



Review

The dengue virus non-structural 1 protein: Risks and benefits

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ABSTRACT

The dengue virus (DENV) non-structural 1 (NS1) protein plays a critical role in viral RNA replication and has a central position in DENV pathogenesis. DENV NS1 is a glycoprotein expressed in infected mammalian cells as soluble monomers that dimerize in the lumen of the endoplasmic reticulum; NS1 is subsequently transported to the cell surface, where it remains membrane associated or is secreted into the extracellular milieu as a hexameric complex. During the last three decades, the DENV NS1 protein has also been intensively investigated as a potential target for vaccines and antiviral drugs. In addition, NS1 is the major diagnostic marker for dengue infection. This review highlights some important issues regarding the role of NS1 in DENV pathogenesis and its biotechnological applications, both as a target for the development of safe and effective vaccines and antiviral drugs and as a tool for the generation of accurate diagnostic methods.

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1. The impact of dengue virus (DENV) on public health

Dengue virus (DENV) belongs to the *Flavivirus* genus of the Flaviviridae family and exists as four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4 (Lindenbach and Rice, 2001). These serotypes are arboviruses (*arthropod-borne viruses*) transmitted by mosquitoes of the *Aedes* genus (Center for Disease Control, 2012). In humans, DENV may cause an acute febrile illness that

is not life threatening, which is called dengue fever (DF), or more severe forms of the disease, known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). These severe forms are life threatening, causing vascular leakage that may lead to death (WHO, 1997). Dengue infection represents a major worldwide public health concern, affecting billions of people living in tropical and subtropical areas (Kalluri et al., 2007; Whitehead et al., 2007; Bhatt et al., 2013); globally, billions of people are exposed to DENV infection, and thousands die each year. A recent report estimates 390 million dengue infections per year, with 96 million leading to sufficient severity to alter the individual's regular routine (Bhatt et al., 2013).

Previous studies estimate that, among the reported apparent dengue infections, at least 500,000 cases result in severe symptoms,

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including DHF and DSS (Gubler and Meltzer, 1999; Pongsumpun et al., 2008; Guzman et al., 2010). The mortality rates of these groups would be approximately 10% for hospitalized patients and approximately 30% for non-hospitalized patients (Gubler and Meltzer, 1999; Pongsumpun et al., 2008). These data clearly point to the urgent need for a more complete understanding of DENV pathogenesis, which could lead to the discovery of new control strategies for this pathogen.

2. Importance of the non-structural 1 (NS1) protein in the DENV life cycle

DENV is an enveloped virus, and its genome is composed of a positive-sense, single-stranded RNA coding for three structural proteins (C, capsid; prM, pre-membrane; and E, envelope) present in the virion and infected cells and seven non-structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) not present in the virion (Whitehead et al., 2007). Most of the DENV proteins contain signal peptides and/or hydrophobic anchors that direct the protein to a precise location in the host cell that is suitable for viral replication (Lindenbach and Rice, 2001). DENV NS1, the focus of this review, is a 43–48-kDa glycoprotein that is expressed in infected mammalian cells as soluble monomers that dimerize in the lumen of the endoplasmic reticulum (Winkler et al., 1988; Flamand et al., 1999; Young et al., 2000; Zhou et al., 2006). The NS1 protein is subsequently transported to the cell surface where the protein remains membrane associated or is released into the extracellular milieu as a hexameric form. The proper processing of the NS1 protein requires a signal sequence in the C-terminus of the DENV envelope glycoprotein (Falgout et al., 1989; Falgout and Markoff, 1995), and this processing was shown to be significantly disturbed following the alteration of N-linked glycosylation sites (Pryor and Wright, 1994; Flamand et al., 1999). Although the functions of DENV NS1 have not yet been fully elucidated, experimental evidence indicates that the protein is involved in RNA replication (Mackenzie et al., 1996; Muylaert et al., 1996; Lindenbach and Rice, 1997; Lindenbach and Rice, 2001; Sampath and Padmanabhan, 2008). The functions of the extracellular forms of DENV NS1 are also not clear, though a specific involvement in pathogenesis and immune evasion mechanisms has been proposed.

