Highlights

- Immunopathogenesis is thought to play a substantial role in DENV disease
- In recent years ZIKV outbreaks causing serious complications have occurred
- These outbreaks are occurring in areas of DENV transmission
- Sequence homology between ZIKV and DENV leads to cross-reactive immunity
- The consequences of ZIKV/DENV cross-reactivity require thorough investigation

The Immunopathology of Dengue and Zika Virus Infections

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Abstract

A large proportion of the world's population live in areas with dengue virus (DENV) transmission resulting in tens of millions of symptomatic dengue cases each year. Serious complications following DENV infection occur more frequently in those suffering from a second or subsequent infection implicating virus-specific immunity as having a role in pathogenesis. In recent years outbreaks of the related Zika virus (ZIKV) have been associated with birth defects and neurological complications. As DENV and ZIKV share a viral vector sequential infections can occur. Given the sequence homology between the two viruses, the generation of cross-reactive immune responses is highly likely. This review examines the role immunopathogenesis plays during DENV infection as well as highlighting recent studies that demonstrate DENV immunity may have an effect on the outcome of ZIKV infection.

Introduction

Since dengue virus (DENV) was first isolated in 1943 the global incidence of disease has increased year on year. Recent estimates suggest that 390 million DENV infections occur each year of which 96 million manifest clinically [1]. Transmission has been reported in 128 countries with a collective population of 3.9 billion, as a result over half the world's population are at risk of contracting dengue [2].

Decades of research into the pathogenesis of dengue have implicated the virus-specific immune response in contributing to severe symptoms. This has complicated the development of a safe and effective vaccine. Sanofi-Pasteur has recently developed a vaccine that has been licensed for use in several countries, but can only be administered to certain age groups. Furthermore, it remains contentious as to whether the vaccine is completely avoiding the induction of harmful immune responses [3,4].

Certain areas of DENV transmission have recently been experiencing outbreaks of the related Zika virus (ZIKV). Whilst initially found to cause relatively mild symptoms, current outbreaks of ZIKV have been linked to birth defects as well as serious neurological complications. As ZIKV and DENV share substantial sequence homology it will be important to establish if cross-reactive immune responses exist and how they might affect the outcome of both diseases.

Dengue virus

DENV is a member of the flavivirus family. It has a single-stranded positive sense RNA genome that is translated into a single polyprotein. This polyprotein is then cleaved into 3 structural proteins; capsid (C), Pre-membrane (PrM) and envelope (E) and 7 non-structural proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5.

DENV is transmitted via the bite of infected *aedes* mosquitoes, whose distribution restricts circulation of the virus to tropical and subtropical regions. DENV exists as four related serotypes (DENV1-4) each of which co-circulates in endemic areas. As a result of this heterogeneity immunity to one serotype can provide only short-lived protection from a subsequent infection with another serotype. Therefore secondary infections are common.

DENV causes a spectrum of disease ranging from dengue fever (DF), a self-limiting febrile illness, to dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS), which can be life threatening without medical intervention. DHF/DSS is characterised by increased capillary permeability leading to plasma leakage. In the absence of fluid replacement therapy, this can result in circulatory insufficiency, shock and organ failure.

Epidemiological evidence has demonstrated that severe complications from DENV infection occur more frequently in patients experiencing a secondary or subsequent infection [5,6]. Furthermore, whilst DHF/DSS are associated with increased viral loads [7], the critical phase of dengue disease manifests when the levels of virus are receding. This suggests that the DENV-specific immune response itself may contribute to disease severity.

The exact mechanism that leads to an increase in vascular permeability during DENV infection is incompletely understood. Aside from the temporal disconnect between the presence of virus and the critical phase of disease, a direct role for virus particles in pathogenesis is further brought into question by the absence of extensive endothelial cell damage[8]. The fall in viral load and the manifestation of severe symptoms instead coincide with an inflammatory 'cytokine storm' again implicating the immune response in pathogenesis.

This cytokine storm results in high circulating levels of many pro-inflammatory mediators such as interferon-γ (IFN-γ), tumour necrosis factor (TNF), soluble TNF receptor 1 (sTNFR1), sTNFR2, CXC-chemokine ligand 8 (CXCL8), CXCL9, CXCL10, CXCL11, CC-chemokine ligand 5 (CCL5) and vascular endothelial growth factor A (VEGFA), as well as the anti-inflammatory cytokine interleukin-10 (IL-10) [7,9-11]. The origins and effects of these molecules during DENV infection are not fully understood but they are thought to underpin a complex interplay between immune cells and other cell types such as endothelial cells that have a role in pathogenesis.

