MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.16, No.2, December 2022, p x-x

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.16.2.x

IgG Subclasses Identification of Immunized Mice Sera with Dengue Tetravalent DNA Vaccine Based on prM-E Genes

BETI ERNAWATI DEWI[1], RIZKA ANDHITIA MENTARI PUTRI[2*], TJAHJANI MIRAWATI SUDIRO[1], AND FITHRIYAH SJATHA[1]

[1]*Department of Microbiology, Medical Faculty, Universitas Indonesia,*
Jalan Pegangsaan Timur 16, Jakarta 10320, Indonesia;
[2]*Master Programme of Biomedical Science, Faculty of Medicine, Universitas Indonesia,*
Jalan Salemba Raya No.6, Jakarta Pusat 10430, Indonesia.

Dengue fever is still a serious health problem in the world. DENV consists of 11 kb of single positive-stranded RNA encoding three structural proteins and seven non-structural proteins. PrM and E proteins are the main targets of the antibody response that rich of epitopes and able to induce protective immunity. There are four DENV serotypes that have similar antigenic structures in the amino acid sequence of protein E. In our previous study, we successfully constructed a recombinant tetravalent DNA vaccine candidate consisting pUMVC4a-based expression plasmid for prM-E protein of all DENV serotypes (pUMD1, pUMD2, pUMD3 and pUMD4). It has been proved that the vaccine candidate was able to induced anti-dengue IgG as well as neutralization antibody to all DENV serotypes. This study aims to determine IgG subclasses of immunized mice with recombinant tetravalent DNA vaccine candidates based on prM-E genes of all serotypes.

Key words: dengue vaccine, DNA vaccine, IgG subclass, recombinant, Tetravalent

Demam berdarah masih menjadi masalah kesehatan dunia. Penyakit yang disebabkan oleh virus dengue (DENV) ini, ditransmisikan oleh *Aedes sp*. DENV terdiri dari 11 kb RNA untai positif tunggal yang mengkode 3 protein struktural dan 7 protein non-struktural. Protein struktural pr-M dan E (*envelope*) merupakan target utama dari respon antibodi yang kaya akan epitope-epitope imunologis dan berkontribusi pada induksi imunitas protektif. Terdapat empat jenis serotipe DENV yang memiliki kesamaan struktur antigenik pada sekuens asam amino protein E. Dalam penelitian sebelumnya, kami telah berhasil mengonstruksi vaksin DNA tetravalent yang terdiri dari plasmid berbasis pUMVC4a dengan insert gen prM-E dari semua serotipe DENV (pUMD1, pUMD2, pUMD3 dan pUMD4). Kandidat vaksin terbukti mampu menginduksi IgG anti-DENV dan antibody netralisasi terhadap semua serotipe DENV. Penelitian ini bertujuan untuk mengetahui subkelas IgG dari mencit yang diimunisasi dengan kandidat vaksin DNA rekombinan berbasis gen sisipan prM-E dari semua serotipe DENV secara tetravalent.

Kata kunci: rekombinan, subkelas IgG, Tetravalent, vaksin dengue, vaksin DNA

Dengue fever (DF) is serious health problem in the world, especially in tropical and subtropical regions. DF is caused by dengue virus (DENV) that transmitted by arthropods, *Aedes sp mosquito.* Several factors such as rainfall, temperature, humidity, level of urbanization and vector control can influence the spread of mosquitoes (Bhatt et al. 2013). DENV distribution has occurred globally, World Health Organization (WHO) estimates there are 390 million DENV infections per year, of which 96 million showed clinical manifestation with disease severity (WHO 2017). Indonesia is the second endemic country with the largest Dengue Hemorrhagic Fever (DHF) case among 30 countries. In 2017, 80% of cities/districts were reported to have dengue fever cases. The case fatality rate (CFR) due to DHF is considered high, although the death rate in 2017 decreased from the previous year. There were 3 provinces with the highest CFR which are Gorontalo (2.18%), North Sulawesi (1.55%) and Southeast Sulawesi (1.47%)(Kemenkes RI 2017).