A recently published review comprehensively explores the physiological role of the DENV NS1 protein and also describes the attempts to utilize this protein as a target for preventive, therapeutic, and diagnostic approaches (Muller and Young, 2013). We strongly recommend that review article to those interested in further details regarding the fascinating history of this protein and the efforts of those dedicated to unveil some of its intriguing features. Herein, we present a considerably shorter review on the DENV NS1 protein in which we offer our own points of view concerning the relevant aspects that continue to raise doubts with regard to the risks and benefits of using this protein, particularly as a target for anti-dengue vaccines.

3. Proposed roles of the NS1 protein in pathogenesis and in immune response evasion

Although the cellular and molecular mechanisms involved in DHF and DSS etiology remain elusive, the current hypotheses associate the major functions of NS1 with the dysfunction of host immune defense and defects in the circulatory system that would be caused by the generation of cross-reactive antibodies, leading to platelet depletion, endothelial cell apoptosis, and complement activation, with damage to host tissues (see Table 1) (Lin et al., 2002; Lin et al., 2006; Kurosu et al., 2007; Martina et al., 2009; Chen et al., 2009; Falconar and Martinez, 2011). Accordingly,

much of the participation of NS1 in DHF and DSS pathogenesis is attributed to autoimmune events (see Fig. 1). The dengue NS1 protein reportedly shares common epitopes with human blood-clotting integrin-adhesin proteins and endothelial cell surface proteins (Falconar, 1997). This molecular mimicry can be attributed to the conservation of ELK/KLE-type motifs in the dengue NS1 protein sequence, which are broadly found in different mammalian proteins (Falconar, 2007). Therefore, antibodies generated against the NS1 protein would cross-react with host proteins and thereby lead to autoimmune damage of the host tissues.

One of the most important proposed autoimmune events involving the NS1 protein is the binding of anti-NS1 antibodies to the platelets and proteins of the coagulation cascade (Fig. 1A). Anti-NS1 antibodies were shown to recognize protein disulfide isomerase on platelets and inhibit platelet aggregation (Cheng et al., 2009). In addition, these antibodies were shown to bind to prothrombin and thrombin and to inhibit prothrombin activation (Lin et al., 2012). Moreover, a study performed in mice showed that antibodies against the NS1 protein bind to human platelets, inhibiting their aggregation and causing a bleeding tendency (Chen et al., 2009); however, these effects disappear after the removal of the C-terminal region of the protein (Chen et al., 2009). Anti-platelet and anti-endothelial cell autoantibodies were also found to be present at higher levels in DHF/DSS patient sera than in DF patient sera (Lin et al., 2001, 2003). In a complementary study, mice infected with a DENV clinical isolate and showing high titers of anti-NS1 antibodies were reported to have significantly compromised coagulation function (Amorim et al., 2012a). Taken together, such studies indicate that anti-NS1 antibodies may be involved in the development of the hemorrhagic events observed in the severe forms of DF, a disease in which platelet numbers and coagulation pathways are often compromised (WHO, 1997; Halstead, 2007; Whitehead et al., 2007).

Another proposed mechanism that could be related to the severity of dengue infection symptoms is related to autoimmune endothelial cell damage (Fig. 1B). The NS1 protein and antibodies generated against this protein bind to the surface of endothelial cells (Lin et al., 2002; Avirutnan et al., 2007). Thus, as a first step in cell damage, NS1 that is bound to the surface of endothelial cells would be a target for anti-NS1 antibodies that would then bind to the NS1 protein and trigger the complement cascade, leading to cell damage. As a second putative damage pathway, it was proposed that anti-NS1 antibodies cross-react with structures present on endothelial cells and induce the production of nitric oxide (NO) (Lin et al., 2002). The production of NO would then lead to the upregulation of the p53 and Bax genes and the downregulation of B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma extra large (Bcl-xL) gene expression, resulting in cytochrome c release and caspase-3 activation and thereby causing endothelial cell apoptosis. Different pathways causing the death and disruption of endothelial cells, such as apoptosis, could be involved in the plasma leakage observed in DSS cases because this tissue is responsible for limiting the permeability of vessels to plasma and blood cells. However, these observations were based on *in vitro* assays and may not necessarily correspond to the *in vivo* conditions, particularly among patients recovering from DSS. In these patients, vascular permeability often reverses rapidly and spontaneously (WHO, 1997; Gubler, 1998; Rajapakse, 2011), suggesting that endothelial destruction is not a feature of DSS. Additional studies on the role of anti-NS1 antibodies in the induction of endothelial tissue damage are required before a definitive conclusion can be drawn concerning this issue.

