New insights into dengue pathogenesis and control

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Preface
Dengue virus poses a major threat to global public health: two-thirds of the world’s population are now at risk from infection by this mosquito-borne virus. Dengue virus causes a spectrum of disease with a small proportion of infected patients developing severe plasma leakage leading to dengue shock syndrome, organ impairment and bleeding. Infection with one of the four distinct viral serotypes results in the development of homotypic immunity to one serotype only. Subsequent infection with a different serotype is associated with an increased risk of developing severe disease, leading to the suggestion that severe disease is triggered by immunopathology. This Review outlines recent advances in the understanding of immunopathology, vaccine development and human monoclonal antibodies against dengue virus.
Introduction

Dengue is a positive sense RNA virus belonging to the Flaviviridae family. There are four distinct serotypes (DENV 1-4) and several genotypes within each serotype. Since the 1950s there has been considerable expansion in the geographic spread of dengue and exponential rise in disease incidence. It is estimated that the annual global incidence is 390 million cases, of which 96 million are clinically apparent. There are several factors that drive this pandemic, including globalization, the spread of the Aedes mosquito vector, inadequately planned urbanization, and the absence of a licensed vaccine or anti-dengue therapeutics.

The clinical phenotype of dengue can vary depending on several factors, of which age, genetic predisposition and background immunity are major determinants. However, most clinical infections result in a self-limiting febrile illness termed dengue fever. The hallmark feature of severe disease is increased capillary permeability, causing plasma leakage, which can lead to haemodynamic compromise and dengue shock syndrome (DSS) (Box 1). If untreated, severe disease can lead to a mortality of up to 20%, but with expert management, primarily careful fluid replacement, this can be reduced to considerably below 1%.

The pathogenesis of severe disease can be explained at least in part through immune mechanisms, of which both antibody enhancement and T cell immunopathology probably have key roles. These theories have served to explain the observation that severe disease is associated with a second dengue infection with a different serotype. However, because of this risk of severe disease in sequential infections, developing a safe vaccine that provides protection against all 4 serotypes has been challenging. There have been several recent advances in tackling and understanding dengue in the past decade, including viral epitope mapping and defining conserved neutralizing antibodies, as well as the publication of the first Phase 3 vaccine efficacy trials. This review focuses on the adaptive immune response to dengue, the challenges of developing a dengue vaccine and new insights from the study of human monoclonal antibodies.

The dengue virus

Dengue virus is a relatively simple positive-sense single stranded RNA virus that is 50 nm in diameter and has three structural proteins; Capsid protein (C) Precursor membrane protein (prM) and Envelope protein (E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and
Studies using X-ray crystallography and cryo-electron microscopy have shed considerable light on structural aspects of the flaviviral life cycle, which are described in detail below as they are of considerable relevance to the description of the antibody response to dengue that follows.3-9

E protein and prM protein form the glycoprotein shell of the virus, with E protein being responsible for host cell binding and entry.10 Dengue E protein has two N-linked glycosylation sites and is divided into three domains I-III(Fig. 1B). During viral assembly in the endoplasmic reticulum, 180 copies of E protein associate in a 1:1 fashion with 180 copies of prM protein to form 60 trimeric (heterohexameric) spikes, which gives “immature virions” their characteristic spiky appearance.10 In the acidic environment of the trans-Golgi network the trimeric spikes reassociate, E protein forms head to tail homodimers with the two prM protein molecules lying across the dimer interface protecting the hydrophobic fusion loop.9 prM protein is thus thought to act as a chaperone to E protein protecting the fusion loop and preventing premature fusion of the immature virion to host cell membranes.

In the trans-Golgi network prM protein is cleaved at a membrane proximal site by host encoded furin protease, which leaves a membrane-anchored M stump and Pr which remains associated with the virion until it is secreted. On secretion from the host cell, Pr falls away to leave the “mature virion”, a smooth structure containing 180 copies of the E protein, arranged into 90 head to tail dimers with icosahedral symmetry around 2, 3 and 5-fold axes (Fig. 1A and D).

In dengue virus, prM protein cleavage is not complete in all the virions, leaving a proportion of intermediate forms, where viral particles contain a varying amount of cleaved and uncleaved prM protein.11, 12-14 prM protein cleavage is more efficient in certain cell types, particularly primary human cells such as dendritic cells compared to virus produced in insect cells or tumour cell lines such as Vero.15, 16 Fully immature dengue viruses, as described above, contain regular trimeric E/prM protein spikes and are non-infectious.17, 18 By contrast, the intermediate forms, some of which are infectious, have a less regular structure, with areas that are spiky and contain prM protein/E trimers or smooth areas that are proposed to contain E protein dimer, with a less well defined boundary zone where there may be a dynamic equilibrium between the two forms.19

Finally, to complete the cycle, virions attach to poorly characterised cellular receptors, proposed to be mediated via domain III of the envelope protein.10 Several host proteins are also involved in viral...
attachment such as heparan sulphate, DC-SIGN, HSP-70, HSP-90, Mannose receptor, CD14, TIM/TAM, and Laminin\textsuperscript{10}. Following endocytosis, acidification in the early endosome triggers a dramatic reorganisation of envelope, from the dimer form to a new trimeric conformation. This exposes the fusion loop at the tips of the trimeric spikes, which then fuse with the endocytic membrane and allow the viral nucleic acid enveloped in the capsid protein to be released into the host cell cytoplasm\textsuperscript{10} (Fig 1A)

**Immune mechanisms**

There are a number of key observations that suggest a role for the immune system in dengue pathogenesis: First, severe dengue is usually, but not exclusively, seen with secondary infection, implying that priming of the adaptive immune system to one dengue serotype leads to disease enhancement following a secondary infection with a different serotype\textsuperscript{2, 21, 22}. Second, the peak in symptoms occurs at a time when virus loads are rapidly decreasing implying that immunopathology may be driven as a consequence of viral control\textsuperscript{23, 24}. Third, higher peak viral loads and/or NS1 (non-structural antigen 1) burden predispose to more severe outcomes when the virus is controlled\textsuperscript{23, 24, 25}. Finally, the peak in symptoms, vascular leak and viral control coincide with a ‘cytokine storm’ in which high circulating levels of many inflammatory mediators such as TNF, sTNFRI, sTNFRII, IFN\textgreek{y}, CXCL9, CXCL10, CXCL11, CXCL8, CCL5 and VEGF as well as the anti-inflammatory cytokine IL-10\textsuperscript{26, 24, 27, 30}. This cytokine storm has been proposed to trigger increased vascular permeability and resolves relatively quickly upon convalescence.

The exact mechanisms underlying the capillary leak are likely to be more complex than a cytokine storm, which is also associated with other diseases with very different clinical phenotypes such as GVHD\textsuperscript{31}, septic shock\textsuperscript{32}, and Influenza infection\textsuperscript{33}. Dengue virus may cause increased vascular permeability through binding to the endothelial glyocalyx layer, which is a negatively charged layer that lines the luminal surface of micro-vessels, providing vital barrier functions to capillaries. Both the virus and the NS1 protein have been shown to bind to Heparan sulfate, a major component of the glyocalyx layer\textsuperscript{14, 35}, which may in turn alter the permeability of capillaries.

