

Functional antibody responses and protection against symptomatic dengue

Corresponding Author: Dr Andrew Teo

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Synopsis: This review article covers the humoral immunity in dengue by discussing the antibody-associated protective or disease-enhancing mechanisms leading to symptomatic dengue infections and severe disease in a subset of infected cases. The review briefly discusses about the current status of dengue vaccines and the antibody response at the beginning followed by the major antigen targets involved in induction of antibody responses. The functional aspects such as virus neutralization potential, affinity and avidity and the effector functions mediated through Fcγ receptor binding, including antibody-dependent enhancement has been discussed. A section on various assays to measure the antibody functions and the critical parameters affecting the read out has also been highlighted.

Major comments:

1. The review discusses antibody response in dengue vaccines both in the first paragraph and before the conclusion which makes it repetitive. Please consider discussing vaccine-induced antibody responses comprehensively in one section. It is important to highlight the issues such as variable neutralizing antibody responses with respect to all the four serotypes, lack of dengue NS proteins in Dengvaxia, which are key for T cell-mediated protection.
2. The abstract says that the “antibody functions and effector mechanisms in DENV infection in the context of both naturally acquired and vaccine-induced immunity will be discussed”. However, there is very little discussion on these aspects in vaccinated cases.
3. There is no section clearly highlighting the “knowledge gaps” in antibody functions. The outstanding questions may be revised as “knowledge gaps” and specific strategies to address these gaps may be discussed.
4. Although the review is about antibody responses, the review would be incomplete without discussion on the cellular immunity and brief mention about the original antigenic sin.
5. Line 343-344 is factually incorrect. It is important to discuss the data generated during the development stages of the three vaccine candidates which culminated in the licensure of these three vaccines.
6. The section “Priorities in evaluating functional antibodies in dengue” is misleading as the section is a description of various assays performed to assess antibody-mediated effector functions.
7. While there is emphasis on ADE, it is important to indicate that only about 10% of the symptomatic dengue cases experience severe disease. It is unlikely that ADE is the only mechanism behind severe disease. There are many reports of severe disease in primary dengue.
8. The review somehow appears to suggest that there are two categories of antibodies, neutralizing antibodies and the Fc-mediated disease enhancing antibodies. It is important to stress that below a certain threshold, the same antibody may turn from being protective to disease-enhancing.

Minor comments

1. In line 20, the authors need to specify that IgGs may lead to ADE in secondary infections only.
2. In line 49, it would be informative to indicate that there is no efficacy data for two serotypes for Qdenga (seronegative) and Butantan-DV under the vaccine-induced immune responses.
3. In Line 66, targets of antibody-mediated immunity against DENV section, the authors have covered different structural domains present on the virus but have not mentioned about the conformational topology associated with mature and

immature virions. Dengue virus breathing exposes broadly neutralizing or weakly neutralizing, the antibodies raised against these regions playing a role in neutralization or infection enhancement. The authors may introduce the concept in the review and also add remarks on virus breathing among cross-reactive viruses influencing antibody mechanisms.

4. In line 83-83, it is suggested that antibodies against immunodominant proteins such as NS5 and NS3 would have protective functions. There is no evidence to support this.

5. References to severe disease need to clarify that these discussions are in the context of secondary dengue

6. Afucosylated antibodies appear to be both beneficial (NK cell activation) and detrimental. This needs to be elaborated.

Are these exclusive processes mediated by different group of antibodies? What is the likely regulatory mechanism favouring one process over the other? The authors should discuss the recent reports on afucosylated antibodies in vaccinated subjects (Sci Transl Med. 2025 September 24; 17(817)).

7. Similarly, how to reconcile the pathogenic associations of anti-NS-1 antibodies if they are also beneficial in clearing NS-1 which is linked to pathogenicity? These associations have very weak mechanistic evidence.

8. Line 384-386 does not appear to be plausible

9. Line 387 is not feasible for a quadrivalent vaccine

10. The authors may also include how antigenic variation or heterogeneity within- and between-serotypes can also influence the antibody functions.

11. The authors may include remarks on how immune exhaustion or comorbid conditions adds to antibody mediated severe disease.