The NS1 protein also appears to contribute to dengue pathogenesis through immune system evasion mechanisms. NS1 was recently shown to antagonize the C4 complement component, leading to the protection of DENV particles from complement-dependent neutralization (Avirutnan et al., 2010; Avirutnan et al., 2011). According to this study, the NS1 protein enhances C4

Table 1

Roles of anti-NS1 protein antibodies in DENV pathogenesis.

Contribution to Pathogenesis	References
Anti-NS1 antibodies recognize protein disulfide isomerase on platelets and inhibit platelet aggregation.	Cheng et al., 2009.
Anti-NS1 antibodies were shown to bind to prothrombin and thrombin and to inhibit prothrombin activation.	Lin et al., 2012.
Anti-NS1 antibodies that target platelets and endothelial cells are present at high levels in DHF/DSS patient sera.	Lin et al., 2001, 2003
Anti-NS1 antibodies may recruit the complement system against endothelial cells.	Avirutnan et al., 2007.
Anti-NS1 antibodies bind to endothelial cells and induce apoptosis.	Lin et al., 2002.
The NS1 protein protects DENV viral particles from complement system-dependent neutralization.	Avirutnan et al., 2010, 2011

cleavage by recruiting and activating the complement-specific protease C1s; NS1 promotes the efficient degradation of C4 into C4b following the binding of C1s to C4 in a complex. The consequence of this evasion mechanism would be the enhancement of dengue viral loads and disease severity (Fig. 1C).

The present literature supports, at least in part, a significant contribution by NS1 to the development of the disease, leading to increases in the viral load, the enhancement of inflammatory responses, tissue damage, and the interference of coagulation pathways. Indeed, NS1 may be involved in all of these pathological events and would thus play an important role in the development of the more severe forms of the disease. Although vaccine approaches using the NS1 protein as a target antigen have shown promising results, doubts have also been raised about the generation of deleterious immune responses.

4. Dengue NS1 protein as a vaccine target

Within the context of the DENV life cycle, the NS1 protein represents a target for vaccine development because infected cells present both the full-length NS1 protein associated with the plasma membrane and NS1 peptides presented by MHC class I molecules. The full-length protein represents a target for antibodies, which may recruit complement proteins or effector cells to kill the infected cell. Additionally, NS1 protein epitopes associated with MHC I molecules are targets for T cells (see Fig. 2). Thus far, a considerable number of subunit vaccine approaches based on the NS1 protein (either DNA, recombinant viruses, or purified proteins) have been investigated under nonclinical conditions (Table 2).

In 1987, it was reported for the first time that immunization with purified NS1 protein recovered from infected Vero cells