NS1 mediated complement activation may play a role in the altered capillary permeability through the generation of anaphylatoxins C3a and C5a and the terminal complement complex SC5b-9\textsuperscript{23}. High plasma and pleural fluid SC5b-9 levels have been demonstrated in patients with dengue shock and patients who were high producers of SC5b-9 had more signs of plasma leakage. NS1 and
complement may have an immune modulating role through a reduced functional capacity of C1s, and C4 thereby altering the classical and lectin complement pathways \(^{36}\).

Several studies suggest a role of mast cells in protection and pathogenesis of dengue. Upon interaction with DENV, mast cells release chemokines recruiting T, NK, and NKT cells to control the infection. On other hand, the interaction also leads to the secretion of mediators such as Chymase contributing to vascular leakage. The level of Chymase is correlated with the severity \(^{37}\).

Overall, the well-established association of severe dengue with secondary infection has led to close study of both T cell and B cell mediated arms of the adaptive immune system, which will be reviewed below.

**The role of T cells.** The association of severe dengue symptoms with rapid decline in viral loads and peaks of pro-inflammatory cytokine secretion have led some to propose a role for the T cell response in driving immunopathology in severe dengue. There has been much study of T cell responses, but the contribution of these responses to pathogenesis and protection remains controversial \(^{38}\). Reports have demonstrated strong T cell responses in dengue-infected individuals and many dengue T cell epitopes have been mapped in humans and mouse models \(^{39-46}\). Responses to the non-structural protein NS3 seems to be relatively immunodominant in humans, although CD8\(^+\) and CD4\(^+\) T cells that respond to epitopes across the whole virus proteome have been observed \(^{39, 41, 44, 46-49, 50}\). The magnitude of the T cell response correlates with disease severity, although during the acute viraemic phase of the disease T cell frequencies in the blood are relatively low, with many undergoing both proliferation and programmed cell death. Following virus clearance, there is an increase in the levels of dengue specific T cells \(^{42, 51-53}\). The low frequency of circulating T cells during viraemia prompted some to question the relevance of the T cell response to dengue infection \(^{52}\). However, a recent study showed that skin blister fluid taken from the patients contained more dengue-specific T cells than in peripheral blood \(^{51}\). This suggested that T cells may migrate from blood to peripheral tissue during an acute infection.

During secondary dengue virus infection, large expansions of responding T cell populations can be seen accounting for up to 20% of circulating T cells \(^{52}\). Because most clinically severe dengue infections occur during a secondary infection \(^2\), there has been much study of crossreactive T cells in dengue \(^{39, 42, 43, 46, 50, 53-55}\). Using double tetramer staining with tetramers containing peptides from either the primary or secondary infecting viruses labelled with different fluorophores we and others
have studied the crossreactivity of CD8$^+$ T cell responses$^{42, 43, 53}$. During a secondary infection, there is a large expansion of T cells that bind to tetramer loaded with peptides derived from the primary dengue infection (Fig. 2). Some of these cells cross react with tetraters loaded with peptide derived from the secondary infecting virus whereas others show lower avidity for the tetramer from the secondary virus and no functional responses to the secondary virus-derived peptides.

The development of a secondary immune response by memory T cells generated to a similar yet distinct epitope encountered during a primary infection was first described for antibody responses to influenza virus infection more than 50 years ago and termed original antigenic sin$^{56}$. Original antigenic sin has been reported for CD8$^+$ T cell responses to lymphocytic choriomeningitis virus (LCMV)$^{57}$ and also appears to operate in dengue virus infection, where some of the dengue-specific T cells that expanded during secondary infection are fully crossreactive between the two serotypes whereas others show preference to the primary infecting serotype$^{42, 43, 50, 58}$. In another study the presence of original antigenic sin was demonstrated in the dengue T cell response but the overall quality of the response did not seem to be compromised. This latter study measured functional responses to peptide stimulation and did not measure non-functional cells with tetramer staining, nor formally show crossreactivity, which was implied based on the similarity of peptide sequences$^{44}$. In summary, whether the expansion of low avidity crossreactive T cells in secondary dengue infection does contribute to disease pathogenesis remains controversial in the absence of a good animal model of disease.

Study of the function of dengue-specific T cells has revealed an interesting difference between mild and severe infections. During severe primary and secondary infections, a high proportion of CD8$^+$ T cells produce the cytokines TNF and IFNγ but fail to display degranulation markers. By contrast, during milder dengue fever illness CD8$^+$ T cells show more degranulation and less production of pro-inflammatory cytokines, which may have a bearing on virus control, disease pathogenesis and immunopathology$^{39}$. CD4$^+$ T cell populations also expand during dengue infection and similarly to CD8$^+$ T cells, the magnitude of the CD4$^+$ T cell response correlates with disease severity$^{39, 46}$. Interestingly, a number of studies have reported the generation of cytotoxic CD4$^+$ T cells directed to dengue$^{39, 46, 55, 59}$.

As mentioned above, the role of T cells in dengue disease pathogenesis and protection is debated. On the one hand, high amplitude T cell responses with the responses skewed to cytokine production and not degranulation described above are associated with, and potentially drive, severe disease$^{42}$. 
On the other hand, T cells may have a role in controlling virus replication. An analysis of a large Sri Lankan cohort has suggested a role for T cells in protecting from dengue as higher amplitude responses against epitopes presented by protective HLA alleles were observed, although most HLA association studies in dengue have been small and not repeated. Depletion and adoptive transfer of CD8+ and CD4+ T cells in mouse models have suggested a role in reducing virus loads and protecting from severe disease. Other investigators who question the importance of T cells in dengue pathogenesis point out that severe dengue can occur during a primary dengue infection in which crossreactive T cells and original antigenic sin would not be operative. However, in primary severe dengue high amplitude and skewed T cell responses are observed similar to secondary infection.

Finally, it has been speculated that the suboptimal efficacy of the recent Sanofi vaccine trial (see later) may in part be driven by the lack of a protective T cell response, as all of the non-structural proteins in this tetravalent vaccine derive from the Yellow Fever virus. We take a balanced view of T cells in dengue: the generation of an early response to dengue may be protective, whereas the late generation of T cell populations that have a proportion of low avidity T cells and are skewed to inflammatory cytokine production in the absence of degranulation may predispose to immunopathology in the presence of high viral or antigen loads and contribute to the cytokine storm and vascular leak (Fig 2).

**Antibody-dependent enhancement.** The theory of antibody dependent enhancement (ADE) attempts to explain why severe disease is usually associated with secondary infections in children and adults. ADE has also been suggested to lead to increased disease severity in infants experiencing primary dengue infection as the levels of passively transferred maternal anti-dengue antibodies fall below neutralising levels during the first year of life. The ADE hypothesis posits that pre-existing heterologous antibodies generated to a primary infection may not be of sufficient avidity or concentration to neutralize a secondarily encountered virus, in which the sequence of envelope protein may vary by 30-35% at the amino acid level. Instead, the virus may be opsonised and targeted for uptake into Fc receptor (FcR)-bearing cells such as monocytes and macrophages, which are major sites of dengue virus replication *in vivo*, and therefore lead to an increase in viral production (Fig 2B). ADE has been subdivided into two phases: extrinsic ADE, which refers to the increase of infection of FcR-bearing cells, and intrinsic ADE in which it is proposed that infection of cells via FcR actually drives increased viral replication mediated by FcR ligation. It has also been shown that dengue viruses interact with Leukocyte immunoglobulin-like receptor B1 leading to
inhibition of the antiviral activity of type I IFN stimulated genes induced through the ligation of DENV-antibody complexes to FcR.