Reviewer #2

(Remarks to the Author)

The manuscript entitled of “Functional antibody responses and protection against symptomatic dengue” gave an intensive review of dengue virus-specific antibodies. Profiles of anti-dengue antibodies were reported in terms of both characteristics (target proteins, serotype cross-reactivities and subclasses) and functions (neutralization, antibody dependent enhancements and Fc dependent effector functions). The reviews covered DENV E protein specific antibodies and DENV NS1 specific antibodies. The authors also proposed the important questions that need to be answered in the field. Although, the manuscript provides comprehensive review of anti-dengue antibodies. Several information is still missing or have to be stated with caution that needs to be discussed and clarified prior to considering for publication.

Major concerns

Part Dengue, vaccination and antibody-mediated immunity

1. Sanofi Pasteur announced that production of Dengvaxia is terminated. This should be included in the manuscript.

2. Line 53... as individuals with high PRNT may still experience symptomatic diseases (Odio, C. D., et al (2025). It would be more precise if the author added that “neutralizing antibody titres that correlated with protection are from the assay which utilize mature dengue viruses” which stated in the cited reference.

Part Targets of antibody-mediated immunity against DV

1. Amino acid sequence homology between dengue serotypes should be included since this largely impact antibody responses in dengue virus.

Part Functional diversity of anti-dengue antibodies

Neutralizing antibodies targeting E protein

Line 105-107 ... More recently, bivalent vaccine formulations targeting both the EDIII domain and the NS1 protein have been shown to elicit strong antigen-specific IgG responses with potent neutralising and ADCC activities against DENV infection (25, 26). The cited references did not demonstrate ADCC activities. Please clarify.

Line 112-113...Although anti-EDIII antibodies can neutralise DENV, they are predominantly serotype-specific and generally present at low titres in dengue patients (ref 27-Sarker, A., Dhama, N. & Gupta, R.D 2023 Front Immunology.1200195). This statement is not exactly precise since there is a publication that demonstrated anti-Dengue EDIII antibodies in dengue infected patients, especially those who have secondary infection are serotype cross-reactive. Please see Midgley, C. M., et al. (2011). An in-depth analysis of original antigenic sin in dengue virus infection. Journal of virology, 85(1), 410–421.

Part Neutralising antibodies targeting quaternary epitopes

Line 123quaternary epitope spanning EDI and EDII of adjacent E protein monomers.. This statement is not true because quaternary epitopes span all three domains of dengue E protein.

Part Antibodies against NS1 proteins – protection or pathology?

It would be strengthening the scientific impact of the manuscript if the author includes recent publication related to DENV NS1 antibodies such as a study demonstrating NS1 antibody levels, subclasses in Thai pediatric cohorts by Noisakran, S. et al. (2025). Anti-Nonstructural Protein 1 Antibody Kinetics and Their Association With Disease Severity in Pediatric Dengue. Open forum infectious diseases, 12(9).

Part Priorities in evaluating functional antibodies in dengue

Line 284-286 ...However, the maturation state of the viruses is influenced by culture conditions such as temperature and duration, which can alter the ratio of immature to mature virions and consequently affects the display of epitopes.... The target cell is also important to obtain mature or immature dengue virion. Please discuss.

Minor concern
Line 326.....Butantan-DV not Butanta-DV

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Medicine initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I thank the authors for considering my suggestions and revising the review. The authors have addressed all my queries.

Reviewer #2

(Remarks to the Author)

The author has addressed all the concerns, and I endorse this manuscript for publication.

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We thank the reviewers for their suggestions to improve the manuscript and we have addressed all suggestions to our best of ability.

Reviewer #1 (Remarks to the Author):

Synopsis: This review article covers the humoral immunity in dengue by discussing the antibody-associated protective or disease-enhancing mechanisms leading to symptomatic dengue infections and severe disease in a subset of infected cases. The review briefly discusses about the current status of dengue vaccines and the antibody response at the beginning followed by the major antigen targets involved in induction of antibody responses. The functional aspects such as virus neutralization potential, affinity and avidity and the effector functions mediated through Fcγ receptor binding, including antibody-dependent enhancement has been discussed. A section on various assays to measure the antibody functions and the critical parameters affecting the read out has also been highlighted.

Major comments:

1. The review discusses antibody response in dengue vaccines both in the first paragraph and before the conclusion which makes it repetitive. Please consider discussing vaccine-induced antibody responses comprehensively in one section. It is important to highlight the issues such as variable neutralizing antibody responses with respect to all the four serotypes, lack of dengue NS proteins in Dengvaxia, which are key for T cell-mediated protection.