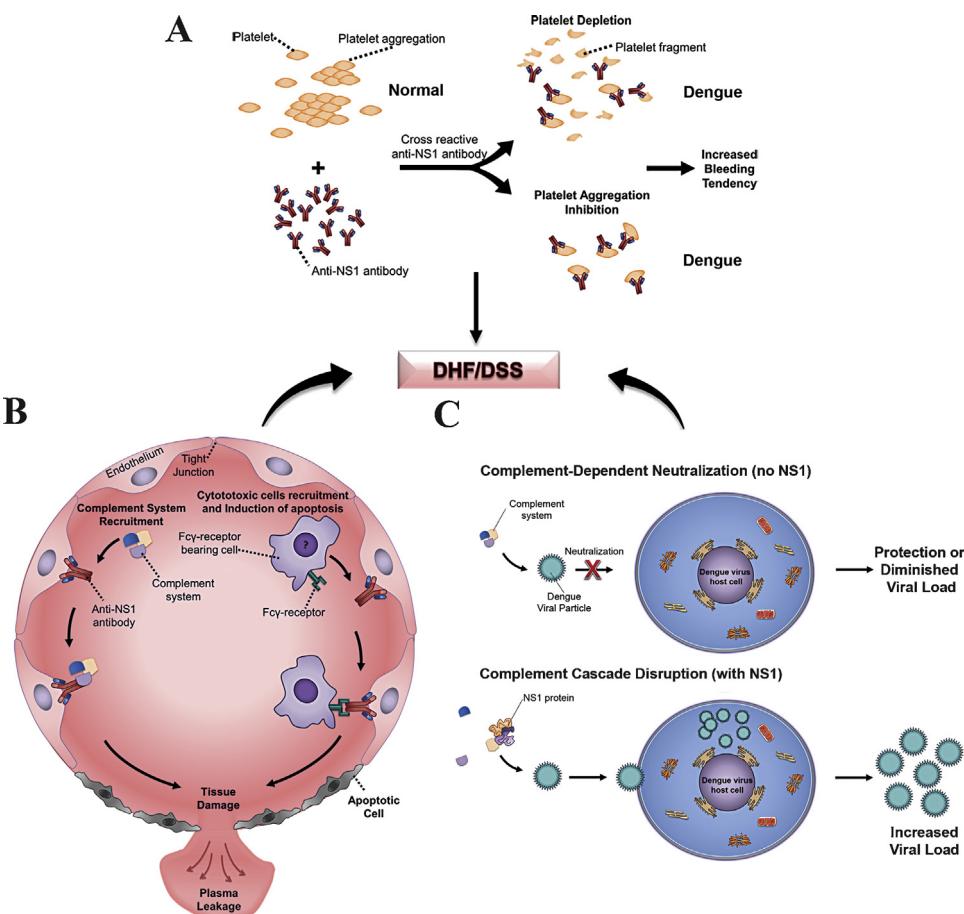


Fig. 1. Involvement of the NS1 protein in DENV pathogenesis. The NS1 protein shares common epitopes with human platelets and endothelial cell surface proteins. (A) This molecular mimicry allows anti-NS1 antibodies to bind to platelets and inhibit their aggregation, which increases bleeding tendency. (B) Anti-NS1 antibodies may also induce autoimmune endothelial cell damage in blood vessels and plasma leakage. (C) In addition, the NS1 protein contributes to an increased viral load by disrupting the complement system, leading to the protection of DENV particles from complement-dependent neutralization. Together, these mechanisms are believed to significantly contribute to the more severe forms of DENV pathogenesis (DHF and DSS).

