosquitos, including Ae aegypti and Ae albopictus. A secondary cycle may also exist since CHIKV or CHIKV antibodies have been detected in cattle, rodents, squirrels, sheep and birds [73, 74]. Vertical transmission can also occur during CHIKV infection. Infrequently described prior to the Réunion island outbreak, little is still known about the exact mechanisms of mother–foetus transmission. Transmission generally arises during childbirth, where it is believed that CHIKV reaches foetal circulation through placental breaches during labour [75]. Some studies estimate the rate for intrapartum transmission between 27.7 and 48.7 % [76, 77]. CHIKV transmission to neonates is associated with significant levels of neurocognitive dysfunction throughout childhood [78].

The recent arrival of CHIKV to the Western Hemisphere categorises it as an emerging pathogen of the twenty-first century. The first official CHIKV case was reported in 1952 in southern Tanzania [79]. In the decades that followed, CHIKV was identified in Africa and the immediate surrounding regions, circulating in both endemic and epidemic patterns. Since 2005, CHIKV has expanded its geographic range, driven by increases in international travel and trade, as well as by adapting to a new mosquito vector [80]. The 2005 epidemic in the islands of the Indian Ocean, namely La Réunion, Mauritius, Comoros, Madagascar and Mayotte, was mainly due to a single mutation allowing the virus to spread via Ae albopictus mosquitoes. The first incidence of autochthonous spread of CHIKV in South and Central America together with the Latin and non-Latin Caribbean islands was documented in 2013 [81]. Additionally, in 2014 local spread of CHIKV was detected in Florida. Since then, the number of CHIKV incidences in the Americas has risen notably [82], and as of October 2016 the Pan American Health Organization has reported more than 194000 suspected CHIKV cases and 107570 confirmed cases throughout North America.

**Clinical features**

Chikungunya infection appears 2–10 days post-exposure, with high fever (>39°C), rash, symmetrical polyarthralgia, myalgia, joint swelling, nausea and headache. Viraemia lasts for approximately 1 week and can reach titres up to $1 \times 10^6$ viral RNA copies per millilitre of blood [83]. During this time, the patient is at risk of spreading the infection to others if competent mosquito vectors are prevalent in the area. Clinical disease improves within 1–2 weeks. Approximately 12–49 % of patients report having chronic pain, defined as lasting upwards of 3 weeks to several years [84]. Reports describing CHIKV chronic arthralgia occurred following the 2005–2006 epidemic in La Réunion, with 36 % of patients reporting the persistence of symptoms 15 months after disease onset, and almost one-quarter reporting at least one relapse of illness following apparent recovery.

During the Réunion outbreak an increased number of atypical CHIKV infections were recorded (total of 614 cases) and a large majority of those patients had underlying medical conditions, including hypertension (54 %), diabetes (39 %), cardiovascular (32 %) and respiratory disorders (16 %), alcoholism (14 %), kidney disease (12 %) and cancer (4 %) [85]. Atypical manifestations of CHIKV infection vary, and include cardiovascular disorders such as heart failure and arrhythmias, pneumonia, skin affections, hepatic dysfunction and pancreatitis. Although rarely reported prior to the Réunion outbreak, encephalitis and neurological sequelae like Guillain–Barré syndrome are also consequences of CHIKV infection. In other reports looking at Indian outbreaks of CHIKV infections, atypical disease also included ocular lesions and complications [86]. Currently there are no specific treatments for CHIKV infections, except for symptomatic management. Importantly, the use of aspirin has been contraindicated, due to contributing to thrombocytopenia and increased bleeding and vomiting [87].

**Pathogenesis**

Extensive viral replication and high viraemia drives the pathogenesis of acute CHIKV infection. Virus dissemination to distal areas, including peripheral joints, muscle, tendons and liver, results in acute inflammation, synovitis and tenosynovitis causing severe and debilitating pain.