ADE can be readily demonstrated in vitro using FcR expressing cells and has been demonstrated to drive higher virus loads in both mouse and primate models of dengue infection. In mouse models, ADE can increase disease severity, leading to vascular leakage and lethality. Studies on FcγRIIA polymorphisms show association of the 131 H/H and H/R alleles with the symptomatic infection whilst the 131 R/R allele is associated with the protection from severe disease.

Antibodies directed against prM protein can facilitate FcR mediated uptake of immature viral particles, which although non-infectious in the absence of antibody, can undergo furin-mediated prM protein cleavage following endocytosis in the host cell rendering them infectious. A similar phenomenon, rendering non-infectious immature particles infectious, has also been demonstrated following ADE driven by antibodies specific for E protein. At sub-neutralizing concentrations almost all antibodies specific for E and prM proteins can be enhancing, with maximum levels of enhancement occurring at roughly half the concentration required for neutralization. In West Nile viral infection, a structurally related flavivirus, it has been estimated that around 15-29 anti-E protein antibodies bound to a virion will promote ADE.

In summary, both antibodies and T cells have been implicated in the generation of protective and pathogenic responses during dengue infection and the optimization of protection and minimization of pathogenic responses are the goal for successful vaccines.

**Dengue Vaccines**

The exponential rise in dengue infections over the past few decades has made the search for a dengue vaccine an imperative, but achieving this goal has proved enormously challenging. Any successful vaccine would need to induce a protective and durable immune response to all four dengue serotypes, preferably with one or two doses, in individuals who have either been unexposed to dengue or had a previous dengue infection. At the same time a vaccine would need to avoid eliciting enhancing or pathogenic immune responses described above.

As primary dengue infection does not give long-term protection to re-infection with the other three viral serotypes, it has been generally held that a vaccine will need to induce protective type
specific responses against all four serotypes mandating a tetravalent formulation. Efforts to develop vaccines have been pursued for almost 50 years beginning in Thailand with work to produce live attenuated virus by serial passage of viral strains representative of the four serotypes. A particular challenge has been to develop attenuated forms of the virus, which are not too virulent to induce overt dengue disease whilst not too over attenuated to be able to incite a protective immune response. Another challenge has been to produce a tetravalent formulation in which all four viruses are delivered together, replicate equally and induce a balanced response against all four serotypes rather than competition between serotypes leading to good responses to some serotypes but poor responses to one or more serotypes.

Recent years have seen significant advances in vaccine development with three live attenuated vaccines currently undergoing clinical trials. There are also a number of other vaccines in preclinical studies. The three live attenuated dengue vaccines currently under investigation are CYD-TDV, NIH Δ30 vaccines, and DENVax (Fig 3).

The most advanced in clinical trials is the Sanofi Pasteur-vaccine CYD-TDV. This is a chimera vaccine using the yellow fever 17D vaccine strain as a backbone, with dengue prM protein and E protein genes replacing those from yellow fever. The vaccine contains a mixture of four recombinant viruses representing each serotype (CYD1-4). Initial clinical trials demonstrated good serological responses to the vaccine, with seropositivity ranging between 66.5 to 100%. However, the overall efficacy (protection from infection) of a Phase IIb trial in Thailand was below expectations with efficacy against dengue 2 the lowest (Table 1). Phase III trials of this vaccine in Asia and Latin America have been more promising, although still showed suboptimal efficacy ranging between 35 and 78% and, again, the efficacies against dengue 2 were lowest. Further analysis revealed that the vaccine gave better protection to vaccinees who were already immune to one or more serotypes prior to vaccination. The protection against severe and haemorrhagic disease has been calculated as being between 80 and 91%.

The early phase of long term follow up of the CYD-TDV vaccine trials have recently reported showing older children in the vaccinated group (age 9-16 years) continuing to benefit. However, in the under 9 year age group, vaccine efficacy was not only lower than older children (44.6% versus 65.6%) but also at 3 years post vaccination there appears to be an increase in hospitalization in this group when compared to control unvaccinated subjects. This increase of infections in the younger age group
may represent disease enhancement, possibly by ADE, in individuals who were dengue naïve at enrolment who have been primed but not protected by the dengue vaccine.

There is now much urgency in the field to understand why the initially promising in vitro correlates of vaccine immunity did not always translate to good in vivo efficacy and to develop more representative in vitro correlates of protection to inform future trials. In the Sabchareon Phase IIb trial of CYD-TDV in Thailand, although the efficacy of the vaccine against dengue 2 was low, the seropositivity to dengue 2 at 1 year after vaccination was high (Table 1). Several reasons are postulated for this mismatch, firstly, the vaccine strain might be a poor immunological match for strains circulating during the study period, although preclinical trials showed the vaccine candidate induced neutralizing antibody activity against a broad range of viral strains. Secondly, T cells may be important for protection and because the CYD1-4 vaccine contains non-structural elements from Yellow Fever virus it may not produce an effective anti-dengue T cell response, Thirdly, the CYD vaccines do not contain DENV NS1 which was suggested to induce protection in mouse models. Finally, the current neutralising assay, PRNT, using virus generated from cell lines may not be a good surrogate for protection in vivo and better, more predictive assays are urgently being sought.

The tetravalent formulation does not mimic the natural situation where infections are overwhelming with a single serotype followed by reinfection months to years later. Although studying sequential infections in the population is difficult, especially after secondary infection, surveillance studies suggest symptomatic cases caused by third or fourth infections are rare. This implies that following secondary infection there is some degree of cross protection to the remaining serotypes. Inducing a balanced immune response to all four serotypes using a tetravalent formulation is clearly a major challenge and heterologous prime boost strategies have been suggested to overcome this. The observation in the recent Sanofi trials that efficacy was better in individuals who had experienced previous dengue exposure compared to the dengue naïve cohort may also be instructive in this regard, implying that the vaccine is able to boost pre-existing dengue immunity but poor at producing a protective response de novo in dengue naïve vaccinees.

Sequential priming and heterologous boosting has been investigated in primates. CYD2 and CYD3 were inherently less immunogenic giving lower numbers of responders than CYD1 and CYD4. Priming with CYD1 and CYD2 followed by boosting at day 56 with CYD3 and CYD4 gave better responses when compared with priming and boosting with tetravalent vaccination, especially with regards to responses to dengue 2. Heterologous priming and boosting has also been investigated in humans,
in which a different bivalent combination of CYD1 and CYD3 were used to prime and CYD2 and CYD4 used for boosting at 15 weeks\textsuperscript{103}. In contrast to the primate study, the sequential bivalent immunization was not superior to the sequential tetravalent immunization. It remains possible that the timing between priming and boosting and the serotype combinations used in these two studies may have influenced the outcome.

Of the other live attenuated vaccine candidates, the NIH Δ30 vaccine Phase I trial showed that seropositivity across all serotypes was over 90% after one dose of vaccine however around 60% of vaccinees had a rash and 73% experienced viraemia\textsuperscript{89, 104}. The LATV, DENVax (Takeda) has undergone a Phase I trial in Colombia\textsuperscript{90} showing more than half of volunteers seroconverted to all four viruses after a single dose. Finally, despite, the TDEN vaccine candidate developed by WRAIR and GlaxoSmithKline inducing responses across the four serotypes in a phase-II trial, development has been stopped\textsuperscript{105}.

Despite the difficulties, there remains a pressing need to pursue vaccine candidates as well as testing whether live attenuated vaccines can be improved to provide a more balanced protection and whether these responses are durable will be determined by longer term follow up of clinical trials.