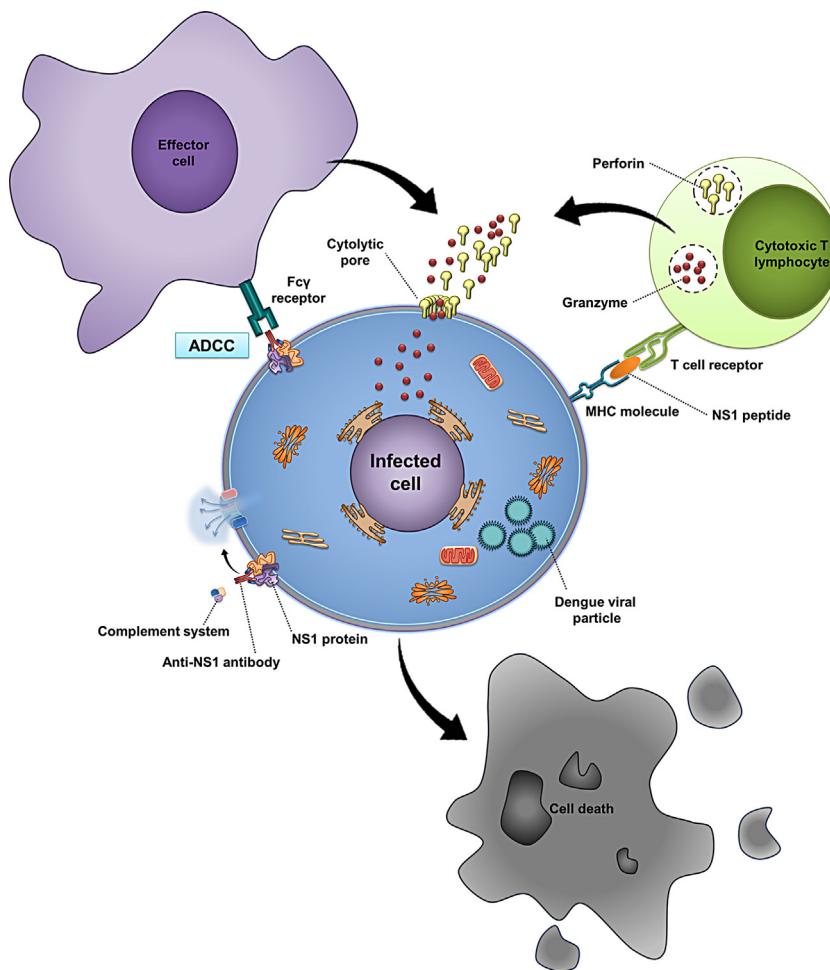


Fig. 2. The DENV NS1 protein is a target for immune responses induced by vaccine approaches. Infected cells present the full-length NS1 protein at the cytoplasmic membrane, which may be targeted by specific antibodies. These antibodies may recruit the complement system or effector cells, which may induce complement-dependent cell lysis or antibody-dependent cell-mediated cytotoxicity (ADCC), respectively. In addition, NS1 protein epitopes may be processed and presented by MHC I receptor molecules on the infected cell surface, representing a target for cytotoxic T cells.

could confer protective immunity to mice (Schlesinger et al., 1987). In the subsequent year, Zhang and colleagues demonstrated that the immunization of mice with dengue structural proteins and a recombinant NS1 protein expressed in eukaryotic cells induced resistance to the encephalitis caused by an intracranial challenge with DENV (Zhang et al., 1988). Additionally, mice passively acquiring anti-NS1 antibodies were also protected against DENV challenge. The most protective anti-NS1 antibodies were also shown to be capable of binding to complement

components (Henchal et al., 1988). Protective immunity was again achieved in 1993 with the use of a DENV NS1 protein produced in eukaryotic cells (Qu et al., 1993). Two years later, a fusion protein comprising NS1 and the envelope glycoprotein expressed in *Escherichia coli* was used in a vaccine formulation and induced protective immunity in mice (Srivastava et al., 1995). Based on these pioneering works, the use of recombinant forms of the NS1 protein appears to be a promising target for vaccines against DENV infection.

Table 2

The DENV NS1 protein as a target for antiviral and vaccine approaches.

Antiviral or vaccine	References
Compound 6-O-butanoyl castanospermine induces the misfolding and accumulation of NS1 in the endoplasmic reticulum of infected cells.	Rathore et al., 2011.
Compound 6-O-butanoyl was found to be protective in lethal challenge experiments performed in mice.	Watanabe et al., 2012.
Compound kotalanol and its de-O-sulfated derivative were shown to decrease DENV replication under <i>in vitro</i> conditions.	Mohan et al., 2012.
NS1-based DNA vaccines induced protective immunity involving both antibody and T cell responses.	Falgout et al., 1990; Wu et al., 2003; Costa et al., 2006, 2007.
NS1-based subunit vaccines induce the generation of antigen-specific serum antibodies and partial or complete protection against DENV infection.	Schlesinger et al., 1987; Henchal et al., 1988; Zhang et al., 1988; Qu et al., 1993; Srivastava et al., 1995; Amorim et al., 2012b; Huang et al., 2013.
NS1 protein targeting to DEC205 ⁺ dendritic cells induced partial protection against DENV infection, which appeared to be largely mediated by T cells.	Henriques et al., 2013.