We thank the reviewer for this recommendation. In the first paragraph, we provided a brief overview, we have removed the repetitive sentence and have updated the text in Section: Dengue vaccines and antibody immunity Line 382

2. The abstract says that the “antibody functions and effector mechanisms in DENV infection in the context of both naturally acquired and vaccine-induced immunity will be discussed”. However, there is very little discussion on these aspects in vaccinated cases.

The vaccine section has been expanded to include a more detailed discussion of antibody responses induced by CYD-TDV, TAK-003 and Butantan-DV Line 382. As studies of vaccine-induced antibody responses have largely focused on neutralising antibodies, with limited information on other antibody functions, the Abstract has also been revised to better reflect the scope of the review: “In this review, antibody functions and effector mechanisms in DENV infection are discussed, with a focus on naturally acquired immunity and neutralising antibody responses following vaccination.” Line 25

3. There is no section clearly highlighting the “knowledge gaps” in antibody functions. The outstanding questions may be revised as “knowledge gaps” and specific strategies to address these gaps may be discussed.

We thank the reviewer for this suggestion. The previous “Outstanding Questions” section has been revised as “Knowledge Gaps and Future Directions” to more clearly highlight gaps in our current understanding of antibody functions in DENV infection, including vaccine-induced antibody responses, Fc-mediated functions and determinants of antibody-mediated protection. Where appropriate, possible approaches to address these gaps have also been included Line 431

4. Although the review is about antibody responses, the review would be incomplete without discussion on the cellular immunity and brief mention about the original antigenic sin.

We thank the reviewer for this suggestion. While cellular immunity is an important component of the immune response to DENV, an extended discussion of cellular responses is beyond the scope of this review, which focuses on antibody-mediated immunity. We have, however, added a brief explanation of original antigenic sin or immune imprinting. Line 42

5. Line 343-344 is factually incorrect. It is important to discuss the data generated during the development stages of the three vaccine candidates which culminated in the licensure of these three vaccines.

We thank the reviewer for pointing out the discrepancy. The statement in line 343-44, was intended to be about the Butantan-DV vaccine for which long term antibody data has not yet been published. We have modified this Line 416

6. The section “Priorities in evaluating functional antibodies in dengue” is misleading as the section is a description of various assays performed to assess antibody-mediated effector functions.

We agree that the previous heading did not accurately reflect the content of the section. The section has therefore been renamed “Methods for evaluating functional antibodies in dengue” to better reflect the assays and approaches discussed. Line 329

7. While there is emphasis on ADE, it is important to indicate that only about 10% of the symptomatic dengue cases experience severe disease. It is unlikely that ADE is the only mechanism behind severe disease. There are many reports of severe disease in primary dengue.

We agree that ADE is not the sole mechanism underlying severe dengue and that severe disease can also occur during primary DENV infection. We have revised the ADE section to clarify this point as follows:

“Although ADE is not the sole mechanism underlying severe dengue, as severe disease can also occur during primary DENV infection, it remains an important contributor to disease pathogenesis, particularly during secondary infection.” Line 247.

8. The review somehow appears to suggest that there are two categories of antibodies, neutralizing antibodies and the Fc-mediated disease enhancing antibodies. It is important to stress that below a certain threshold, the same antibody may turn from being protective to disease-enhancing.

We would like to clarify that the review does not intend to categorise neutralising and Fc-mediated antibodies as separate protective and disease-enhancing antibody populations. Rather, the Fc-mediated antibody functions are discussed in the context of both protective immunity and disease pathogenesis. We agree that the functional activity of an antibody (e.g. neutralising antibody) may vary depending on its concentration. We have therefore revised the ADE section to clarify that

neutralising antibodies have also been shown to enhance DENV infection at sub-neutralising concentrations. Line 250

Minor comments

1. In line 20, the authors need to specify that IgGs may lead to ADE in secondary infections only.
We have revised the relevant abstract sentence to specify "...and may also contribute to antibody-dependent enhancement in a secondary heterotypic infection." Line 20
2. In line 49, it would be informative to indicate that there is no efficacy data for two serotypes for Qdenga (seronegative) and Butantan-DV under the vaccine-induced immune responses.