**New insights from monoclonal antibodies**

A large number of mouse antibodies have been generated against dengue and the epitopes for several of these have been mapped by systematic or random mutagenesis to the envelope protein\textsuperscript{106-115}. More recently human monoclonal antibodies have been generated from dengue infected patients; the main epitopes recognised by monoclonal antibodies are described in this section (Table 2).

**Antibodies targeting the fusion loop epitope of E protein.** The fusion loop epitope (FLE) is a major epitope targeted by antibodies produced in both humans and mice, monoclonal antibodies specific for the FLE are exemplified by mAb E53, originally generated to West Nile virus in mice, but which is fully crossreactive to dengue viruses\textsuperscript{116}. Mutation of W101 of E protein blocks the binding of antibodies directed to the FLE and mutation of this residue has been shown to reduce the reactivity of polyclonal human serum\textsuperscript{117-119} FLE mAb show good neutralisation of dengue viruses grown in insect cells, which contain high levels of prM protein\textsuperscript{16}. However, FLE antibodies bind better in the presence of prM protein, which by virtue of changing the virion architecture better exposes the FLE\textsuperscript{13}. The binding and neutralisation of FLE mAb to more mature virions produced in primary human
cells, which contain lower levels of prM protein, is not so efficient with neutralisation titres typically failing to exceed 60-80% even at high concentrations\(^\text{16}\). Antibodies to the FLE although commonly generated in humans and often crossreactive between serotypes may not therefore be the ideal response to target by vaccines. Another antibody, 1C19, recognising the BC loop (amino acid 73,78 and 79) close to the FLE potently neutralised all 4 serotypes suggesting the epitope may be a good target for vaccines. However, it is not clear whether the epitope for 1C19 is prM dependent in common as the epitope for 1C19 lies close to the FLE\(^\text{120}\).

**E Protein Domain III.** Antibodies targeting E protein Domain III have been frequently isolated from mice and\(^\text{73}\) are among the most potent antibodies described to dengue with 50% *in vitro* neutralisation (NT50) levels in the low picomolar range\(^\text{73, 107-114}\). These antibodies are frequently dengue serotype specific although the EDIII binding mAb 4E11 has been engineered to bind to and potently neutralize all four dengue virus serotypes\(^\text{121}\). The epitopes for some EDIII binding mAb have been mapped in detail by mutagenesis, crystallography and cryo-electron microscopy. Monoclonal antibody E16 is an example of this class of antibody which binds to the lateral ridge of EDIII of WNV\(^\text{73}\). E16 shows potent neutralisation of West Nile virus both *in vitro* and *in vivo* in both prophylactic and therapeutic settings\(^\text{122}\). Another EDIII antibody 1A1D2 binds to the A strand (amino acid 305-312) of dengue EDIII\(^\text{123}\). In contrast to the lateral ridge of EDIII, the A strand is more conserved among the dengue viruses allowing 1A1D2 to cross-react between dengue serotypes 1,2 and 3\(^\text{106}\).

Interestingly, the epitope for 1A1D2 is not fully exposed in the smooth mature form of the dengue virion and cryo-electron microscopy has shown that a temperature-dependent conformational change, allowing EDIII to hinge up from its flat orientation in the smooth mature virion, facilitates mAb 1A1D2 binding\(^\text{123}\) (Fig. 3). Because of the potency of the EDIII-specific mAb and the general serotype specificity of the response, EDIII has been proposed as a potential dengue immunogen in a variety of vaccine formulations. However, the contribution of EDIII to the human antibody response appears to be more limited: it has been shown that the depletion of EDIII-specific antibodies from human immune serum does not reduce its neutralisation potential *in vitro* and *in vivo*\(^\text{79, 124}\).

**PrM protein and virus maturity.** As described above immature dengue particles contain prM, which acts as a chaperone for E protein preventing premature fusion to host cell membranes before virus release. prM protein is cleaved by furin protease in the Golgi and falls away when mature virus particles are released from cells\(^\text{8-10}\). However, prM cleavage is frequently incomplete with a range of
partially mature virus forms produced with intermediate levels of prM\textsuperscript{11}. This produces a challenge for the host immune response as mature and immature virus particles present markedly different structural determinants at the virion surface. The human host will first be infected by virus injected following an insect bite which have a high prM content but then virus production will be driven from human cells with potentially a lower prM content, suggested by studies of primary human cells infected with dengue, although the prM content of dengue in ex vivo samples has not been measured\textsuperscript{15, 16}. The ability to neutralize more mature, low-prM containing viruses, is not possessed by a number of anti-dengue antibodies such as those targeting the FLE or prM\textsuperscript{15, 16}.

**Alternative conformations of the virion surface.** The binding of antibodies to viruses is a complex process, in which the packing of E protein and prM protein into the virion lattice will affect the accessibility of the target epitopes by the antibodies. Several antibodies that bind well to recombinant monomeric envelope protein fail to show good binding or neutralization of intact virions because their epitopes are poorly exposed\textsuperscript{14, 17, 73, 123, 125}. The binding of such antibodies may be enhanced by the prM protein content of the virions as described above for the anti-FLE antibodies, but may also be enhanced by prolonged incubation or at increased temperature\textsuperscript{126}. This has led to the proposition that the traditional view of the virion as an invariant/rigid smooth or spiky entity, which is necessarily imposed by the solution of cryo-EM structures, is not accurate. Instead, it is proposed that the virion is a dynamic entity that is capable of adopting many different conformations with different thermodynamic stabilities often referred to as virion breathing\textsuperscript{123, 127, 128}. In this regard, an alternative “bumpy” form of DENV2 virus particles has been recently described in which there is disruption of the regular packing of the E protein dimers at the virion surface at 37\degree C\textsuperscript{127, 128}, which has implications for antibody binding as shown by cryo-EM structures of mAb 2D22 binding to DENV2 particles where the valency of binding to the bumpy form of the virus was lower than that to the more regular form\textsuperscript{129}. The display of an ‘ensemble’ of different E protein and virion conformations can be continuously sampled and captured by antibodies explaining the increase in binding of antibodies in a temperature and time dependent\textsuperscript{12, 126}.

**Human monoclonal antibodies:** The study of antibody responses in humans has been revolutionised by the description of various techniques that have allowed the generation of large numbers of human monoclonal antibodies. Three main techniques have been used; expansion and EBV immortalisation of memory B cells; single cell sorting, cDNA cloning and antibody expression from sorted plasmablasts isolated from acutely infected individuals and finally, the optimization by electrofusion of traditional hybridoma technology to make antibodies from memory B cells\textsuperscript{130-132}. 


**Antibodies to precursor membrane protein.** PrM protein-specific antibodies appear to be a major component of the memory B cell response to dengue; these antibodies show poor neutralization (maximum 30-50%) even at high concentration\textsuperscript{15, 133-136}. PrM protein-specific antibodies do not bind to fully mature virions which do not contain prM protein, whereas many partially mature particles do not contain a high enough density of prM protein to allow neutralisation but yet may be sufficient to promote ADE\textsuperscript{15, 18}. We have speculated that the inefficient cleavage of prM protein may be an immune evasion strategy, leading to the generation of poorly neutralising antibodies directed to prM protein and to the FLE, which form a major component of the human antibody response\textsuperscript{15, 16, 117}. The high frequency, low potency and high ADE potential of antibodies directed to prM protein has implications for vaccine design; all attenuated vaccines at an advanced stage of development contain prM protein, the ideal vaccine would focus responses to the E protein and the prM protein component of the response be minimized if the potential for ADE in vaccines is to be reduced. One possible route to this would be the generation of attenuated chimeric viruses with prM protein derived from third party flaviviruses such as Japanese encephalitis or West Nile virus where the anti-prM protein response cross reacts poorly with dengue\textsuperscript{15, 137}.

