Anti-Idiotype Antibody against Pre-Membrane-Specific Antibody as an Adjunct to Current Dengue Vaccination Strategy

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Abstract:
Dengue is a rapidly emerging vector-borne viral disease of humans transmitted by mosquitoes of the genus *Aedes*. Dengue viruses are divided into five antigenically distinct serotypes, DENV-1 to -5. The disease is endemic in over 130 countries, placing almost half of the world's population at risk. Clinical disease presents as either a mild self-limiting infection or severe complications. Recovery from primary infection by one serotype provides life-long immunity against reinfection by that particular serotype whereas with subsequent infections by other serotypes the risk of developing severe dengue is increased. In contrast to previous understanding that immature dengue virus particles are non-infective it was shown recently that they become highly infectious in the presence of antibodies raised to the pre-membrane protein, prM, of the virion. While no licensed dengue treatment is currently available, several prototype vaccines are being evaluated in clinical studies. Most of these vaccine candidates contain native dengue prM, the presence of which can have the opposite effect to that desired by making immature dengue particles infective. This occurs through a mechanism of prM-specific antibody-dependent enhancement of infection. Hence, in order to safeguard patient welfare when designing future dengue vaccine constructs, provision of another anti-idiotype antibody that binds to and blocks the pathogen-activating region of anti-prM antibody, thus rendering it inactive, should be considered as an adjunct therapy. This strategy would have a potentially significant benefit by reducing cases of secondary infection, which is the major cause of dengue morbidity and mortality.

Keywords: dengue; virus; immunity; vaccine; adjunct; serotype; antibody; idiotype.

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Introduction

Dengue is one of the most common arthropod-borne emerging viral infections in the world, endemic throughout South East Asia, Africa, the Americas, Western Pacific and Eastern Mediterranean [1]. Dengue virus (DENV) belongs to the *Flavivirus* genus of the Flaviviridae family which also includes West Nile, Yellow Fever, Japanese Encephalitis and tick-borne encephalitis viruses [2]. Based on differences in antigen neutralization tests DENV is divided into five different serotypes, DENV-1 to DENV-5 [3], the last of which was discovered only very recently [4,5]. Unlike DENV 1-4 which circulate between humans and *Aedes* mosquitoes, DENV-5 follows a sylvatic cycle involving non-human primates and the vector [5]. From this evidence, it may be assumed that further uncharacterized dengue serotypes are circulating in the forest environment and a comprehensive phylogenetic analysis is required to reveal them.

Global burden

The spread of dengue in recent decades has been so rapid that today over 130 nations are officially endemic for the disease, such that 40% of the world's population lives under threat of acquiring infection. With 50-100 million clinical cases reported annually, mostly in tropical and sub-tropical regions, the incidence of dengue has escalated 30-fold over the last 50 years [6]. This is attributed not only to a gradual extension of the geographical range of the mosquito vector that is facilitated by global warming but also to periodic explosive epidemics of infection. An estimated 500,000 people with severe dengue require hospitalization each year, leading to at least 20,000 deaths, a large proportion of which are of children, [6,7]. The true numbers are probably far worse, since significant underreporting and misclassification of dengue cases have been documented [8].

Spectrum of disease

Dengue is transmitted mainly by *Aedes aegypti* and *A. albopictus* mosquitoes, which show a preference for biting during daylight [9]. Infection causes a severe influenza-like illness and sometimes leads to a potentially lethal complication called severe dengue. This occurs most often in Asian and Latin American countries where it has become a leading cause of juvenile hospitalization and mortality [6]. As for many viral infections including influenza, recovery from infection by one DENV serotype provides life-long sterilizing immunity to that particular serotype. Of note, however, following recovery cross-immunity to the other four serotypes is only partial and temporary [10]. Importantly, perhaps paradoxically any subsequent infections by heterologous serotypes actually increase significantly an individual's risk of developing severe dengue [11]. Severe manifestations include dengue haemorrhagic fever or dengue shock syndrome, which are marked by severe abdominal pain, persistent bloody vomiting, rapid breathing, bleeding gums, fatigue and restlessness. Such a condition may be fatal due to plasma leakage, fluid accumulation, respiratory distress, severe bleeding and/or organ impairment. In some regions, in patients with severe dengue fatality rates can exceed 10% but may be reduced to below 1% with proper case management [7].