A macaque model of infection, which recapitulates some important features of human CHIKV disease, demonstrated that the spleen (red pulp), lymph nodes (cortex), liver, necrotic muscle fibres and synovial tissues displayed significant mononuclear/macrophage accumulation, suggesting organ damage was attributable to the mononuclear phagocytic host systems. Biopsy, serum and cerebrospinal fluid (CSF) analyses of acutely infected, intensive care unit patients confirm the presence of viral inclusions in liver macrophages (Kupffer cells) and myocytes [88]. This is supported by analysis of CSF samples extracted from meningoencephalitic animals, which confirmed the presence of activated CD14+CD16+HLA-DR+ monocyte/macrophage population of cells. Together these findings confirm a cause–effect relationship of the myeloid monocyte/macrophage cell systems to the inflammatory pathogenesis of CHIKV [89].

Since the increase in incidence of global CHIKV transmission and disease, more knowledge has been gained about CHIKV pathogenesis and sequelae like Guillain–Barré syndrome. Brain autopsies also display neuropathology such as oedematous frontal/occipital cortices, haemorrhagic
subarachnoid space and demyelinated subcortical white matter. Activated microglial cells (but not microglial nodules) were dispersed in the grey matter and diencephalon regions [90].

Mechanism of disease

Once bitten by a mosquito, chikungunya infects local cells including fibroblasts, melanocytes and endothelial cells. Resident skin immune cells serve as an important first defence against chikungunya infection. Immune cell populations within the skin include numerous types of DCs, monocytes and lymphocytes. It has shown that the dendritic cell immunoreceptor (DCIR) expressed on DCs, as well as monocytes, macrophages and neutrophils, plays an important role in CHIKV pathogenesis (see Fig. 2). Importantly, increased DCIR expression was linked to chronic inflammation associated with rheumatoid arthritis and myocardial infarction in humans and with rheumatoid arthritis in mouse models [91]. Using in vivo and in vitro experiments, it was shown that CHIKV infection leads to DCIR-modulated DC responses and that the absence of DCIR leads to significantly enhanced CHIKV pathology [92]. It was therefore suggested that targeting the inflammatory pathways that DCIR modulates may be beneficial in the treatment of CHIKV and other alphavirus-induced inflammatory diseases. In addition, the presence of injected mosquito saliva assists in augmenting CHIKV pathology, via triggering the down regulation of pro-inflammatory cytokines and upregulating anti-inflammatory cytokines, such as IL-4 and IL-10. Together this contributes to increased viral load [93].

Early work indicated that monocyte-derived macrophages were permissive to CHIKV in vitro [94]. This is supported by extensive studies defining macrophages as the dominant immune infiltrate at the site of CHIKV infection. Studies by Her et al. have shown that monocytes isolated from patients acutely infected with CHIKV harbour infective virus. Whereas research using a macaque model has shown that macrophages are the prominent cell reservoirs of chronic CHIKV infection, with CD68+ macrophages remaining infected for up to 3 months [89], demonstrating critical roles for macrophages in both acute and chronic disease. This is further supported by the finding of CHIKV RNA and protein inside synovial macrophages 18 months after the initial infection, also suggesting macrophages may be long-term reservoirs of infection [72].

Additional in vitro experiments with whole human blood or purified monocytes also indicate monocytes and myeloid DCs are targets for CHIKV [95]. Some myeloid DCs (4%) were shown to have become positive with CHIKV antigen following phagocytosis of viral antigen. In an in vitro system, the release of apoptotic blebs from infected cells has been shown to facilitate the spread of CHIKV to uninected neighbouring cells including macrophages [96]. It was shown that, phagocytosis of CHIKV-containing blebs contributed to the spread of CHIKV.

In an adult mouse model of CHIKV disease, arthritis was associated with a prolific influx of mononuclear lymphocytes [97]. Widespread infiltration of macrophages into synovium and surrounding connective tissues was observed from day 7 post-infection and until late post-infection. Macrophage deficient mice (depleted via clondronate liposome) were reported to have reduced foot swelling, but suffered an extended viraemia thereby demonstrating a role for macrophages in arthritic disease but also in viral clearance [97]. This data is in agreement with what had previously been seen in primates where monocytic infiltrates are seen after CHIKV infection and depletion of macrophages ameliorates disease [89]. In a supporting study, mice lacking the chemokine (C-C motif) ligand 2 (CCL-2) receptor (regulator of macrophage polarisation) resulted in the recruitment of neutrophils followed by eosinophils, rather than macrophages, and exacerbated arthritic disease [98]. This also affected changes in the expression of inflammatory mediators, including an up regulation of chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL2, granulocyte-stimulating factor, IL-1β, and down regulation of IL-10. These factors are thought to explain the shift from infiltrating macrophages to neutrophils and eosinophils.