**Antibodies targeting quaternary epitopes on the virion.** Some of the most potent human antibodies bind to conformational and quaternary determinants that are only reproduced on intact dengue virions\textsuperscript{16, 138-141}. One well characterised epitope, the so called “herring bone epitope”, was originally described by cryo-EM of mAb-CR4354 binding West Nile virus and has also been described for the DENV1 specific mAb-HM14c10\textsuperscript{138, 142}. These antibodies bind a similar epitope which bridges between two adjacent head to tail E protein dimers that form a herringbone like conformation on the mature virion (Fig. 4A). Several other conformationally sensitive antibodies have been mapped by cryo-EM; mAb-1F4 binds to a single E protein monomer, but only in the context of the virion, whereas mAb-5J7 binds across three adjacent E protein monomers with a major component of the interaction across the hinge between EDI and EDII of the central E protein monomer\textsuperscript{139, 140}.

The conformational antibodies described above show potent neutralisation but are nevertheless serotype specific. We have recently described the cloning of a large series of anti-E mAb from dengue infected patients\textsuperscript{16}. One third of the antibodies bind to a complex epitope present only on intact virions. Interestingly, although most of the panel of human antibodies showed relatively good neutralisation of high-prM protein-containing viruses produced in insect cells, only the mAb binding
to the complex epitope on intact virions could fully neutralise low prM protein containing virions produced in primary human cells.

The broadly neutralizing antibodies bind to the basic repeating envelope dimers making up the virion surface lattice and the epitope is termed the E protein dimer epitope EDE (Fig. 4B-C)\textsuperscript{16, 141}. The antibodies bind in a valley formed between the two monomers of E protein making up the 90 head to tail dimers of the virion and overlaps with the footprint on E protein where prM protein sits on the immature dengue virus particle generated as it passes through the acidic trans-Golgi network\textsuperscript{8}. The antibodies make contact with a number of conserved amino acid side chains and main chain atoms in the E protein peptide backbone explaining the cross reactivity and broad neutralization of the four virus serotypes. Another recently reported mAb 2D22 binds to envelope dimers although the epitope is slightly shifted toward EDIII compared to the epitope described above leading 2D22 to be specific to DENV2 and not broadly reactive\textsuperscript{129}.

The discovery of the EDE opens up a number of interesting future possibilities in dengue. Current vaccination strategies use tetravalent formulations with the aim of raising single serotype specific responses against all four serotypes. The demonstration that potent and broadly neutralising antibodies are produced in dengue infection means that the generation of such antibodies should be a goal for the next generation vaccines. Furthermore, it may be possible to design a universal, rather than tetravalent formulation to achieve this response, or that heterologous prime boost strategies may be used. Importantly, as the response is limited to the E protein dimer it opens the way for subunit vaccines consisting of E protein dimers alone. To achieve this, the E protein dimer will need to be stabilised as E protein only naturally forms dimers at relatively high protein concentrations. A similar situation has been observed with respiratory syncytial virus where potent neutralising antibodies have been shown to bind to the trimeric pre-fusion forms of the fusion protein\textsuperscript{143}. Efforts to stabilise a soluble F trimer have been successful either by cavity-filling hydrophobic substitution or by covalent linkage of the F monomer allowing the generation of a novel subunit immunogen capable of inducing a neutralising response against the F protein trimer in murine and primate models\textsuperscript{143}.

As described here the neutralization of dengue viruses is a complex process with a number of different virion forms produced during infection. Successful vaccines need to target potently neutralising epitopes such as those found on Domain III or the quaternary epitopes described above and minimize the targeting of poorly neutralizing epitopes such as those on prM or the FLE.
Conclusion and future direction

Despite nearly 50 years of work we still do not have a fully efficacious dengue vaccine and it remains to be determined whether the Sanofi CYD-LATV will be licensed and be deployed in endemic countries. However, the burden of dengue continues to increase and despite promising advances in vector control strategies and an increasingly active search for dengue antivirals an effective vaccine is seen by many to be the only realistic strategy to control the spread of the disease and reduce the burden it has on the healthcare systems in endemic countries.

There are a number of second-generation live attenuated vaccines approaching larger scale clinical trials. The challenge of producing protection against all four virus serotypes using a tetravalent formulation is formidable and heterologous prime–boost strategies, which mimic natural infection but have given conflicting results, may be further evaluated. The recent description of potent human neutralising antibodies in dengue gives insight into the sort of responses that should be targeted by vaccines. However, major challenges remain in how to preferentially boost responses to these complex quaternary epitopes. Finally, the recent Sanofi trials have demonstrated the need for robust in vitro correlates of protection, which would guide the development of future vaccine trials and there is now interest in developing human dengue challenge studies to guide future vaccine development.
Box 1

**Dengue clinical manifestations and treatment**

- The majority of patients infected with one of the 4 dengue virus serotypes are either asymptomatic or present with a self-resolving febrile illness, dengue fever, lasting 4-8 days.
- Key clinical manifestations include high fever, headache, retro-orbital pain, muscle pains, and other well recognised signs include; rashes, abdominal pain, vomiting and mucosal bleeding.
- The defining feature of severe disease is increased capillary permeability, causing plasma leakage which can occasionally lead to shock and death\(^\text{144}\).
- Other severe manifestations include haemorrhage and organ impairment, which includes hepatitis, myocarditis and encephalitis.
- Clinical symptoms vary by age of the host, with children being more at risk of shock and adults more likely to develop organ impairment and bleeding\(^\text{145}\).
- Treatment of dengue is supportive, in the form of intravenous fluids for patients identified to have haemodynamic compromise from plasma leak and those with those unable to tolerate oral rehydration.
- There have been several recent clinical trials investigating novel anti-viral and immune modulators however as yet none have demonstrated any clinical benefit in dengue\(^\text{146}\).

References


**Highlighted References**

5 Zhang et al: This paper describes the arrangement of prM and E to form the spiky trimers on immature dengue virions.

6. Kuhn et al: The first structure of a mature flavivirus, the dengue virion is shown consisting of 90 E dimers arranging into a smooth virus particle.

7. Modis et al: This paper shows the envelope protein structure changing from dimer to trimer after fusion.

8. Li, L et al. Key study demonstrating the structure and linkage of the PrM protein to the E protein.

15. Dejnirattisai et al: The study on human monoclonal antibodies revealed that there were high proportion of anti-prM antibodies. These antibodies did not effectively neutralized dengue virus but potently enhance the infection (antibody-dependent enhancement).

16. Dejnirattisai et al: This paper demonstrates mAbs against a previously unknown epitope; the envelope dimer epitope (EDE), are broadly neutralizing across the dengue serotypes.

42. Mongkolsapaya et al: These papers describe original antigenic sin in T cell responses to dengue infection.

44. Weiskopf et al: This paper shows cross reactive T cells from the first infection were expanded during the secondary infection, original antigenic sin. But the overall quality of the responses did not seem to be compromised.