However, as mentioned in the previous section, the cross-reactivity of anti-NS1 antibodies with proteins of the coagulation cascade (Falzarano, 1997) and its ability to cause platelet depletion and endothelial tissue damage (Lin et al., 2001; Lin et al., 2002; Lin et al., 2003; Lin et al., 2006) have resulted in a loss of interest in the use of NS1 in the development of vaccine formulations. Nonetheless, data obtained with experimental recombinant viruses and DNA-based vaccines have further confirmed that DENV NS1 is a promising and safe antigen target (Falgout et al., 1990; Wu et al., 2003; Costa et al., 2006, 2007).

DNA and recombinant virus-based vaccines were shown to induce protective immunity that involved both antibody and T cell responses (Falgout et al., 1990; Wu et al., 2003; Costa et al., 2006, 2007). In contrast, the protective immunity generated in mice immunized with purified NS1 protein appeared to be mainly based on the generation of antigen-specific serum antibodies (Schlesinger et al., 1987; Zhang et al., 1988; Henchal et al., 1988; Srivastava et al., 1995; Amorim et al., 2012b; Huang et al., 2013). As the immune mechanisms leading to protection in these reports have not been elucidated to date, investigations involving the depletion of specific immunological cell populations and the passive transfer of antibodies would be important to determine the precise function that each branch of the immune system plays in the generation of protective and deleterious immune responses. Although such studies could provide new insight into the immune mechanisms induced by immunization with the DENV NS1 protein, considerable caution should be taken in interpreting any link to protection mediated by different arms of the immune response in an animal model using intracranial challenge. Indeed, this challenge route is highly artificial and does not reproduce the disease typically observed in humans, even though it is widely employed due to the lack of a suitable animal model to evaluate anti-DENV vaccine candidates.

Interestingly, none of these studies reported autoimmune events following immunization with NS1-based vaccines. In fact, our own results demonstrate that the immunization of mice with recombinant NS1 co-administered with different adjuvants did not induce hematological disturbances or the alteration of coagulation pathways that could lead to a bleeding tendency in the vaccinated animals (Amorim et al., 2012b). In addition, none of the biochemical markers applied for evaluating the possible tissue damage and inflammatory reactions caused by immunization with the recombinant NS1 protein were altered. Thus, it remains unclear how anti-NS1 antibodies may be linked to the generation of autoimmune responses and how the factors involved in the generation of immune responses may lead to the exacerbation of or protection against the more severe symptoms of the disease. Anti-DENV vaccines based on inactivated viruses do not express the NS1 protein; such viruses do not replicate, and DENV non-structural proteins are therefore not presented to the immune system (Whitehead et al., 2007). Conversely, DENV attenuated vaccines do express the NS1 protein, though the role of NS1-specific immune responses in the protection induced by such vaccine formulations needs to be determined.

In fact, we recently demonstrated that targeting the DENV NS1 protein to the DEC205 receptor-bearing dendritic cell (DC) population confers CD4⁺ and CD8⁺ T cell-mediated protection (Henriques et al., 2013). Additionally, the NS1 protein genetically fused to a monoclonal antibody specific for DEC205⁺ DCs was properly targeted to this presenting cell population, which is known to cross-present exogenous antigens on MHC class I molecules (den Haan et al., 2000; Pooley et al., 2001; den Haan and Bevan, 2002). Experiments performed to specifically deplete CD8⁺ or CD4⁺ T lymphocytes showed that the immunological protective state involves both of these lymphocyte populations (Henriques et al., 2013). We previously demonstrated that the partial protection achieved after immunization with a recombinant NS1 protein can mainly

be ascribed to antigen-specific antibodies (Amorim et al., 2012b), which is in accordance with the demonstration that anti-NS1 antibodies passively administered to mice could protect animals intracranially challenged with DENV by supporting the binding of complement components (Henchal et al., 1988).