M2 macrophages, stimulated by IL-4/IL-13, are associated with anti-parasitic activity, promotion of tissue remodeling, tissue repair, tumour progression and immunoregulatory functions [99]. Activation of M2 or M2-like macrophages can occur in a variety of tissue types, including damaged musculoskeletal tissue, and this response can occur despite the presence of an infectious pathogen. Arginase 1, a marker for M2 macrophages, was found to be dramatically induced in mice following CHIKV infection. Authors believe that M2 macrophages may be responsible for pathology seen in alphavirus-infected mice [100]. Another similar study reports that CHIKV infection triggers the recruitment of immunosuppressive arginase 1 and inducible nitric oxide synthase 2 (iNOS) expressing monocytes/macrophages, which attenuate T-cell-mediated viral elimination, contributing to chronic CHIKV pathogenesis [101].

Another mechanism contributing to CHIKV disease is via the cytokine MCP-1/CCL2. MCP-1 is a key player in the migration and infiltration of monocytes/macrophages, memory T lymphocytes, and NK cells to the site of infection [102]. High levels of MCP-1 have been found in acute CHIKV patients, in NHP models (serum) and mouse models (tissues). A descriptive study characterising the biomarkers in CHIKV patients, reported that the expression levels of inflammatory cytokines, including MCP-1, correlated to the severity of a patient’s symptoms (chronic versus acute) in association with CHIKV resolution [103]. Patients with acute CHIKV often were found to have elevated serum concentrations of IL-1, IL-6 and IL-10. In contrast, chronic disease was associated with variable elevations in MCP-1, IL-6, IL-8, macrophage inflammatory protein-1α (MIP-1α) and macrophage inflammatory protein-1β (MIP-1β) inflammatory proteins. The use of Bindarit (anti-MCP...
antibody) for the treatment of CHIKV-infected mice protected against joint and muscle damage, supporting the importance of MCP-1 in CHIKV pathogenesis [104]. Further prominent anti-viral cytokines produced after CHIKV infection include type-I IFNs. Expression of type I IFNs is triggered following engagement of pattern recognition receptors with antigen. Infected patients were found to have significantly higher levels of IFNs. As CHIKV is incapable of directly engaging pattern-recognition receptors this suggest that induction of IFN expression is via an indirect pathway [105]. Fibroblasts are the suggested source of the type-I IFNs and more work is needed to determine which IFN-stimulated gene in fibroblasts actively controls CHIKV replication.

**Summary and future directions**

The common consensus is that the pathogenesis of CHIKV disease is predominantly immune mediated. The long-term arthralgia experienced in some patients may be explained in part by the chronic activation of an inflammatory state by macrophages persistently infected with CHIKV. These monocytes and/or macrophages act as Trojan horses, for the delivery of virus into peripheral tissues leading to persistent infection. This is thought to be due to the early escape of CHIKV from blood monocytes and relocation to synovial macrophages [95]. In addition, macrophage-derived host factors like TNF-α, IFN-γ and MCP-1 appear to contribute to arthritis in susceptible hosts.

**DENGUE VIRUS**

**Background**

To date, DENV is the most prevalent arbovirus disease, with an incidence rate that has increased 30-fold over the past 50 years [106]. The World Health Organization estimates that between 50 to 100 million individuals become infected with DENV every year [106]. It is of significant public health concern as there are currently no licensed vaccines or specific therapeutics for use against DENV. Lifelong immunity is conferred once a person contracts an infection from one of the four serotypes. However, immunity is specific and a secondary infection to a heterologous serotype places the patient at a higher risk of developing one of two severe and often fatal illnesses called dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). This is the result of antibody-dependent enhancement (ADE) as reviewed by Halstead [107]. This mechanism of ADE makes developing a vaccine, which would be efficient against all
serotypes in one formulation, especially challenging. The main species of mosquitoes, which transmit DENV are *Ae aegypti* and *Ae albopictus*.