45. Yauch et al: This paper shows the protective role of CD8 T cells in a murine model of dengue infection
48. Zompi et al.: This paper demonstrates that both cross reactive antibodies and T cells protected mice from secondary heterotypic dengue infection.

51. Rivino et al: This study demonstrates that dengue-specific T cells migrate to peripheral tissues such as the skin during the acute phase of infection.

66. Simmons et al and 68 Kliks et al: These studies demonstrate that maternal antibodies contribute to the development of the severe disease in infants experiencing primary dengue infection.


78. Rodenhuis-Zybert, I.A. et al. An interesting paper demonstrating immature dengue virions can be driven to become infectious through ADE in secondary infections.

80. Pierson et al 2007: This study measured the number of antibodies required for neutralisation and enhancement of infection.

93. Villar et al and 94. Capending et al: First ever phase 3 dengue vaccine studies, with an overall efficacy of 56.5% in Asian children and 64.7 % in Latin America, with serotype variation.

95. Hadinegoro et al: Three year follow-up of the CYD-TDV vaccine in 35,000 participants, confirming better efficacies in children >9 years old, with lower vaccine efficacy in children less than 9 years and increased hospitalisation in this age group.

105. Thomas and Rothman: A good review on the dengue vaccine development.

110. Sukupolvi-Petty et al: one of several studies from this group defined the epitopes recognized by protective antibodies. Furthermore the viral strains/genotypes had an influence on neutralizing potency.

120 Smith et al: Characterization of a panel of human monoclonal antibodies and identify an antibody recognizing all 4 dengue serotypes with potent neutralizing activity. Its epitope was on the bc loop of a monomeric E.

123. Lok, S. M: This interesting paper demonstrates the crystal structure of the mAb 1A1D2 binding to the viral E protein, which reorganizes to expose hidden epitopes suggesting that the virus can "breathe" to accommodate antibody binding.

129 Fibriansah et al, 138. Teoh et al, 139. Fibriansah et al, and 140. Fibriansah et al: These EM studies show the binding of potent serotype specific anti-dengue antibodies recognizing
conformation dependent epitopes. All but one recognize epitopes across 3 E monomers of the two adjacent dimers on viral particle.

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Glossary terms

• **Aedes mosquito vector:** The dengue virus is transmitted through the bite of infected Aedine mosquitoes; the primary vector being *Aedes aegypti* followed by *Aedes albopictus*.

• **Cytokine storm:** Excessive production of pro-inflammatory cytokines occurs during acute dengue infection and has been proposed to drive the vascular leak.

• **Original antigenic sin:** This refers to the immune response to challenge with a second pathogen with similar yet distinct sequences. Upon challenge, rather than making an entirely new response to the secondary antigen, crossreactive and potentially suboptimal memory cells generated to the primary challenge are expanded. Original antigenic sin was first described for antibody responses but has also been demonstrated in T cell responses.

• **Heterologous prime boost strategies:** This refers to a vaccination strategy whereby different immunogens are given sequentially to boost and focus an immune response to a given antigen. This can be done by utilising a variety of platforms; viral, DNA or protein, which share a common antigen being given in sequence.

• **TNF:** Tumour necrosis factor is an inflammatory cytokine

• **sTNFRI and sTNFRII:** Soluble forms of Tumour necrosis factor receptor type I and II

• **IFN-γ:** Interferon gamma

• **CXCL9, CXCL10 and CXCL11:** chemokine ligand 9, 10 and 11 belong to the CXC motif chemokine family which are chemoattractants induced by IFN-γ. Their receptor is CXCR3 expressed on several cells such as T cells and NK cells.

• **CXCL8:** Chemokine ligand 8 belongs to the CXC motif chemokine family. It is produced from many cell types such as macrophages and endothelial cells. There are many receptors for
this chemokine such as CXCR1 and CXCR2. CXCL8 is also known as IL-8 and considered a proinflammatory cytokine in several diseases.

- **CCL5**: Chemokine ligand 5, also known as RANTES, is a member of the CC motif chemokine family. It attracts T cells to inflammatory sites.

- **VEGF**: Vascular endothelial growth factor produced by several cell types, for example monocytes and endothelial cells. It is involved in angiogenesis and vascular permeability.

- **CLA**: Cutaneous lymphocyte-associated antigen

**Summary**

1. Dengue has four distinct viral serotypes and infection with one serotype results in the development of homotypic immunity. Subsequent infection with a different serotype is associated with an increased risk of developing severe disease, leading to the suggestion that severe disease is triggered by immunopathology.

2. Both T cell and B cell mediated arms of the adaptive immune system are thought to be involved in the immunopathology of severe dengue. Both antibody dependent enhancement and a skewed T cell response through original antigenic may play a role in disease pathogenesis in secondary infections.

3. There are several vaccine candidate in development, the most advanced in clinical trials is the Sanofi Pasteur-vaccine CYD-TDV. A recent 3 year follow-up study demonstrated an overall vaccine efficacy of 65%, with lower efficacies and higher hospitalizations in children less than 9 years old.

4. A large number of dengue monoclonal antibodies have been recently been described with antibodies targeting E protein Domain III being amongst the most potently neutralising, but often serotype specific. Recently however a broadly neutralizing antibody directed towards the E protein dimer epitope (EDE) have been discovered.

5. Antibodies directed against prM protein can facilitate FcR mediated uptake of immature viral particles, which although non-infectious in the absence of antibody, can undergo prM cleavage following endocytosis in the host cell rendering them infectious.

6. Future vaccines need to target potently neutralising epitopes such as those found on Domain E III or the quaternary epitopes such as EDE and minimize poorly neutralizing and potentially enhancing prM or FLE antibodies.
Figure legends

**Figure 1A DENV life cycle** (1) The dengue viral replication process begins when the virion attaches directly to a diverse group of cellular receptors or the Fc part of the dengue immune complex attaches to Fc receptor on the target cells and (2) subsequently enters the cell by receptor-mediated endocytosis. (3) Acidification of the endosomal vesicles triggers conformational changes in the virion resulting in an irreversible trimerization of the viral E protein. This exposes the fusion protein and mediates the fusion between the viral and the endosomal membranes, allowing the release of nucleocapsid into the cytoplasm. The viral RNA is released into the cytoplasm and presented to rough endoplasmic reticulum (rER). (4) Here, viral RNA is translated into a single polyprotein which is processed by viral and host proteases. (5) After the viral replication complex is synthesized, viral RNA translation switches off and RNA synthesis begins by the transcription of an antisense viral RNA followed by the amplification of viral RNA. (6) The newly synthesized RNA is subsequently packaged by Capsid protein forming a nucleocapsid. (7) Virus assembly occurs on the surface of the ER when nucleocapsid buds into the ER lumen resulting in non-infectious, immature viral particles. (8) Immature viral particles are transported through the Golgi into the trans-Golgi network (TGN), where acidification induces conformational changes of the virion exposing the furin cleavage sites. Afterwards the host protease furin cleaves between pr and M proteins, with the pr remaining associated until the virion is released to the neutral extracellular milieu. Most notably, the cleavage of the dengue virus between pr and M proteins was consistently found ineffective in many cell types resulting in the mixture of maturation levels of dengue virions, (9) which are subsequently released from infected cells by exocytosis pathway.

**Figure 1B-D Dengue virus structure** B) The structure of E dimer shows domain I, II and III in red, yellow and blue. Balls and sticks represent two glycan in red and yellow (reproduced with permission from Modis et al) C) The structure of immature dengue particle shows the arrangement of E and prM in light blue (reproduced with permission from Mackenzie NM et al) D) the mature dengue virus shows E arranged into a smooth particle (reproduced with permission from Kuhn et al).