DENV belongs to the Flaviviridae family, which is a single-stranded positive-sense enveloped RNA viruses. It is an spherical virus and 40 to 50 nm in diameters. The RNA genome consists of approximately 11 kb nucleotides that encode large polyproteins containing three structural proteins (capsid ©, prM, and envelope (E)) and seven non-structural proteins (NS1, NS2, NS3, NS4A, NS4B and NS5). DENV is divided into four serotypes, DENV-1, DENV-2, DENV-3 and DENV-4. These share about 70% amino acid sequence identity in E protein, that cross-reactive anti genetically. The E

*Corresponding author: Phone: +62-081215300246; E-mail: rizkaandhitiap@gmail.com

protein is the outer part of flaviviruses which contributes for adhesion, membrane fusion, and virus assembly. It is the main immunogen that determines tropism and virulence, and also become the main target of antibodies that stimulates the formation of neutralizing and protective antibodies. It has three domain which are EDI, EDII and EDIII. The prM protein is a chaperon protein that helps prevent E protein from error folding through the cell secretion pathway (Gallichotte *et al.* 2015; Fahimi *et al.* 2018).

Currently, there are several dengue vaccines that is still under development. One type of vaccine that potential for dengue vaccine is recombinant DNA vaccine. It is a vaccine that genetically engineered, containing the gene of interest inserted into plasmid. Compared to conventional vaccines based on protein/peptide, DNA vaccine is considered to be non-virulent, easy manufacturing, cost-efficient and high in stability (Stachyra, Góra-Sochacka and Sirko 2014). Konishi *et al.* (2006), have succesfully constructed a dengue tetravalent DNA vaccine consisting of pcDNA3-based plasmids expressing prM-E genes of the four serotypes and was evaluated in mouse model. (Konishi, Kosugi and Imoto 2006). The result did not show any detectable interference and could induced neutralizing antibody responses to all serotypes. Sjatha *et al.* (2014) constructed chimeric DNA vaccines consist of DENV DIII on a JEV prM-E backbone. The result showed that their vaccine candidate induced strong neutralizing antibody responses with reduced ADE activity (Sjatha *et al.* 2014).

In our previous study, we have successfully constructed recombinant DNA tetravalent vaccine candidate consisting pUMVC4a-based expression plasmid for prM-E protein of all DENV serotypes (pUMD1, pUMD2, pUMD3 and pUMD4). The DENV strains used are strains that circulate in Indonesia (Yunita 2012; Rachmayanti 2013; Putri 2015). From in vivo study, showed lower viremia titers for almost 70 times compared with unimmunized mice (Putri 2015). Furthermore, it has been proved that vaccine candidate capable to induce anti-dengue IgG as well as neutralization antibody to all DENV serotypes. Rodrigo *et al.* (2009) suggest that DENV neutralization is modulated by combined Fc region of IgG subclasses virion avidity of binding and FcγR preference. Sjatha *et al.* (2014) in their study, suggest that isotypic profile of antibody related to their affinity to complements affect antibody responses in mouse sera. To examine this possibility, this study assesses the profile of subtype IgG of the vaccine candidate in mice model.

MATERIALS AND METHODS

Ethics Statement and Mice Experiments. Ten Balb/c mice were divided into two groups: (1) five mice were immunized with pUMVC4a as control and (2) five mice were immunized with the dengue tetravalent vaccine candidate (100 µg/mouse) by intramuscular injection using needle-free jet injector (ShimaJET: Shimadzu, Kyoto, Japan). Mice were vaccinated three times, within three-week interval. Blood were drawn before vaccination, two weeks post vaccination as well as termination blood. All protocols within this experiment have been ethically approved by Health Research Ethics Committee of the Faculty of Medicine Universitas Indonesia-RSCM number: KET.550/UN2.F1/ETIK/PPM.00.02/2019. The experimental animal was carried out in the animal care facility of the Department of Microbiology, Faculty of Medicine, Universitas Indonesia with a closed cage system and ad libitum feed-drink.

Viruses. The virus used as antigen for in-house indirect ELISA was dengue virus serotype-2 (DENV-2) NGC strain harvested at 5 d.p.i with viral titer was 3.5×10^7 FFU mL^{-1}.

Propagation *Eschericia coli* DH5α Harboring Plasmid. *Eschericia coli* DH5α competent carrying recombinant plasmids (pUMD1, pUMD2, pUMD3 and pUMD4) was propagated in 5 mL of liquid Luria Bertani (LB) medium containing kanamycin (50 µg mL^{-1}) and incubated at 37 °C in a shaker incubator (200 rpm) for 18-20 hours. After bacterial growth occurs, 4 mL of liquid culture of each plasmid was replanted in 200 mL of liquid LB medium containing kanamycin (50 µg mL^{-1}) and incubated at 37 °C in a shaker incubator (200x rpm) for 18-20 hours. Each of the culture then was harvested using centrifuge at 4000 rpm for 5 minutes in 4 °C.