Immunopathogenesis

The adaptive immune response, inflammatory mediators and autoimmunity are all important aspects of the immunopathogenesis that characterizes dengue infection [11,12]. From an epidemiological perspective, the presence of pre-existing heterologous antibodies which fail to neutralize the prevailing infecting serotype is a major contributing cause for the development of the noted serious consequences of secondary DENV infection [13]. These sub-neutralizing immunoglobulin (Ig)G antibodies enhance phagocytosis of opsonized virus particles by cells of the reticuloendothelial system via both Fc receptor-gamma (FcγR)-dependent and -independent mechanisms [14,15]. The recently postulated 'intrinsic antibody-dependent enhancement (ADE) hypothesis' proposes that the high viral load and antibody levels that are observed in dengue patients may be attributed to FcγR-mediated internalization of DENV virions suppressing innate immunity, increasing interleukin-10 production and polarizing $CD4^+$ T lymphocyte responses towards a predominant type 2 cytokine profile [16,17]. As predicted by the hypothesis of original antigenic sin, secondary DENV infection shows a skewed expansion of T cells with low affinity for the currently infecting serotype and high affinity for the previously infected serotype [18,19]. Antibodies mediate anti-viral protection via both direct neutralization and indirect effector functions such as opsonization [20]. While the primary target of DENV for neutralizing antibody is envelope (E) protein, antibodies specific to pre-membrane (prM) protein have also been identified [12]. Neutralization is likened to a 'multiple hit' phenomenon in which virus inactivation occurs once the number of antibodies bound to a virion exceeds a required threshold [21].

Virus structure and life cycle

Each DENV particle has a lipid-enveloped spherical structure, 40-50 nm in diameter, containing positive sense single-stranded RNA [10,22]. The nucleocapsid core comprises capsid protein (C) and a single molecule of RNA approximately 10.7 kilobases in length. An open reading frame encodes a precursor polypeptide that is processed by host and viral proteases to produce membrane (M), E and C structural proteins as well as seven non-structural (NS) proteins, NS1, 2a, 2b, 3, 4a, 4b and 5 [10].

prM, a precursor protein found in intracellular immature virions, acts as a chaperone that is involved in folding of E protein. It undergoes furin cleavage in the trans-Golgi network to form M protein in mature virions [22]. During the process of synthesis, prM and E proteins form heterodimers that are oriented into the lumen of the endoplasmic reticulum, where they associate into trimers, an interaction that is thought to induce budding of nascent virus particles [23,24]. E protein is organized into three domains: domain (D)I comprises a beta-barrel structure formed by linked domains II and III; DII is a stretch of 13 conserved hydrophobic residues creating an internal fusion loop; DIII is an Ig-like fold that is involved in virion host cell entry [24].

The pseudo icosahedral structure of E protein displays three distinct chemical environments defined by proximity to 2-, 3- or 5-fold axes of symmetry [22]. Steric constraints imposed by adjacent E proteins on a virus particle may cause differences in access of antibody to epitopes in each environment [25]. This may result in differing numbers of sites available for binding. Antibodies may bind to a small fraction of accessible epitopes, leading to low site occupancy. Poorly exposed epitopes may require complete occupancy for neutralization whereas some epitopes may not be available for binding without the required stoichiometry [25,26].

The arrangement of E proteins on the surface of a DENV particle determines the accessibility of epitopes and thereby influences the neutralization potency of specific antibodies. Dramatic changes in conformation and arrangement of E proteins on a virion during its life cycle modulate antibody binding and function [26,27].

A pressing need for vaccine development

As there are no specific therapeutics and prevention is currently limited to vector control measures, dengue is increasingly having a significant impact on global public health. In the absence of a licensed vaccine, the World Health Organization (WHO) recommends prevention of dengue through an integrated vector control program involving mosquito habitat removal, use of insecticides, surveillance and case management. An efficacious vaccine is seen as a key component of a future strategy. To this end, vaccine development is a high priority which WHO supports through technical guidance and expert advice [28, 29].

The first dengue candidate vaccine was evaluated in a rodent model in the 1940s [30]. Since then, prototype vaccines have been continually under development [31]. Several molecular biological technologies are now being implemented using live-attenuated virus, purified inactivated virus, recombinant subunits, virus-like particles and plasmid- or viral-based platforms. All these approaches are at different stages of pre-clinical and clinical development and each has its advantages and disadvantages [3,32,33].