Based on these observations, it appears that the protective immunity induced against DENV NS1 is multifactorial. NS1-associated protective immunity would involve CD4⁺ T lymphocytes capable of secreting antiviral cytokines, such as interferon- γ , and CD8⁺ T lymphocytes that recognize and kill infected cells presenting NS1 epitopes associated with MHC I molecules. In addition, anti-NS1 antibodies would recognize the full-length NS1 protein exposed on surface of infected cells and deploy the complement cascade pathway or Fc γ -receptor-bearing cells capable of killing the infected cells. Once the precise protection mechanisms specific for the NS1 protein that are induced during DENV infection are elucidated, other NS1-based vaccine formulations could be designed to induce similar immune response patterns. Regardless, any efforts in this field will significantly improve the rational design of anti-DENV vaccines.

5. Dengue NS1 as a target for antiviral drugs

As mentioned above, NS1 is important in DENV RNA replication and thus represents a putative target for chemotherapy. Current reported examples of antiviral drugs targeting the NS1 protein are related to interference with the proper N-glycosylation of the protein, which is required for its biological activity (Table 2). Accordingly, sulfonium-ion glycosidase inhibitors have been studied with the aim of decreasing DENV replication. Recently, it was reported that the compound 6-O-butanoyl castanospermine was capable of inducing the accumulation of misfolded proteins in the endoplasmic reticulum of infected cells, leading to decreased viral replication. In addition, this same compound, the antiviral effect of which is attributed to inhibition of the NS1 protein N-glycosylation, was shown to be protective under *in vivo* conditions in mice (Whitby et al., 2005; Rathore et al., 2011; Watanabe et al., 2012). In addition, two mammalian naturally occurring intestinal α -glucosidase inhibitors, kotalanol and its de-O-sulfated derivative, were shown to decrease DENV replication under *in vitro* conditions (Mohan et al., 2012). These reports clearly show that natural molecules produced by mammalian metabolism may target the DENV NS1 protein.

6. Roles of NS1 in dengue diagnosis

DF has a broad spectrum of clinical symptoms, some of which are mostly similar to the symptoms induced by different acute infections or illnesses; hence, an accurate differential diagnosis is challenging. The detection of DENV particles in cell cultures has the highest specificity yet involves a relatively risky, laborious, and time-consuming procedure. In addition, the period during which DENV particles circulate in the blood is brief (Vaughn et al., 1997). Although the detection of the viral genome provides a rapid, sensitive diagnosis, such an assay is labor intensive and expensive, and the sensitivity of such genetic assays decreases after the onset of symptoms due to the low viremia during this period (Lanciotti et al., 1992). Alternatively, serological assays based on the detection of IgM and/or IgG using enzyme-linked immunosorbent assays (ELISAs) have been described and represent recommended diagnostic alternatives for DENV infections (TDR/WHO, 2009; PAHO, 1994; Groen et al., 2000). However, cross-reactivity with other flaviviruses due to prior infection or immunization with flavivirus-based live vaccines has been demonstrated to interfere with the results (Koraka et al., 2002).

The NS1 protein is secreted by DENV-infected cells, and the soluble forms of the protein can be detected in the bloodstream from the first day after the onset of symptoms until day 9, a time when the clinical phase of the disease is complete, with NS1 being detected in levels up to 15 µg/ml (Young et al., 2000). This protein can also be detected during periods in which the viral RNA is not detectable by RT-PCR and IgM antibodies specific for structural proteins are not yet circulating (Alcon et al., 2002). However, in a secondary DENV infection, the immune complexes formed by NS1 and antibodies increase rapidly via an anamnestic immune response; thus, the antigen is rarely found at 5–7 days after the onset of symptoms in secondary DENV-infected patients (Vazquez et al., 2010).