We have updated according in the section "Dengue vaccines and antibody immunity" Line 382

3. In Line 66, targets of antibody-mediated immunity against DENV section, the authors have covered different structural domains present on the virus but have not mentioned about the conformational topology associated with mature and immature virions. Dengue virus breathing exposes broadly neutralizing or weakly neutralizing, the antibodies raised against these regions playing a role in neutralization or infection enhancement. The authors may introduce the concept in the review and also add remarks on virus breathing among cross-reactive viruses influencing antibody mechanisms.
We have updated the texts accordingly, Line 66 - 87

4. In line 83-83, it is suggested that antibodies against immunodominant proteins such as NS5 and NS3 would have protective functions. There is no evidence to support this.

We thank the reviewer for this comment. We agree that the previous wording did not adequately distinguish between antibody targets associated with protection and those with other functional outcomes. The sentence has therefore been revised "Although, antibodies against immunodominant proteins such as E and NS1 are established correlates of protection against symptomatic dengue, the protective efficacy of antibodies against other NS proteins (NS3 and NS5) remains less defined." Line 92

5. References to severe disease need to clarify that these discussions are in the context of secondary dengue

We have revised the relevant sections to clarify that associations with severe disease are discussed in the context of secondary DENV infection where supported by the cited studies. Line 158 - PMID: 26209381; Line 240 - PMID: 34083490, PMID: 28126818; Line 277 - PMID: 30531923

6. Afucosylated antibodies appear to be both beneficial (NK cell activation) and detrimental. This needs to be elaborated. Are these exclusive processes mediated by different group of antibodies? What is the likely regulatory mechanism favouring one process over the other? The authors should discuss the recent reports on afucosylated antibodies in vaccinated subjects (Sci Transl Med. 2025 September 24; 17(817)).

We thank the reviewer for this important comment. We have expanded this section to clarify that "While afucosylated IgG may enhance NK cell-mediated ADCC, its contribution to protection during DENV infection remains unclear. Whether the opposing effects of afucosylated antibodies are mediated by distinct antibody populations or influenced by antibody neutralising capacity and the

FcγRIIIa-expressing cell type also remains unknown. As suggested, we have incorporated the recent study by Ashraf *et al.* (2025), which links elevated IgG1 afucosylation with increased susceptibility to DENV infection following Dengvaxia vaccination and provides evidence for FcγRIIIa-dependent enhancement of viral replication”. Line 246-255

7. Similarly, how to reconcile the pathogenic associations of anti-NS-1 antibodies if they are also beneficial in clearing NS-1 which is linked to pathogenicity? These associations have very weak mechanistic evidence.

We agree that the mechanisms determining the protective versus pathogenic effects of anti-NS1 antibodies remain unclear. We have revised the section to distinguish experimentally demonstrated mechanisms from clinical associations and to clarify that a potential role in reducing circulating NS1 does not necessarily translate into clinical protection. We also discuss epitope specificity as a potential contributor to these divergent effects, while acknowledging that this remains to be established in human dengue. Line 256

8. Line 384-386 does not appear to be plausible

While antibodies to reduce transmission to vector is less studied, there are several pieces of evidence to suggest this mechanism. We highlighted this as a Knowledge gaps. Line 434 – PMID: 31675360, PMID: 24550728

9. Line 387 is not feasible for a quadrivalent vaccine

This sentence is only intended to speculate about a hypothesis, from lessons we learned from COVID-19 vaccinations - PMID: 36195473. We have excluded this sentence for clarity.

10. The authors may also include how antigenic variation or heterogeneity within- and between-serotypes can also influence the antibody functions.

We have briefly addressed both inter- and intra-serotype variation, noting that sequence variation between DENV serotypes contributes to cross-reactive antibody responses, while genetic variation within individual serotypes can affect antibody neutralisation. A more detailed discussion of DENV antigenic heterogeneity is beyond the scope of this review, which focuses primarily on antibody functions associated with protection and pathogenesis. Line 69

11. The authors may include remarks on how immune exhaustion or comorbid conditions adds to antibody mediated severe disease

We thank the reviewer for this suggestion. While immune exhaustion and comorbidities may influence dengue disease outcome, these topics extend beyond the scope of this review, which focuses on antibody functions associated with protection and pathogenesis. We therefore have not expanded these topics, as doing so would substantially broaden the scope of the review.

Reviewer #2 (Remarks to the Author):

The manuscript entitled of “Functional antibody responses and protection against symptomatic

dengue” gave an intensive review of dengue virus-specific antibodies. Profiles of anti-dengue antibodies were reported in terms of both characteristics (target proteins, serotype cross-reactivities and subclasses) and functions (neutralization, antibody dependent enhancements and Fc dependent effector functions). The reviews covered DENV E protein specific antibodies and DENV NS1 specific antibodies. The authors also proposed the important questions that need to be answered in the field. Although, the manuscript provides comprehensive review of anti-dengue antibodies. Several information is still missing or have to be stated with caution that needs to be discussed and clarified prior to considering for publication.