Since the first isolation of DENV in Nagasaki, Japan, the geographical distribution of DENV has reached most tropical and sub-tropical regions. Before 1970, only nine countries had experienced severe dengue epidemics. Dengue is now endemic in more than 100 countries in the Americas, Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific [108]. In particular, 75% of the current global burden of dengue is located in the South-East Asia and Western Pacific regions [109]. Historically, dengue outbreaks often occurred in urban areas, with a high population density enabling ease of transmission. However, outbreaks in Cambodia (2007) indicate that DENV has also migrated to rural regions [109].

**Clinical features**

DENV presents with a large spectrum of clinical signs, which had been previously classified in categories such as acute febrile illness (dengue fever, DF), DHF and DSS. Due to changes in DENV epidemiology, alterations were made to the WHO classification to improve diagnosis and subsequent management of DENV [110]. Currently, DENV illness is classified as severe dengue (SD) or non-severe dengue [111]. The incubation for DENV is approximately 5–7 days. Most patients will recover from mild, self-limiting fever without complications. However, some patients progress to severe disease and require urgent medical intervention. This is more often the case for babies and children. A child is 15-fold more likely to die from a secondary DENV-2 (one of the four serotypes of DENV) infection than an adult. It is noteworthy that the elderly are also more susceptible to severe or fatal infections. Overall, secondary dengue infection is the most significant risk factor for SD. Symptomatic red flags of SD include abdominal pain, vomiting, oedema, muscosal bleeding, lethargy and hepatomegaly [111]. A study of 1484 patient medical records found that gastrointestinal presentations (in particular abdominal pain, melena, hepatomegaly, hæmatemesis, nausea/vomiting and diarrhoea) are often indicative of SD infections [112]. Signs and symptoms of SD progress quickly through three phases: febrile, critical and recovery [111]. In the febrile stage, patients present with a sudden onset of fever, myalgia, arthralgia, rash, severe headache and retro-orbital pain. The critical stage, between days 3–7 post-infection, is associated with a petechial rash, thrombocytopenia and vascular leakage syndrome (increased vascular permeability, hæpovælema, hypotension and shock) [106, 111]. Atypical presentations of DENV illness in children (N=254) commonly include lymphadenopathy (41.7%), splenomegaly (21.2%), biphasic fever (18.1%), hepatitis (11.4%), febrile diarrhoea (6.3%), refractory shock (2.4%) and impaired consciousness (1.9%) [113]. In particular, impaired consciousness was a sign of poor prognosis, as it was present in 66.6% of deaths observed [113]. Treatment of SD is generally supportive, consisting of intravenous fluid replacement, pain management with analgesics, and bed rest. In some cases, blood transfusions and oxygen therapy may also be required.

**Pathogenesis**

Recent literature has demonstrated a relationship between pathogenicity of DENV and the myeloid monocyte/macrophage cell system. DENV structural proteins and negative-sense viral RNA have been identified in lymph node macrophages, spleen, lung, liver and monocytes present in clotted blood of patients with fatal DENV infection [114]. Furthermore, the replication of DENV in monocytes, macrophages and DCs in lymph nodes as well as the spleen has been demonstrated through the staining of DENV-2 non-structural protein 3, in human autopsies [115].

Organ lesions of four fatal DF cases in Brazil were analysed using optical and EM to quantify the degree of organ damage [116]. In all lesions examined, histopathological changes characteristic of severe DF were noted. In patients with existing co-morbidities such as diabetes, mononuclear infiltrates resulting in hyaline membrane formation were found in the lungs and acute tubular necrosis was identified in the kidneys. Myocarditis and haematological disturbances (e.g. thrombus formation, loss of endothelium) was noted. Spleen pathology (lymphoid follicle atrophy) associated with a decreased T cell number was also observed. Detection of DENV antigens (non-structural protein 3) demonstrated that DENV replicated in a large range of tissues including hepatocytes, cardiac fibres, type II pneumocytes, monocytes, macrophages and endothelial cells.