Limited progress to date

Of the several strategies that have been evaluated in clinical studies, the most advanced is a live-attenuated vaccine. The first such candidate to enter phase I and II clinical trials was tested in Thai adults and children. Neutralizing antibody was not detected in those volunteers who failed to seroconvert to all four then known dengue serotypes [34]. In addition, unacceptable reactogenicity in some individuals ruled out further clinical testing. Secondary infection by a heterologous DENV serotype is the greatest risk factor for acquisition of life-threatening dengue haemorrhagic fever. Thus, the major challenge facing vaccinologists is to achieve pan-serotype immunity without triggering associated pathology [3,35].

Currently, a live-attenuated tetravalent vaccine based on chimeric yellow fever-dengue virus (CYD-TDV) has progressed to phase III efficacy trials [36]. This preparation contains four recombinant viruses (CYD-1 to -4), each of which expresses the dengue prM and E proteins of one of four dengue serotypes together with the non-structural and capsid proteins of the attenuated yellow fever (YF) vaccine virus YF-17D [37].

Antibody-dependent enhancement of secondary infection

Recent investigations have shown the human anti-DENV immune response to be dominated by prM-specific antibodies during both primary and secondary infections [38]. This anti-prM IgG is highly cross-reactive and non-neutralizing. Importantly, when forming a complex with immature DENV (imDENV), antibodies to prM have the capacity to render normally non-infectious imDENV particles highly infectious [39,40].
Antibodies directed against E and prM proteins appear to play a dual role in controlling viral infection as they can both neutralize and enhance infectivity of DENV particles [41,42]. Although most potently neutralizing antibodies have been mapped to DIII of E protein [43], their role in protection against disease has been questioned given that only low levels of DIII-specific antibodies are produced during natural infection. Nevertheless, when produced even at high concentration, DI/DII-reactive antibodies fail to neutralize mature virions, presumably due to steric hindrance [44]. It is thought that antibodies to prM facilitate efficient binding and internalization of a virus-immune complex in the host cell, typically keratinocytes, the predominant cell type in human skin, whereupon intracellular furin cleaves prM to M, thereby activating the membrane fusion potential of E protein [40]. Circulating titres of anti-prM antibody are higher in patients experiencing a secondary dengue infection [26]. Since anti-prM antibodies are very cross-reactive and weakly neutralizing even at high concentrations among all DENV serotypes, immature virions promote ADE of dengue infection more effectively than do fully mature DENV particles [45]. The discovery of the infective potential of immature virions in the presence of antibodies not only suggests incomplete prM cleavage during natural infection but also implies that this is utilized by the virus as a mechanism to evade host humoral immunity [45]. During primary infection, in an absence of antibodies immature particles fail to bind efficiently to potential host cells and therefore are of minor importance in disease pathogenesis. In contrast, in secondary infection cross-reactive and weakly neutralizing anti-prM IgG plays a crucial role in development of ADE and thus acts to promote severe dengue [46].

Current vaccines hold potential to exacerbate infection

Native dengue prM is a component of most contemporary vaccine strategies, for instance attenuation naturally or by recombination, chemical inactivation, yellow fever-dengue virus chimeras, DNA- or subunit protein-based vaccines [47,48]. Since extremely cross-reactive and infection-enhancing prM-specific antibodies appear to dominate the anti-DENV immune response in humans, this raises concern over the safety of vaccines that do incorporate native dengue prM sequences [38]. It is therefore prescient to reconsider vaccine design in order to eliminate ADE activities that may be induced by infection-enhancing epitopes on prM to which an individual is exposed through existing immunization protocols [41].

A vaccination strategy to block prM-specific antibody

Vaccine candidates that lack prM proteins, such as those based on soluble recombinant E protein or E domain subunits, are set to assume an increased importance. A cryptic epitope on the E protein that locates to the DI/DII junction is inaccessible to antibody binding in a native virus particle but may become exposed if E is not folded correctly [41].
Although few epitopes have been mapped to prM, a novel infection-enhancing epitope first recognized by monoclonal antibody shows an epitope-reactive antibody with broad cross-reactivity and poor neutralizing activity against each DENV serotype [49]. As a consequence, a blocking antibody that binds to the idiotype of anti-prM antibody to render it inactive should be considered as a design feature of next generation dengue vaccines. This antibody adjunct strategy may achieve a significant outcome in reducing dengue morbidity and mortality.

Conclusions

Generation of anti-prM antibodies that enhance DENV infection may not be completely avoided even with immunization strategies employing E protein alone or subunits of E proteins [39]. When administered passively (in those who have already acquired primary infection or reside in endemic areas), a blocking antibody can bind effectively to the antigen-binding site (ADE-enhancing region) of anti-prM IgG and thus reduce its capacity to induce ADE of dengue infection. Hence, this proposed adjunct therapy provides a newly refined vaccine strategy. In turn, such a rationale offers renewed hope of an efficacious vaccination program to prevent severe dengue being realized in the foreseeable future.