The role of DENV-specific antibodies in pathogenesis

Virion-specific antibodies

The most well characterised mechanism of immunopathology during DENV infection involves antibodies specific for proteins on the virion surface. PrM and E-specific antibodies generated during a primary infection may be poorly neutralising during a subsequent infection due to antigenic variation and insufficient titre (Figure 1).

This can lead to the opsonisation of the virus allowing for increased entry and replication in Fc receptor bearing cells such as macrophages, a major site of DENV replication. This mechanism of antibody dependent enhancement (ADE) has been demonstrated both *in vitro* [12-15] and *in vivo* [16-19]. In addition to enhancing viral replication, the interaction between opsonised virus and Fc receptors can lead to the induction of cytokines [20] that could further recruit inflammatory immune cells and contribute to the cytokine storm.

NS1-specific antibodies

Whilst NS1 does not form part of the dengue virion it is found on the surface of infected cells as well as in a secreted hexameric form. Soluble NS1 can disrupt endothelial cell monolayer integrity as well as inducing inflammatory cytokine release via an interaction with TLR4 [21-23]. Immunisation with NS1 has been shown to protect against these effects [21,24-27]. Immunogens to generate NS1-specific antibodies have been suggested as an alternative approach to vaccine design that would avoid ADE. However caution is required as they have also been implicated in disease pathogenesis.

Cross-reactivity between NS1-specific antibodies and proteins expressed on the surface of endothelial cells [28] as well as platelets [29] has been demonstrated. This could lead to both endothelial cell damage as well as platelet dysfunction and depletion both of which could contribute to the plasma leakage and thrombocytopenia that are characteristic of severe dengue. In addition the interaction between NS1-specific antibodies and endothelial cells could lead to enhanced viral replication [30] and the secretion of inflammatory cytokines [31].

Maternally derived antibodies

During the first year of life infants experiencing primary DENV infections can develop severe complications. It is suggested to that this is result of passively transferred maternal DENV-specific antibodies that have fallen below neutralising levels and are capable of mediating ADE [32-35]. Maternally derived DENV-specific antibodies may have the potential to cause ADE following ZIKV infection of young children. Similarly, passively transferred maternal ZIKV-specific antibodies could impact on DENV infection. This would have implications for vaccine design.

The role of DENV-specific T cells in pathogenesis

Given the temporal association of severe dengue symptoms with viral clearance and pro-inflammatory cytokine secretion it follows that virus-specific T cells could play a role in disease pathogenesis. Both CD4+ and CD8+ T cells are highly activated during acute infection and the magnitude of the overall response correlates with disease severity [36-39]. During secondary DENV infections virus-specific T cells expand rapidly and can constitute up to 20% of T cells in circulation [40].

Cross-reactivity towards multiple serotypes is a common feature of the DENV-specific T cell response. Some T cell populations are fully cross-reactive to antigens of the primary and secondary infecting viruses [39]. However, CD8+ T cells that preferentially recognise epitopes derived from the primary infecting serotype have been identified [39]. The expansion of cells generated during a primary infection but displaying a low avidity for the current infecting serotype may result in delayed viral clearance and higher viral loads.

In addition, the functionality of DENV-specific T cells has been observed to differ between mild and severe disease [36]. T cells isolated from DF patients were found to degranulate and produce lower levels of proinflammatory cytokines whilst T cells from DHF patients produced higher levels of inflammatory cytokines and were less effective at degranulation. This could have a detrimental effect on virus control as well as enhancing immunopathogenesis.

However, studies have shown DENV-specific T cells may be protective in both humans [41] and mice [42-44]. Moreover, it has been suggested that the suboptimal efficacy of the Sanofi-Pasteur DENV vaccine may be because it is not promoting a full anti-DENV T cell response[45]. This vaccine is comprised of the structural proteins of DENV inserted into the non-structural proteins of an attenuated yellow fever virus (YFV). The NS-specific T cells generated by this vaccine are consequently specific for YFV. Therefore, the role of virus-specific T cells in dengue pathogenesis remains contentious.

Zika virus

ZIKV was first isolated from a febrile non-human primate in the Zika Forest of Uganda in 1947 [46]. Originally believed to cause only a mild, self-limiting febrile illness similar to DF [47] recent outbreaks of ZIKV infection have been associated with more severe disease outcomes. An increase in ZIKV transmission in Brazil in 2015 was coincident with a rise in the occurrence of microcephaly [48,49], a birth defect resulting in a reduction in head size, causing several complications including developmental delay. In addition, ZIKV infection has also been associated with the neurological autoimmune disease, Guillain-Barré syndrome [50].