**Mechanism of disease**

Numerous primary cells, such as DCs, monocytes, macrophages, lymphocytes, fibroblasts, endothelial and epithelial cells have been used to study DENV pathogenesis *in vitro*. There are many challenges to developing an animal model to study DENV as NHPs are not generally affected by epidemic strains of DENV. Currently, there are two main models utilised to study DENV pathogenesis: AG129 mice, which are deficient in αβγ-IFN and mouse-adapted dengue strains whereby the virus is mutated and serially passaged in normal mice. It is apparent that both DENV models have limitations. AG129 mice lack type I and type II IFNs, which play a crucial role in normal antiviral responses. Adapted dengue strains accumulate mutations, and therefore no longer accurately reflects parental virus behaviour. Despite these limitations, results obtained in mouse models can be used to guide research using human participants and adds further weight to results obtained from *in vitro* experiments. Further detail concerning the advantages and disadvantages of current mouse models for DENV can be found in the following review articles [117]. Recently, the NHP black-tufted marmoset (*Callithrix penicillata*) subcutaneously infected with DENV-3 has been shown to mimic human DENV infection and its potential as a feasible experimental model for DENV infection has been highlighted [118].
Monocytes, including DCs and macrophages, play a critical role in DENV pathogenesis [119]. The dermis, where blood vessels are located, has been identified as the main site of early DENV replication. Resident CD103+ classical DCs and macrophages are initially infected following breach of mucosal surfaces via the virus. Assisting in effective infection is the presence of mosquito saliva, injected into the host skin during the blood meal. Schmid et al. (2016) demonstrated that injection of mosquito salivary extract into the dermis of mice lacking the IFN α/β receptor enhanced dengue pathogenesis in the presence of cross reactive antibodies [120]. Salivary gland extract also increases viral load, increased antibody-enhanced DC and macrophage infection by DENV and boosted their migration to local draining lymph nodes [121]. Monocytes and Ly6C+ CD11b+ monocyte-derived DCs recruited to the site of infection have also been demonstrated to harbour virus (120). In addition, both CD16+ and CD16– human blood monocytes have been shown to be susceptible to DENV infection [122].

The literature demonstrates that the depletion of macrophages in mouse models of DENV resulted in increased infectivity with DENV, which indicates that macrophages have a key role in host virus control [123]. Similarly, mast cell-deficient Kit (W-sh/W-sh) mice displayed a prolonged bleeding time and increased macrophage infiltration as well as a two- to threefold increase of DENV antigen at the intra-dermal inoculation site when compared to DENV-infected wild-type mice [124]. In a NHP model, CD14+ and CD16+ monocytes have been shown to promote plasmablast differentiation, which produce DENV-neutralising antibodies [125]. In addition, DF patients exhibited lower expression of complement receptor-3 (CR3) (CD11b), CR4 (CD11c) and CD59 on monocytes when compared to healthy controls. In fact, patients who produced high levels of terminal complement complex SC5b-9 were identified to have more severe vascular leakage [126]. These results highlight the key role of mast cells and tissue macrophages in opposing DENV replication in human tissues.

Monocytes have been shown to also have a role in amplifying the immunological response to DENV. Once viral entry and uncoating has occurred in monocytes, large numbers of viral proteins are synthesised in the host endoplasmic reticulum (ER) before transport to the Golgi apparatus and subsequent exocytosis. The sudden increase in unfolded proteins activates numerous ER stress responses, which in turn contributes to inducing apoptosis [127]. Furthermore, the anti-DENV IFN-γ response is regulated by type I IFN and IL-18 in a TCR-independent manner. In vitro studies of cells extracted from patients presenting with acute DF demonstrated that monocytes aid in ridding DENV infection in DCs by enhancing the γδ T cell responses and they also lead to an increased secretion of IL-18 [128]. Cultures that did not have monocytes exhibited a lower IL-18 concentration in supernatant and lower IFN-γ secretion from γδT cells [128]. Other studies have observed the maturation of DENV non-structural protein 4B in monocytes increases the secretion of pro-inflammatory chemokines and cytokines (IL-6, IL-8, IP-10, TNF-α, IFN-γ) critical in the pathogenesis of DHF [129]. In addition, patients infected with DENV were seen to have higher levels of platelet-monocyte aggregates in blood samples that were shown to signal for elevated expression of specific pro-inflammatory cytokines (IL-1β, IL-8, IL-10 and MCP-1) [130]. Interestingly, monocytes and macrophages exposed to DENV in neonates and the elderly have exhibited lower cytokine expression, production of NO, lipid peroxidation and enzymatic/nonenzymatic anti-oxidative responses than that of young adults [131]. This indicates an immunosuppressive condition and suggests variations in pathogenesis at these ages. A study of paediatric patients in New Delhi found that while CD14+ and CD16+ monocytes increased early in the infection, CD14+ monocytes were the major cells infected by DENV and produced elevated amounts of the anti-inflammatory cytokine IL-10 [132]. This increase in IL-10 was noted as one distinct marker of DENV infection severity in paediatric patients.