Identification of pUMD1 and pUMD3. Colony PCR was performed to confirm the gen insert of recombinant plasmids of pUMD1 and pUMD3. A small amount of cells from a single colony of *E. coli* DH5α competent carrying plasmids were picked up by using sterilized toothpick and resuspended into 50 µL distillated water. A 2 µL suspension was used as template in PCR reactions. PCR reactions were performed in 25 µL volume following PCR Master Mix Kit (Qiagen, Catalog No. 201443) standard procedure.

Identification of pUMD2 and pUMD4. Confirmation of pUMD2 and pUMD4 was performed using restriction enzymes digestion method. Enzymes that used in this study were XbaI (Thermo Fisher,

Table 1 Primers used in this study

Primers	Sequences	Description
D1 2063 s	5'-TACATCGTGGTAGGAGCAGG-3'	DENV-1
D1 2510 c	5'-GCTCTGTCCAGGTGTGAACT-3'	
DV3KF-eco	5'-CGCGATATCACCATGAACAGGAGACGCAGAACT-3'	DENV-3
DV3KR	5'-TGCTCTAGATTAGGCCTGCACCATAACTCC-3'	

Catalog No. 0682) for pUMD2, while pUMD4 were used double digest with two restriction enzyme BamHI (Thermo Fisher, Catalog No. ER0051) and XbaI (Thermo Fisher, Catalog No. 0682). Reaction were performed: 1.5 µL of Tango buffer (10x), 0.5 µL of XbaI (10 u µL^{-1}), 8 µL of nuclease free water, and 2µL (100 ng µL^{-1}) pure plasmid of pUMD2, then incubated for 2 hours. For pUMD4, the reaction were perfomed: 1.5 µL of Tango buffer (10x), 0.25 µL of XbaI (10 u µL^{-1}), 0.25 µL of BamHI (10 u µL^{1}), 9 µL of nuclease free water, and 4µL pure plasmid of pUMD4 then incubated for 1 hour.

In vitro Expression Analysis of Protein E. The expression of prM-E protein of pUMD1, pUMD2, pUMD3 and pUMD4 was confirmed in Vero cells using Lipofectamine LTX with Plus Reagents (Invitrogen). In brief, Vero cells were grown in 24 well-plate to 80% confluence. First, each plasmid was diluted to 10 ng µL^{-1} in Opti-MEM medium (Gibco-BRL) 10 ng µL^{-1}. Plasmids were incubated with 1 µL reagent plus for 5 minutes (room temperature). Next, 3 µL Lipofectamine-LTX was added into the mixture and incubated for 30 minutes (room temperature). The mixture then added to the cells and incubated for another 48 hours at 37 °C. After incubation, cells were fixed using 500 µL/well absolute ethanol and incubated for 1 hour at 4 °C. Then, results were visualized with immunostaining method.

Plasmid Isolation. Plasmid isolation was performed based on Hispeed® Plasmid Midi Kit (Qiagen) with modification. Pure DNA plasmid were confirmed by gel electrophoresis then stored at -30 °C. The concentration of pure DNA plasmid were measured with spectrophotometer (Nanodrop 2000c, ThermoFisher, Catalog No. ND-2000).

Immunization of Mice. Five 4-6 week-old of BALB/c mice per immunization group were immunized three times into quadriceps muscle with 100 µg of candidate plasmid (tetravalent) or pUMVC4a using needle-free injector (Shimajet, Japan). Immunization was carried out within three-weeks interval, retro-orbital blood was drawn two-weeks post immunization as well as termination blood. Pre-immunization blood of all groups were also taken. Blood samples were centrifuge at 3000 rpm 4 °C for 10 min, then collected sera were stored at -30 °C until further examination.

IgG Subclasses Measurement with In-house Indirect ELISA. Pre-immunization and termination sera from both groups were measured for IgG1, IgG2a, IgG2b and IgG3. In this study, we were only using DENV-2 (NGC) as coating antigen on ELISA plates. DENV-2 (3.5 x 10^7 FFU mL^{-1}) were diluted into 1:10 in coating buffer. In brief, DENV-2 were coated in 96 well ELISA plate (NUNC, Thermofisher Catalog No. 44-2404-21) at 4 °C overnight. Followed by blocking with 100 µL of 5% (w/v) skim milk then incubated at 37 °C for 1 hour. Then, plates were washed using PBS containing 0.05% Tween-20. A 100 µL of serum samples (diluted 1:50) were added into the plates and incubated at 37 °C for 1 hour. After incubation plates were washed then 100 µL each of diluted anti mouse-IgG subclasses (1:1000; Biologend) were added into the plates then reincubated at 37 °C for 1 hour. Plates were washed and 100 µL of diluted (1:1000) HRP-avidin were added into the plates and incubated at for 1 hour. After incubation, plates were washed and 50 µl of TMB substrate (1-Step Ultra TMB-ELISA Substrate Solution, Catalog No: 34028) was added followed by incubation in dark with gentle agitation for 20 minutes at 37 °C . Finally, 20 µL of stop solution was added and absorbancies were measured at 450 nm.