**Figure 2 T Cell responses.** A) The phenomenon of original antigenic sin (OAS) occurs when preferential memory cell activation in the presence of sequence variation leads to cross-reactive T cells generated during a primary infection dominating during a secondary infection. If there is a high proportion of low avidity cross reactive T cells and are skewed to inflammation cytokine production without cytotoxicity, it may predispose to immunopathology. B) Double tetramer staining of PBMC taken from a dengue-infected patient showed original antigenic sin in T cell response in
heterologous secondary infection (modified with permission from Mongkolsapaya et al42 C) Many cross reactive T cells and T cells having high avidity to the first infection showed poor responses to the secondary infecting serotype compared to the presumed primary infecting serotype (modified with permission from Mongkolsapaya et al43. Figure 2D Adaptive immune responses and dengue immunopathogenesis. The mixture of newly produced dengue virions can be neutralised by the optimum concentration of anti-DENV antibodies. In addition, DENV proteins (E/prM/NS1), presented on the surface of virus infected cells, or soluble NS1 can be recognised by dengue specific antibodies resulting in complement dependent cell lysis and complement activation by classical pathway or antibody dependent cell cytotoxicity (ADCC) mediated by NK cells. sNS1 can also bind to heparan sulfate, a major component of the endothelial glyocalyx layer that regulates capillary permeability. The subsequent recognition by anti-NS1 antibodies triggers complement activation and anaphylatoxins which may contribute to the disruption of this layer and vascular leak.

Moreover, the viral proteins can be intracellularly processed into small peptides presented on MHC class I or MHC class II which are recognised by DENV specific CD8 T cells or CD4 T cells, respectively. The recognitions of MHC-peptide complexes by dengue serotype specific T cells or “good” dengue serotype cross reactive T cells result in target cell lysis with or without cytokine production whilst the recognitions of these by “bad” dengue serotype cross reactive T cells cause only cytokine production. Instead of the viral blocking, subneutralising concentrations of anti-E and/or anti-prM can bind to the dengue virus mediating dengue virus infection in Fc receptors bearing target cells such as monocytes and macrophages resulting in the increase inviral load by the process of antibody dependent enhancement (ADE). The cytokines secreted by dengue infected cells and cytokines produced from T cells and NK cells which can be amplified by the high viral load causing the cytokine storm and plasma leakage in dengue infected patients.

Figure 3 Dengue vaccines

1) The Sanofi Pasteur vaccine contains four chimeric live flaviviruses, each derived from genome of the yellow fever virus 17D vaccine strain with the precursor membrane (prM) and envelope (E) gene segments replaced by the corresponding gene segments of each of the four dengue virus serotypes (DENV-1 to DENV-4).

2) The US National Institutes of Health (NIH) vaccine contains a mixture of four recombinant dengue virus genomes; the DENV-2 component is a chimeric dengue virus derived from a DENV-4 genome with pre-M and E gene segments replaced by those of DENV-2. The vaccine were attenuated by deleting 30 nucleotide (Δ30) from the 3’UTR of DENV genome.
3) DenVax vaccine from Takeda contains a mixture of four recombinant DENV-2 genomes; each derived from genome of an attenuated DEN-2 virus with prM and E gene segment replaced by the corresponding gene segment of the DENV-1, DENV-3 and DENV-4.

Figure 4 Quaternary Epitopes. A) The cryo-EM structure shows HM14c10 binds the epitope between 2 E monomers of adjacent dimers (reproduced with permission from Teoh et al\textsuperscript{138}. B) the structure of and C) the cryo-EM reconstruction of DENV-2 particle in complex with anti-EDE mAb demonstrate the epitope of anti-EDE antibody is across 2 E within 1 dimer. The colour scale in C indicates the radial depth from inside (red) to the outer shell (yellow, green and blue) (reproduced with permission from Dejnirattisai et al\textsuperscript{16} and Rouvinski et al\textsuperscript{141}. Domain I, II and III of E protein are indicated in red, yellow and blue. On top view, grey and green ovals showed the binding areas of heavy and light chains of the anti-EDE mAb.
<table>
<thead>
<tr>
<th>Vaccine candidate</th>
<th>Phase</th>
<th>Trial Country</th>
<th>Age</th>
<th>Trial design</th>
<th>Results</th>
<th>Side effects</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>CYD-TDV (Sanofi-pasteur)</td>
<td>Phase III</td>
<td>Latin America</td>
<td>Children 9-16 yrs</td>
<td>Vaccination at 0, 6, 12 months Follow up 25 months after final vaccination Incidence density dengue 2.9/100 person years</td>
<td>Overall efficacy 60.8% [52-68] Efficacy against • DENV 1 50.3% [29.1-65.2] • DENV 2 42.3% [14.0-61.1] • DENV 3 74.0% [61.9-82.4] • DENV 4 77.0% [60.2-88.0] Seropositive at baseline 83.7% Seronegative at baseline 43.2% severe disease 91.7% [31.4-99.8] DHF 90% [10.7-99.8]</td>
<td>4 SAE** • Asthma • Urticaria • Acute peripheral polyneuropathy and viral meningitis • Seizure No long term sequelae</td>
<td>Villar et al\textsuperscript{92}</td>
</tr>
<tr>
<td>CYD-TDV (Sanofi-pasteur)</td>
<td>Phase III</td>
<td>Asia Pacific</td>
<td>Children 2-14 yrs</td>
<td>Vaccination at 0, 6, 12 months Follow up 25 months after final vaccination Incidence density dengue 4.7%</td>
<td>Overall efficacy 56.5% [43.8-66.4] Efficacy against • 2-5 yrs 33.7% • 6-11 yrs 59.5% • 12-14 yrs 74% • DENV 1 50.0% [24.6-66.8] • DENV 2 35.0% [-9.2-61.0] • DENV 3 75.3% [52.9-90.8] • DENV 4 66.7% [54.5-87.0] Seropositive at baseline 74.3% Seronegative at baseline 35.5% Against severe disease 80.38% Against DHF 88.5%</td>
<td>1 SAE</td>
<td>Acute disseminated encephalomyelitis with no long term sequelae</td>
</tr>
<tr>
<td>CYD-TDV (Sanofi-pasteur)</td>
<td>Phase IIb</td>
<td>Thailand</td>
<td>Children 4-11 yrs</td>
<td>CYD-TDV at 0, 6, 12 months</td>
<td>Overall Efficacy 30.2% [-13.4-56.6] Efficacy against</td>
<td>No attributable SAE</td>
<td>Sabchareon et al\textsuperscript{92}</td>
</tr>
<tr>
<td>Vaccine Type</td>
<td>Phase</td>
<td>Location</td>
<td>Age Group</td>
<td>Details</td>
<td>Efficacy</td>
<td>Adverse Events</td>
<td>References</td>
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<tr>
<td>TDEN (WRAIR/GSK)</td>
<td>Phase II</td>
<td>Thailand</td>
<td>Adults 20-24</td>
<td>Pre-vaccination: 76.5% (F17) and 78.9% (F19) seropositive to all 4 serotypes Post-vaccination: 97.1% F17 and 100% F19 seropositive to all 4 serotypes</td>
<td>Viremia 5/80 Rash &gt;50% body in 5%</td>
<td>Watanaveeradej et al.</td>
<td></td>
</tr>
<tr>
<td>NIH live attenuated tetravalent vaccine (NIH LATV Δ30)***</td>
<td>Phase I</td>
<td>USA</td>
<td>Adults 18-50yrs</td>
<td>TV003 was the best formula with 45% of vaccinees had neutralizing antibodies against all 4 serotypes.</td>
<td>64% rash Viraemia in 73% of vaccinees</td>
<td>Durbin et al.</td>
<td></td>
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<tr>
<td>DENVax (Takeda)***</td>
<td>Phase I</td>
<td>Colombia</td>
<td>Adults 18-45yrs</td>
<td>71% seroconverted to all 4 serotype in the intradermal group Neutralizing antibody titre to DENV 2 was the highest</td>
<td>No SAE Overall viraemia found ranging 43% (low dose) to 85% (high dose)</td>
<td>Osorio et al.</td>
<td></td>
</tr>
<tr>
<td>Soluble E protein (Merck)</td>
<td>In Phase I</td>
<td></td>
<td></td>
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<tr>
<td>Purified inactivated virus (WRAIR)</td>
<td>In Phase I</td>
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<tr>
<td>DNA expressing prM and E (NMRC)</td>
<td>In Phase I</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