The evaluation of NS1 as a diagnostic marker in early studies (Young et al., 2000; Alcon et al., 2002) led to the development of a large number of dengue diagnostic kits and approaches using this protein as the analyte (Chaiyaratana et al., 2009; Lemes et al., 2005; Fry et al., 2011; Blacksell et al., 2012; Muller et al., 2012). Circulating NS1 is also a useful target for the rapid and early diagnosis of DENV infection; hence, NS1 capture ELISA may also be a good confirmatory test for the early detection of DENV (Zainah et al., 2009). Although the diagnosis of DENV acute infection using NS1 strips has been shown to be a good first-line test for DF (Dussart et al., 2008), some reports have shown that sensitivity can be different for DENV serotype-2 infections (Chaterji et al., 2011; Hang et al., 2009; Ramirez et al., 2009). To improve the sensitivity of dengue diagnostic kits, some authors have hypothesized that a combination of approaches to detect the circulating antigen and anti-NS1 antibodies will increase the sensitivity and reliability of DENV infection diagnosis (Zainah et al., 2009; Hang et al., 2009; Ramirez et al., 2009). In addition, it was shown that the combination of dengue NS1 antigen detection with anti-glycoprotein E IgM and IgG serology can significantly increase the sensitivity of acute dengue diagnosis (Fry et al., 2011), thereby enabling the diagnosis of early acute positive samples and making this test more useful for the clinical screening of DENV infection cases.

7. Concluding remarks

The dichotomy of considering the DENV NS1 protein a major player in pathogenesis and a promising vaccine antigen still persists after almost three decades of debate. Furthermore, it remains unclear why anti-NS1 antibody levels are usually detectable at high levels for many months after DENV infection, though there is no evidence of clinical abnormalities related to these antibodies in individuals who have recovered from the infection. Most of the above-mentioned studies reporting evidence of deleterious autoimmunity mechanisms with anti-NS1 antibodies were conducted *in vitro*, with no correlations with the observations in DENV-infected patients. In addition, the reports showing that anti-NS1 antibodies cross-react with platelets and interfere with the coagulation pathway were generally under highly inflammatory conditions, both in humans and mice. It is important to note that highly inflammatory conditions, such as those generated in mice after the administration of several doses of the protein in the presence of Freund's adjuvant or in human DHF cases, may result in the induction of antibodies with altered properties that could be deleterious to the host metabolism. Moreover, elevated anti-NS1 antibody levels in patients with DHF appear to reflect enhanced viremia, which is significantly higher in the severe forms of the disease (Vaughn et al., 2000). An elevated viremia will obviously result in the increased expression of NS1 and thus in elevated anti-NS1 antibody levels. Regardless, this condition is not found in patients with DF, which may explain why anti-NS1 antibodies capable of cross-reacting with platelets and proteins of the coagulation

cascade are found only in patients with severe dengue infections. Although cross-reactivity has been observed *in vitro*, it is not clear how anti-NS1 antibodies contribute to DHF in humans, as significant levels of these antibodies are found many months after severe dengue infection in patients who have recovered, with no evidence of hemorrhagic events. In addition, we found that high levels of anti-NS1 antibodies in mice immunized with the NS1 protein do not induce any hematological disturbance (Amorim et al., 2012b).

The NS1 protein is one of the most conserved proteins among the four DENV serotypes. In this review, we discuss several studies that have demonstrated that DENV replication might be disrupted by targeting NS1. Despite conflicting evidence regarding the generation of autoimmune responses, there is growing interest in the use of the NS1 protein as a target for anti-DENV vaccines. Indeed, recent evidence on the generation of NS1-dependent protective immunity based on recombinant proteins or DNA vaccines, without the concomitant triggering of autoimmune responses, has inspired renewed interest in the development of safe and effective anti-DENV vaccines. Additional advances in the production of recombinant NS1 protein, with structural and immunological features similar to the features of the protein produced by the virus, will help in the development of drugs and vaccines that may interfere with viral replication. Along these lines, the conserved nature of NS1 among the four DENV types and the design of a consensus version of the protein could further boost vaccine research focused on the proper control of the disease caused by the four DENV serotypes.

In conclusion, we believe that, in a period in which new preventive approaches against dengue fever are urgently needed and investigated by companies and research institutes, the NS1 protein should be considered an important player. Within this context, as previously noted, the DENV NS1 may play an important role in the discovery of innovative, safe, and effective approaches for the control of this disease and may help us to better understand DENV biology.

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