RESULTS

Identification of Plasmid Recombinant. PCR colony was performed to confirm the insert of prM-E genes corresponds to its construction in pUMD1 and

Table 2 Mean of IgG subclasses titre tetravalent and pUMVC4a groups

Antibody	Tetravalent Group (Mean ± SD)	pUMVC4a Group (Mean ± SD)
IgG1	0.920±0.203	0.490±0.051
IgG2a	1.004±0.154	0.409±0.108
IgG2b	0.281±0.022	0.144±0.029
IgG3	0.392±0.069	0.346±0.079

pUMD3. The product that was expected for pUMD1 was 448 bp, while pUMD3 was 2009 bp (Putri 2015). As shown in Fig 1, the presence of prM-E were detected both in pUMD1 and pUMD3.

The inserted gene for pUMD2 and pUMD4 were confirmed using double digest with enzyme restriction method. The insert gene products that was expected for pUMD2 and pUMD4 was around ±2000 bp according to the gene map illustration shown in Fig 2 (Yunita 2012; Rachmayanti 2013). As shown in Fig 3, the presence of prM-E genes insert were detected both in pUMD2 and pUMD4.

Expression of prM-E Protein in Vero Cells. Transfection of plasmids pUMD1, pUMD2, pUMD3 and pUMD4 was performed to analyze the protein expression in Vero cells for 48 hours. Then immunostaining were performed to confirm the ability of the recombinant plasmids. We used antibody from DENV infected patients that may contain antibody to E protein to analyze prM-E protein expression of candidate vaccine. The Vero cells that were transfected with pUMD1, pUMD2, pUMD3, pUMD4 were stained brown indicating positive results (Fig 4). In this experiment we used pUMVC4a vector plasmids as negative control and show no cells were stained indicating there was no E protein expression. Also, it was confirmed that the transfection and staining process was done correctly.

IgG Subclasses Titer. Termination individual sera from both tetravalent and pUMVC4a groups were profiled to measure the IgG subclasses titers. Then, the mean groups titer of each group for anti-DENV-2 IgG1, IgG2a and IgG2b were satistically analyzed with independent t-test, while IgG3 was analyzed with Mann-whitney U test. The mean groups titer is shown in Table 2. As seen in Fig 5, the mean titers of IgG1, IgG2a and IgG2b showed significant difference ($p<0.05$), while IgG3 did not. Also, the results showed that IgG2a was the main subclass induced in immunized mice followed by IgG1, IgG2b and IgG3. The IgG2a titer in tetravalent group was 2.5 fold higher than the pUMVC4a control group, while other subclasses were 2 fold higher in recognizing DENV-2. The results indicates that vaccine candidates was able to induce humoral immune response in mice.

DISCUSSION

In our previous study, we have successfully constructed and proven our recombinant plasmids were able to induced humoral response in mice. We used prM-E as insert genes to pUMVC4a as recombinant plasmid. The prM-E proteins are the main targets of the antibody response to DENV infection. The glycoprotein E is rich in immunological epitopes and contributes to the induction of protective immunity. In line with Sjatha *et al* (2014), suggest that the antibody response to serotype-specific DIII of protein E, resulted in reduced cross neutralization and did not increase virus infection or in other words there are less possibility of ADE (Sjatha *et al.* 2014).

In this study, we administered the vaccine by intramuscular injection which targets mycocytes and keratynocytes directly, including antigen presenting cells (APCs). Theoritically, after DNA is internalized, the DNA will translocate to the nucleus to be transcribed and followed by translation in the cytoplasm, expressed into proteins that have been converted into peptide chains. In addition, intramuscular administration of DNA vaccines can be targeted directly at APCs located around the injection site. In this pathway, the encoded antigen will be expressed and after transcription, the antigen peptide will be presented by MHC class I and II against APC. Then the APC will migrate to the lymph nodes and induce CD8+ and CD4+ T helper cells. In other words, these interactions will initiate humoral and cellular immune responses (Abbas *et al.* 2010; Yam-Puc *et al.* 2016).