*95% CI: 95% confidential interval

**SAE: Serious adverse event

*** under phase II trial
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody name</th>
<th>Cross reactivity</th>
<th>NT50 ug/ml</th>
<th>In vivo protection</th>
<th>Structure number</th>
<th>accession number</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>prM</td>
<td>several mAbs</td>
<td>DENV1-4*</td>
<td></td>
<td>weak (hardly provide fully neutralisation)</td>
<td>-</td>
<td>-</td>
<td>Beltramellopp, Dejnirattisai, De Alwis, Smith</td>
</tr>
<tr>
<td>Envelope fusion loop (amino acid 98-113)</td>
<td>several mAbs</td>
<td>DENV1-4*</td>
<td>0.016 - &gt;10ug/ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Williams, Smith, Dejnirattisai, Smith, Beltramellopp</td>
</tr>
<tr>
<td>Envelope bc loop domain II (amino acid 73,78,79)</td>
<td>1C19</td>
<td>DENV1-4</td>
<td>0.01-0.05</td>
<td>showing prophylactic activity against DENV 1 and DEVN 2 on AG129 mice***</td>
<td>-</td>
<td>-</td>
<td>Smith</td>
</tr>
<tr>
<td>Envelope domain III</td>
<td>serotype specific and cross reactive</td>
<td>DENV1</td>
<td>strong (mostly type-specific) to weak (cross reactive) &lt;0.07-&gt;10</td>
<td>-</td>
<td>-</td>
<td>Beltramellopp, De Alwis, Smith</td>
<td></td>
</tr>
<tr>
<td>Quaternary epitopes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Envelope herringbone epitope (DI, DI/II hinge and DIII)**</td>
<td>HM14c10</td>
<td>DENV1</td>
<td>0.005-1.503</td>
<td>showing both prophylactic and therapeutic activity against DENV 1 on AG129 mice***</td>
<td>EMD-5268</td>
<td>Teoh</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Characteristic of human monoclonal antibodies

* NT50: Neutralisation T50
** Secreted proteins
*** AG129 mice: Animal model for protective activity
<table>
<thead>
<tr>
<th>Envelope harrington binding epitope (D/DII hinge, DII fusion loop and DIII)</th>
<th>5J7</th>
<th>DENV3</th>
<th>0.1</th>
<th>-</th>
<th>EMD-5935</th>
<th>de Alwis(^{150}), Smith(^{135}), Fibrinsah(^{140})</th>
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</thead>
<tbody>
<tr>
<td>Envelope (the epitope is on the monomer DI, DII/III hinge, DIII in the context of virion only)</td>
<td>1F4</td>
<td>DENV1</td>
<td>0.11</td>
<td>showing prophylactic activity against DENV 1 on AG129 mice***</td>
<td>EMD-2442 and PDB 4C2</td>
<td>de Alwis(^{150}), Fibrinsah(^{149})</td>
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<tr>
<td>Envelope dimer epitope (EDE) (domain I, II and III)</td>
<td>752-2 C8</td>
<td>DENV1-4</td>
<td>0.59-0.17</td>
<td>-</td>
<td>PDB 4UTA</td>
<td>Dejnirattisai(^{13}), Rouvinski(^{141})</td>
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<tr>
<td>Envelope dimer epitope (EDE) (domain I, II and III)</td>
<td>753(3) C10</td>
<td>DENV1-4</td>
<td>0.012-0.28</td>
<td>-</td>
<td>PDB 4UT9</td>
<td>Dejnirattisai(^{16}), Rouvinski(^{141})</td>
</tr>
<tr>
<td>Envelope dimer epitope (EDE) (domain I, II and III)</td>
<td>747 B7</td>
<td>DENV1-4</td>
<td>0.015-1.27</td>
<td>-</td>
<td>PDB 4UT6</td>
<td>Dejnirattisai(^{16}), Rouvinski(^{141})</td>
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<tr>
<td>Envelope dimer epitope (EDE) (domain I, II and III)</td>
<td>747(4) A11</td>
<td>DENV1-4</td>
<td>0.011-1.17</td>
<td>-</td>
<td>PDB 4UT7</td>
<td>Dejnirattisai(^{16}), Rouvinski(^{141})</td>
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<tr>
<td>Envelope dimer epitope (EDE) (domain I, II and III)</td>
<td>2D22</td>
<td>DENV2</td>
<td>0.08</td>
<td>showing both prophylactic and therapeutic activity against DENV 2 on AG129 mice***</td>
<td>EMD-2967, EMD-2996, EMD-2997, EMD-2998, and EMD-2999</td>
<td>de alwis(^{146}), Fibrinsah(^{18})</td>
</tr>
</tbody>
</table>

- characteristic of majority
- ** DI, DII and DIII for domain I, II and III of E protein
- *** Ag129 mice: mice lack Type I and type II interferon receptors
Mature particle at neutral pH

Immature particle at neutral pH

Figure 1B-D
Effective cross-reactive T cell

DENV serotype A infection

DENV serotype B infection

DENV serotype A infection followed by DENV serotype B infection

Ineffective cross-reactive T cell (producing inflammatory cytokine without cytotoxicity)

Efficient target cell killing

Inflamatory cytokines

DENV serotype A – infected cell

DENV serotype B – infected cell

High proportion of ineffective cross reactive T cells: inefficient in control viral infection

High proportion of effective cross reactive T cells: efficient in control viral infection

Primary infection: Den 1

Secondary infection: Den 2

Figure 2A-C
CYD-TYD (from SanofiPasteur)

DENV1 5' C prM E Nonstructural genes 3'
DENV2 5' C prM E Nonstructural genes 3'
DENV3 5' C prM E Nonstructural genes 3'
DENV4 5' C prM E Nonstructural genes 3'

Live attenuated Δ30 Vaccine (from NIH)

DENV1 5' C prM E Nonstructural genes Δ30 3'
DENV2 5' C prM E Nonstructural genes Δ30 3'
DENV3 5' C prM E Nonstructural genes Δ30 3'
DENV4 5' C prM E Nonstructural genes Δ30 3'

Figure 3
DENVax (from Takeda)

![Diagram showing the genome organization of DENV1, DENV2, DENV3, and DENV4](image)

**Figure 3 continued**
Figure 4