Competing Interests

The authors have declared no competing interests.

References

1. Ratnam I, Leder K, Black J, Torresi J (2013). Dengue fever and international travel. *J Travel Med* 20: 384-393
2. Simmons CP, Farrar JJ, Nguyen VV, Wills B (2012). Dengue. *N Engl J Med* 366: 1423-1432
3. Ansari S, Taylor-Robinson AW (2014). Strategic approaches to multivalent vaccine development against dengue virus infection. *Ann Vaccines Immunization* 1: 1005
4. Normile D (2013). Surprising new dengue virus throws a spanner in disease control efforts. *Science* 342: 415
5. Mustafa MS, Rasotgi V, Jain S, Gupta V (2015). Discovery of fifth serotype of dengue virus (DENV-5): a new public health dilemma in dengue control. *Med J Armed Forces India* 71: 67-70
6. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, *et al.* (2013). The global distribution and burden of dengue. *Nature* 496: 504-507
7. Whitehorn J, Farrar J (2010). Dengue. *Br Med Bull* 95: 161-173
8. World Health Organization (2012). Global strategy for dengue prevention and control 2012–2020. Available from: http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf. [Last accessed 6 June 2015]
9. Scott TW, Amerasinghe PH, Morrison AC, Lorenz LH, Clark GG, *et al.* (2000). Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. *J Med Entomol* 37: 89-101

10. Burke DS, Monath TP (2001). Flavivirus. In: Knipe DM, Howley PM, eds., Field Virology, 4 th ed. Lippincott Williams & Wilkins, Philadelphia, pp. 852-921
11. Rothman AL (2004). Dengue: defining protective versus pathologic immunity. *J Clin Invest* 113: 946-51
12. Wahala WMPB, Silva AM (2011). The human antibody response to dengue virus infection. *Viruses* 3: 2374-2395
13. Tirado SM, Yoon KJ (2003). Antibody-dependent enhancement of virus infection and disease. *Viral Immunol* 16: 69-86
14. Halstead SB (2003). Neutralization and antibody-dependent enhancement of dengue viruses. *Adv Virus Res* 60: 421-467
15. Huang KJ, Yang YC, Lin YS, Huang JH, Liu HS, *et al.* (2006). The dual-specific binding of dengue virus and target cells for the antibody-dependent enhancement of dengue virus infection. *J Immunol* 176: 2825-2832
16. Halstead SB, Mahalingam S, Marovich MA, Ubol S, Mosser DM (2010). Intrinsic antibody dependent enhancement of microbial infection in macrophages: disease regulation by immune complexes. *Lancet Infect Dis* 10: 712-722
17. Tsai TT, Chuang YJ, Lin YS, Chang CP, Wan SW, *et al.* (2014). Antibody-dependent enhancement infection facilitates dengue virus-regulated signaling of IL-10 production in monocytes. *PLoS Negl Trop Dis* 8: e3320
18. Mongkolsapaya J, Dejnirattisai W, Xu XN, Vasanawathana S, Tangthawornchaikul N, *et al.* (2003). Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat Med* 9: 921-927
19. Rothman AL (2009). T lymphocyte responses to heterologous secondary dengue virus infections. *Ann NY Acad Sci* 1171: 36-41
20. Sanyal S, Taylor-Robinson AW (2013). Host-virus interactions in dengue infection indicate targets for detection and therapeutic interventions. *Immunity Diseases* 1, a7 1-4
21. Dowd KA, Jost CA, Durbin AP, Whitehead SS, Pierson TC (2011). A dynamic landscape for antibody binding modulates antibody-mediated neutralization of West Nile virus. *PLoS Pathog* 7: e1002111
22. Heinz FX, Stiasny K (2012). Flaviviruses and their antigenic structure. *J Clin Virol* 55: 289-95
23. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Chacon IV, *et al.* (1999). Dengue virus structural differences that correlate with pathogenesis. *J Virol* 73: 4738-4747
24. Clyde K, Kyle JL, Harris E (2006). Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. *J Virol* 80: 11418–11431
25. Stiasny K, Kiermayr S, Holzmann H, Heinz FX (2006). Cryptic properties of a cluster of dominant flavivirus cross-reactive antigenic sites. *J Virol* 80: 9557-9568
26. Kuhn RJ, Dowd KA, Beth Post C, Pierson TC (2015). Shake, rattle, and roll: impact of the dynamics of flavivirus particles on their interactions with the host. *Virology* 479-480: 508-517
27. Izabela A, Rodenhuis Z, Wilschut J, Jolanda MS (2010). Dengue virus life cycle: viral and host factors modulating infectivity. *Cell Mol Life Sci* 67: 2773-2786
28. World Health Organization (2015). Dengue vaccine research. Available from: http://www.who.int/immunization/research/development/dengue_vaccines/en/. [Last accessed 6 June 2015]
29. Gubler DJ (2015). The partnership for dengue control - a new global alliance for the prevention and control of dengue. *Vaccine* 33: 1233
30. Sabin AB, Schlesinger RW (1945). Production of immunity to dengue with virus modified by propagation in mice. *Science* 101: 640-642
31. Gyawali N, Taylor-Robinson AW (2014). Vaccine development against dengue, a viral disease of increasing significance to global public health. *Immunity Diseases* 2, a9 1-4