Once DENV infection has been established, monocytes contribute to the development of classical signs of vasculature leakage. DENV2 infection of human primary monocytes triggers vascular endothelial growth factor expression via the TLR3, IFN-β promoter stimulator 1 signalling pathways [133]. MIF has a critical role in DENV pathogenesis, as illustrated by studies where MIF knock-out mice exhibited less severe clinical disease. As reviewed by Chiang et al., MIF has been shown to contribute to vascular leakage, DENV replication and the expression of adhesion and coagulation molecules on MIF-stimulated monocytes and endothelial cells, resulting in heightened inflammatory states during DHF [134]. Monocytes have also been observed to contribute significantly to ADE, whereby cells with Fcγ receptors are invaded by DENV with the help of cross-reactive anti-DENV antibodies [135, 136]. ADE-affected monocytes were observed to increase TNF-α and IFN-α cytokine production and co-stimulatory marker expression (CD86 and CD40) thereby contributing to the pathogenesis of DENV [135]. In fact, ADE-DENV-infected monocytes produced significantly higher levels of pro-inflammatory cytokines (namely, IL-6, IL-12p70, TNF-α, prostaglandin E2) than cells that were directly infected by DENV [137]. This response allows for the disruption of tight junctions via degrading apical-junction complex proteins and loss of transepithelial electrical resistance. In vivo studies using a mouse model suggests that this results in vasculature plasma leakage that is characteristic of DHF [137].

Summary and future directions

A thorough review of the current literature indicates that monocytes have a critical role in DENV pathogenesis. The current hypothesis suggests that monocytes play a role in host viral control, by initially secreting DENV-neutralising antibodies or suppressing the expression of receptors/domains key to initiating the pro-inflammatory state. However, once DENV infection and activation of monocytes occurs, they are stimulated to release pro-inflammatory...
cytokines, which causes an amplification of the immune response and results in vascular leakage, a hallmark feature of DHF.

Therapeutic strategies for DENV are still in preliminary stages and there have been no major successes in the field thus far. This is thought to be a product of limitations inherent in mouse models, which prevent accurate portrayal of the DENV immunological response in humans. Furthermore, the process of ADE in reoccurring DENV infections is a major concern for vaccine development. Despite these challenges, recent advances in understanding the molecular mechanisms and pathogenesis of DENV have aided the development of new novel therapeutic strategies. These are reviewed in detail in the following paper [138]. In particular, silencing of cell surface receptors and the main components of the clathrin-mediated endocytosis pathway (clathrin heavy polypeptide and human dynamin 2) on CD-14 monocytes using RNA interference has shown to be effective in reducing DENV entry and subsequent infection [139]. Other pharmacologicals have been grouped into the following classes of fusion inhibitors, glycosidase inhibitors, carbohydrate-binding agents and Heparan mimetics and have varied success in blocking DENV entry. Overall, therapeutic strategies that target the role of monocytes in DENV infection is key to combating the life-threatening effects of vascular leakage in DSS.

ZIKA VIRUS

Background

While zika virus (ZIKV) infection itself is usually mild and self-resolving, infection during pregnancy has been linked to the development of microcephaly and other serious neurological birth defects [140]. The main species of mosquitoes that transmit ZIKV are *Ae aegypti* and *albopictus* mosquitoes [141]. However, sexual transmission of ZIKV has also been recorded [142]. Perinatal transmission was identified during the French Polynesia 2013–2014 outbreak with PCR confirming the presence of ZIKV in breast milk [143].