Guillain-Barré syndrome (GBS) is characterised by paralysis caused by neurological damage. Although GBS is a multi-faceted disease that can have distinct etiologies the most common is auto-reactive antibodies often triggered by an infectious agent [51]. The molecular mechanism behind GBS has been well elucidated for some pathogens such as *Campylobacter jejuni* whereby sugars comprising the bacterial lipopolysaccharide (LPS) elicit antibodies that cross-react to sugar components of GM1 gangliosides expressed on neuronal membranes [52]. Such mechanisms are yet

to be determined for ZIKV and it is still unclear whether GBS triggered by ZIKV is the result of direct viral damage to neural cells or the result of the generation of auto-antibodies.

Like DENV, ZIKV is an arthropod-borne virus belonging to the flavivirus family and is transmitted by the bite of *aedes* mosquitoes [53]. In contrast to DENV, ZIKV can also be transmitted by the aforementioned perinatal route as well as via sexual contact [54,55]. The two viruses share sequence homology of around 43% across the polyprotein and are structurally very similar. These apparent similarities between DENV and ZIKV give rise to important questions. Can pre-existing DENV-specific immune responses cross-react with ZIKV? And if so, what role might these responses play in Zika disease pathogenesis or protection?

ADE and ZIKV

As ZIKV appears to exist as a single serotype [56] multiple infections such as those caused by DENV are not thought to occur. However, as discussed above ZIKV and DENV share the same vector and individuals living in endemic areas have been found to experience sequential infections with both viruses [57]. Furthermore, cross-reactive antibodies can be generated [57-63]. It is of importance to understand what impact this may have on the outcome of both infections.

Studies have shown that DENV-specific antibodies are able to enhance infection of Fc receptor bearing cells with ZIKV *in vitro* [58,60,64,65]. In a mouse model of ZIKV infection adoptive transfer of DENV convalescent plasma enhanced pathogenesis [65]. However, whether or not this mechanism is at play during natural infection requires further research. DENV-specific antibodies are capable of neutralising ZIKV infection *in vitro* [57-59,62]. Furthermore, a monoclonal antibody isolated from a dengue patient has been found to protect mice from a lethal ZIKV challenge [62].

One fundamental question that is yet to be answered and is central to whether or not DENV-specific antibodies can mediate ADE during ZIKV infection is which cell types provide the major site of replication for the virus. If Zika predominantly replicates in non-Fc receptor bearing cells then DENV-specific antibodies are unlikely to have a major impact on pathogenesis. There is mounting evidence to suggest that the tissue tropism of ZIKV is distinct from that of DENV [66].

ZIKV and DENV cross-reactive T cells

Studies investigating ZIKV-specific T cells are sparse at present but have found evidence of cross-reactivity to DENV. One study involving humanised mice identified T cells specific for several epitopes that were either ZIKV-specific or cross-reactive between ZIKV and DENV. Both types of T cells were found to be protective following ZIKV challenge [67].

A study involving ZIKV infected humans found memory T cells specific for NS1 and E to be poorly cross-reactive with DENV even in those individuals that had experienced a previous DENV infection [57]. However, *in silico* analysis has identified T cell reactivity conserved across several flaviviruses including ZIKV predominantly found in the NS3 and NS5 proteins [68]. Substantial further research is required to establish the role T cells play during ZIKV infections and whether or not DENV-cross-reactive T cells can contribute to pathogenesis or protection.

Conclusion

Immunopathogenesis is a substantial contributing factor to DENV disease. Due to their co-circulation individuals can be exposed to both DENV and ZIKV potentially resulting in sequential infections. The sequence homology of the viruses is likely to lead to the generation of cross-reactive immune responses, some of which have already been identified. A DENV vaccine is currently licensed for use in several countries experiencing ZIKV outbreaks. Furthermore, much effort is being applied to the development of a ZIKV vaccine. Gaining a comprehensive understanding of the effect

DENV-immunity has during ZIKV infection and vice versa is crucial for the design of safe vaccine strategies.

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Figure legends

Figure 1 Cross-reactive DENV/ZIKV-specific immune responses have the potential to affect the outcome of both diseases. Immunopathogenesis has been implicated in contributing to the severe symptoms associated with DENV infection. Epidemiological evidence shows poor disease outcomes are associated with secondary infections and mechanisms involving both antibodies and T cells have been investigated. Due to the co-circulation and genetic relatedness of ZIKV and DENV it is of importance to understand what impact immune responses to one of these viruses will have on the outcome of infection with the other.

Primary DENV infection

DENV-specific immune response: Antibodies T cells

Secondary DENV infection

Neutralising antibodies
Effective T cells

Protection

Pathogenesis

Enhancing antibodies
suboptimal T cells

ZIKV infection

Cross-recognition of ZIKV by DENV-specific immunity

Protection or pathogenesis?

ZIKV infection

ZIKV-specific immune response: Antibodies T cells

DENV infection

Cross-recognition of DENV by ZIKV-specific immunity

Protection or pathogenesis?