32. Perng GC, Lei H-Y, Lin Y-S, Chokephaibulkit (2011). Dengue vaccines: challenge and confrontation. *World J Vacc* 1: 109-130

33. Rodriguez-Barraquer I, Mier-y-Teran-Romero L, Schwartz IB, Burke DS, Cummings DA (2014). Potential opportunities and perils of imperfect dengue vaccines. *Vaccine* 32: 514-520

34. Sanchez V, Gimenez S, Tomlinson B, Chan PK, Thomas GN, *et al.* (2006). Innate and adaptive cellular immunity in flavivirus-naïve human recipients of a live-attenuated dengue serotype 3 vaccine produced in Vero cells (VDV3). *Vaccine* 24: 4914-4926

35. Chokephaibulkit K, Perng GC (2013). Challenges for the formulation of a universal vaccine against dengue. *Exp Biol Med* 238: 566-578

36. Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, *et al.* (2014). Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 384: 1358-1365

37. Guy B, Barrere B, Malinowski C, Saville M, Teyssou R, *et al.* (2011). From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine* 29: 7229-7241

38. Dejnirattisai W, Jumnainsong A, Onsirisakul N, Fitton P, Vasanawathana S, *et al.* (2010). Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 328: 745-748

39. Beltramello M, Williams KL, Simmons CP, Macagno A, Simonelli L, *et al.* (2010). The human immune response to Dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity. *Cell Host Microbe* 8: 271-283

40. Rodenhuis-Zybert IA, van der Schaar HM, da Silva Voorham JM, van der Ende-Metselaar H, Lei HY, *et al.* (2010). Immature dengue virus: a veiled pathogen? *PLoS Pathog* 6: e1000718

41. Chan AHY, Tan HC, Chow AY, Lim APC, Lok SM, *et al.* (2012). A human PrM antibody that recognizes a novel cryptic epitope on dengue E glycoprotein. *PLoS One* 7: e33451

42. Schaar HM, Wilschut JC, Smit JM (2009). Role of antibodies in controlling dengue virus infection. *Immunobiology* 214: 613-629

43. Thompson BS, Moesker B, Smit JM, Wilschut J, Diamond MS, *et al.* (2009). A therapeutic antibody against West Nile virus neutralizes infection by blocking fusion within endosomes. *PLoS Pathog* 5: e1000453

44. Wahala WM, Kraus AA, Haymore LB, Accavitti-Loper MA, de Silva AM (2009). Dengue virus neutralization by human immune sera: role of envelope protein domain III-reactive antibody. *Virology* 392: 103-113

45. Zybert IAR, Wilschut J, Smit JM (2011). Partial maturation: an immune-evasion strategy of dengue virus? *Trends Microbiol* 19: 248-254

46. de Alwis R, Williams KL, Schmid MA, Lai CY, Patel B, *et al.* (2014). Dengue viruses are enhanced by distinct populations of serotype cross-reactive antibodies in human immune sera. *PLoS Pathog* 10: e1004386

47. Durbin AP, Whitehead SS (2010). Dengue vaccine candidates in development. *Curr Top Microbiol Immunol* 338: 129-143

48. Wan SW, Lin CF, Wang S, Chen YH, Yeh TM, *et al.* (2013). Current progress in dengue vaccines. *J Biomed Sci* 20: 37

49. Luo YY, Feng JJ, Zhou JM, Yu ZZ, Fang DY, *et al.* (2013). Identification of a novel infection-enhancing epitope on dengue prM using a dengue cross-reacting monoclonal antibody. *BMC Microbiol* 13: 194