The World Health Organization predicts that ZIKV will continue to spread across many more countries and is, therefore, of great public health significance [140]. ZIKV was first detected in 1947 in the Zika Forest in Uganda during a yellow fever surveillance of Rhesus monkeys. The following year, it was isolated from a pool of *Ae africanus* mosquitoes [144]. The first case of ZIKV in humans was identified in Uganda, 1952, and soon spread to Egypt, India, Malaysia, Thailand, Vietnam and the Philippines as reviewed by Malone et al. [145]. The first large outbreak of disease caused by ZIKV infection was reported from the Island of Yap in 2007 located in the Caroline Islands, which are part of the Federated States of Micronesia [146]. ZIKV caused another large epidemic in French Polynesia in 2013. More than 400 laboratory cases were confirmed and the epidemic spread across the Pacific Ocean to Brazil, Suriname and Columbia [147] Since 2015, the prevalence of ZIKV has been steadily increasing, with the first confirmed case of ZIKV infection in Brazil. Since that time, it has been responsible for over 1.5 million cases in Brazil alone and has spread rapidly across to 26 countries in the Americas [148]. Most recently, on 1 February 2016 ZIKV was been declared a Public Health Emergency of International Concern due to an increasing number of outbreaks [149]. Currently, ZIKV is distributed over Africa, the Americas, Asia and the Pacific. Since 2007, a total of 64 countries and territories have reported transmission of ZIKV. It is hypothesised that the fast rate of ZIKV transmission over the Americas is due to both a lack of host immunological recognition of the virus as well as the widespread presence of *Ae* mosquitoes in the environment.

Clinical features

The incubation period for ZIKV is between 3–12 days and signs and symptoms are often mild, resembling a less severe DENV infection. Symptoms include fever, arthralgia, conjunctivitis and maculopapular rash, which lasts for up to 1 week. While ZIKV disease is rarely fatal, medically compromised patients are at risk, as highlighted by the death of a patient with sickle cell disease [150]. At present, there is no vaccine or pharmaceutical treatment available for ZIKV. Current best practice guidelines recommend supportive therapy, including bed rest, analgesia and fluid replacement.

Complications of ZIKV is highlighted in the data from the French Polynesia epidemic, with 73 cases of Guillain–Barré syndrome among other neurological conditions [151]. Between 2014 and 2015, during the Brazilian ZIKV epidemic, there was a reported 20-fold increase in the cases of microcephaly. The causative link between ZIKV infection in pregnant women and microcephaly has been speculated by numerous sources [152]. The highest risk of foetal malformation is observed when ZIKV infection occurs during the first trimester of pregnancy [152]. Furthermore, the use of ultrasound imaging has revealed the presence of calcifications on the foetal brain and placenta [153]. Foetal autopsy upon one elective abortion showed disruption and inflammation in the native cortical architecture with reverse transcription (RT)-PCR confirming the presence of ZIKV in the brain tissue [154].

Pathogenesis

ZIKV is detected in human blood samples from the day of infection. It has been demonstrated that the strain of ZIKV responsible for the recent epidemic in French Polynesia invades human dermal fibroblasts, epidermal keratinocytes and immature DCs [155]. Subsequently, this resulted in the formation of autophagosomes, which enhances ZIKV replication in the host. Furthermore, ZIKV RNA has been detected in the amniotic fluid of newborns with microcephaly thereby increasing widespread public concern [156].

Mechanism of disease

Primary cells, such as human skin and neural stem cells, have been used to study ZIKV pathogenesis in vitro. Similar to DENV, it has been demonstrated that immature skin DCs are also permissive to ZIKV infection, with 50% of
human immature DCs generated *in vitro* expressing the viral envelope protein after exposure to ZIKV [155]. Hamel *et al.* also showed that like DENV, entry receptors such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), and proteins from the TIM and TAM family have a major role in facilitating viral entry into target cells. Furthermore, the production of IFNα, β and γ by ZIKV-infected skin fibroblasts was noted to significantly decrease the viral titre in the supernatants of these cells, thereby indicating that cells such as macrophages and monocytes, which produce these cytokines will also contain antiviral properties against ZIKV [155].