Major concerns

Part Dengue, vaccination and antibody-mediated immunity

1. Sanofi Pasteur announced that production of Dengvaxia is terminated. This should be included in the manuscript.

We have updated this in under the section “Dengue vaccines and immunity” Line 394

2. Line 53... as individuals with high PRNT may still experience symptomatic diseases (Odio, C. D., et al (2025). It would be more precise if the author added that “neutralizing antibody titres that correlated with protection are from the assay which utilize mature dengue viruses” which stated in the cited reference.

We thank the reviewer for this comment. It now reads “... with high PRNT, mature dengue virions may still experience symptomatic dengue. Line 52

Part Targets of antibody-mediated immunity against DV

1. Amino acid sequence homology between dengue serotypes should be included since this largely impact antibody responses in dengue virus.

The reviewer has pointed out an important point, we have included in “The four DENV serotypes share approximately 65-70% amino acid sequence identity, contributing to cross-reactive antibody responses that may mediate protective neutralisation or, under certain conditions, ADE.”. Line 68

Part Functional diversity of anti-dengue antibodies

Neutralizing antibodies targeting E protein

Line 105-107 ... More recently, bivalent vaccine formulations targeting both the EDIII domain and the NS1 protein have been shown to elicit strong antigen-specific IgG responses with potent neutralising and ADCC activities against DENV infection (25, 26). The cited references did not demonstrate ADCC activities. Please clarify.

We thank the reviewer for identifying this error. “ADCC” was a typographical error and has been corrected to “complement-dependent cytotoxicity,” which accurately reflects the activity demonstrated in the cited study Line 119

Line 112-113...Although anti-EDIII antibodies can neutralise DENV, they are predominantly serotype-specific and generally present at low titres in dengue patients (ref 27-Sarker, A., Dhama, N. & Gupta, R.D 2023 Front Immunology.1200195). This statement is not exactly precise since there is a publication that demonstrated anti-Dengue EDIII antibodies in dengue infected patients, especially those who have secondary infection are serotype cross-reactive. Please see Midgley, C. M., et al. (2011). An in-depth analysis of original antigenic sin in dengue virus infection. Journal of virology, 85(1), 410–421.

We thank the reviewer for this comment. We have revised the text to acknowledge that anti-EDIII antibodies can exhibit cross-reactivity across DENV serotypes, particularly during secondary infection and have cited the publication. Line 126

Part Neutralising antibodies targeting quaternary epitopes

Line 123quaternary epitope spanning EDI and EDII of adjacent E protein monomers..
This statement is not true because quaternary epitopes span all three domains of dengue E protein. We have corrected the text to clarify that quaternary epitopes can span all three E protein domains (EDI, EDII and EDIII) across adjacent E protein monomers. Line 137

Part Antibodies against NS1 proteins – protection or pathology?

It would be strengthening the scientific impact of the manuscript if the author includes recent publication related to DENV NS1 antibodies such as a study demonstrating NS1 antibody levels, subclasses in Thai pediatric cohorts by Noisakran, S. et al. (2025). Anti-Nonstructural Protein 1 Antibody Kinetics and Their Association With Disease Severity in Pediatric Dengue. Open forum infectious diseases, 12(9).

We have now incorporated the study by Noisakran *et al.* (2025) and included their findings that anti-NS1 antibody isotypes and IgG subclasses were differentially associated with disease severity, with higher anti-NS1 IgG1, IgG4 and IgA levels observed in patients with dengue haemorrhagic fever, whereas anti-NS1 IgM levels were higher in patients with dengue fever. Line 272

Part Priorities in evaluating functional antibodies in dengue

Line 284-286 ...However, the maturation state of the viruses is influenced by culture conditions such as temperature and duration, which can alter the ratio of immature to mature virions and consequently affects the display of epitopes.... The target cell is also important to obtain mature or immature dengue virion. Please discuss.

We have revised the text to include the cell line used for virus propagation as an additional factor

that can influence DENV virion maturation, thereby affecting epitope display and antibody recognition. Line 314

Minor concern

Line 326.....Butantan-DV not Butanta-DV

This correction has been done in the revised manuscript.