There have also been two reports of ZIKV transmission by blood transfusion suggesting susceptibility of blood cells to ZIKV infection. In fact, one person donated platelets by apheresis and the leukocyte depleted platelet units were irradiated with 25 Gy and transfused in two patients 2 days later. The donor developed a cutaneous rash, retro-orbital pain, and pain in both knees. Upon testing, the donor was found positive for CHIKV and DENV but tested positive for ZIKV, by RT-PCR, 2 weeks later. Routine tests revealed that the two recipients were negative on PCR assays for CHIKV, DENV and ZIKV prior to transfusion, but tested positive for ZIKV on day 6 (patient 1) and 23 (patient 2) post transfusion. Taken together, this suggests that platelets are susceptible to ZIKV infection and can also play a role in viral dissemination [157].

In a more recent study, Murray *et al.* found that erythrocytes are also permissive to ZIKV infection. A patient returning from Honduras developed signs of ZIKV infection including rash, fever, headache and conjunctivitis. After assessment, ZIKV RNA was found in serum up to day 8 after onset of illness and in body fluids including vaginal secretions up to day 14 post onset of symptoms. Interestingly, whole blood samples remained positive up to day 81. Ficoll was used to separate PBMCs and erythrocytes and only the erythrocyte fraction was positive for ZIKV RNA suggesting they are permissive to long-term viral infection [158].

The increased expression of ZIKV has resulted in the development of animal models to better characterise the pathogenesis of ZIKV disease. In particular, mice deficient in IFN-α and -β or IFN-α, -β and -γ (Ag129) were found to be susceptible to ZIKV infection [159, 160]. Aliota *et al.* identified that while there was no tissue damage observed in the majority of tissues examined, the most significant histopathological finding associated with ZIKV infection was observed in the brain [159]. In particular, neutrophil infiltration with abnormal neurons and glia were identified in hippocampal sections, increasing in density adjacent to the choroid plexus. Furthermore, the brain meninges were also noted to have increased numbers of neutrophils and mononuclear cells [159].

In another report, subcutaneous infection of newborn Swiss mice with a Brazilian strain of ZIKV leads to rare microglial cells in the spinal cord exhibiting positive immunolabelling for viral infection, whilst neurons remained negative [161]. There is much debate as to the origin of microglial cells, however, there seems to be a general consensus that microglia are derived from two sources: first, from an early embryonic source in the yolk sac; and, secondly, from myeloid precursors that subsequently take up residence in the central nervous system during embryonic development, forming a stable self-renewing population through adulthood effectively making them a myeloid cell [162].

**Summary and future directions**

ZIKV is of significant public concern due to its links with Guillain–Barré syndrome and severe neurological defects such as microcephaly in newborns of mothers infected with ZIKV. While the exact mechanism of ZIKV disease is still being investigated, it is likely that myeloid cells will play some role in its pathogenesis. This is due to ZIKV’s close relation to DENV with a similar albeit less severe form of clinical presentation. As described here, several myeloid cells such as erythrocytes, microglia and DCs have shown to be permissive for ZIKV infection. With increasing research, our current knowledge gaps in regards to disease mechanisms will inevitably improve. Currently, there are no vaccinations available, though clinical trials of a DNA vaccine commenced in 2017. Until a vaccine becomes available, prevention from ZIKV infection through mosquito bites or sexual intercourse is the best form of treatment.

**Conclusion**

EBOV, CHIKV, DENV and ZIKV have increased significantly in incidence over recent years. With the exception of EBOV, all other viruses are mosquito-transmitted and have been involved in numerous outbreaks worldwide. DENV and ZIKV are *Flaviviruses* while CHIKV is an *Alphavirus*. There has been a growing body of evidence that host myeloid cells are major players in the pathogenesis of these emerging zoonotic viral diseases. For the viruses discussed in this review myeloid cells, in particular macrophages, have been shown to play critical roles in the mechanism of disease. Whether it is by being a source of virus replication and amplification, induction of apoptosis/cell death or by amplifying the inflammatory response, macrophages play a significant role in the disease process and a thorough understanding of their role will provide new targets and investigation avenues for future therapeutic strategies. Given the large health burden of these infectious diseases, it is imperative that further research be continued in this area to eventually develop successful vaccination and treatment strategies.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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