The IgG subclasses profile result was shown that the highest antibody induced by the tetravalent vaccine was IgG2a followed by IgG1, IgG2b and IgG3. It is known that the production of IgG2a, IgG2b and IgG3 is stimulated by Th1 immune response whereas IgG1 is

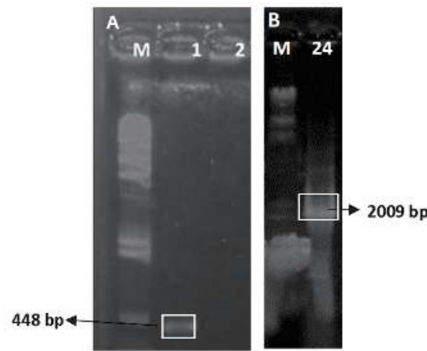

Fig 1 Colony PCR screening for pUMD1 and pUMD3. (A) M: λ Hind/III, 1-3: recombinant pUMD1, K-:negative control; (B) M: λ Hind/III. 24: recombinant pUMD3, K+: positive control for pUMD3 insert cloned to pUMVC4a. White box showed target band.

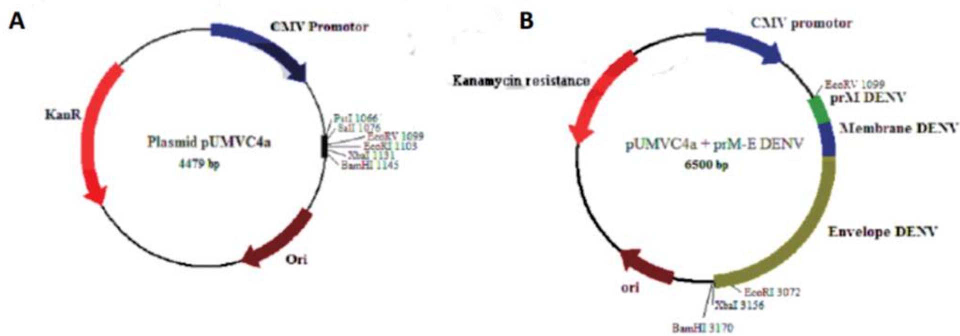

Fig 2 Illustration of plasmid construction for pUMD1, pUMD2, pUMD3 and pUMD4. (A) pUMVC4a vector plasmid, (B) plasmid recombinant.

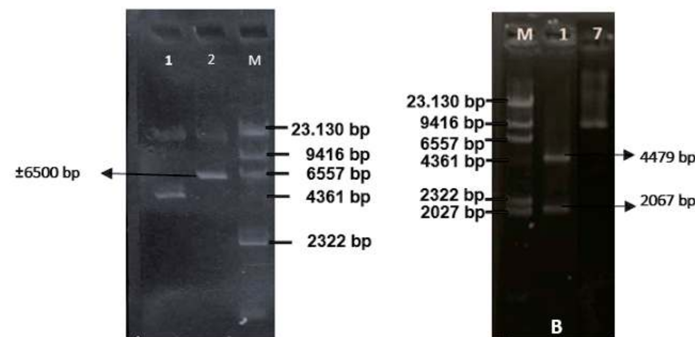

Fig 3 Screening for pUMD2 and pUMD4 using restriction enzyme digestion method (A) M: λ Hind/III, 1: pUMD2, 2: pUMVC4a; (B) M: λ Hind/III, 1: pUMD4, 7: pUMVC4a.

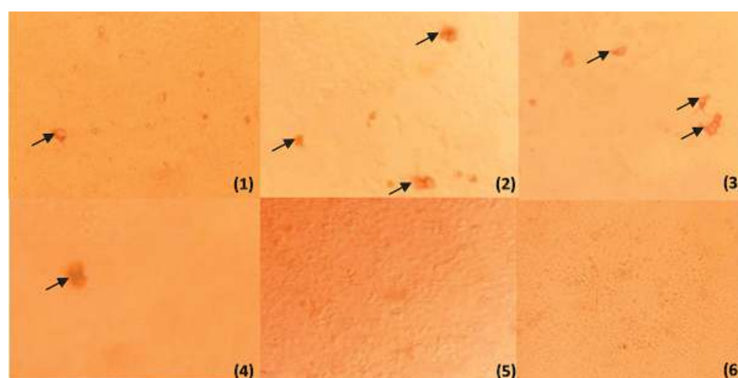

Fig 4 Expression of prM-E protein in Vero cells using HRP detection system, positive results were indicated by brown stain (1) Vero cells transfected with pUMD1; (2) Vero cells transfected with pUMD2; (3) Vero cells transfected with pUMD3; (4) Vero cells transfected with pUMD4; (5) Vero cells transfected with pUMVC; (6) negative control (not transfected).

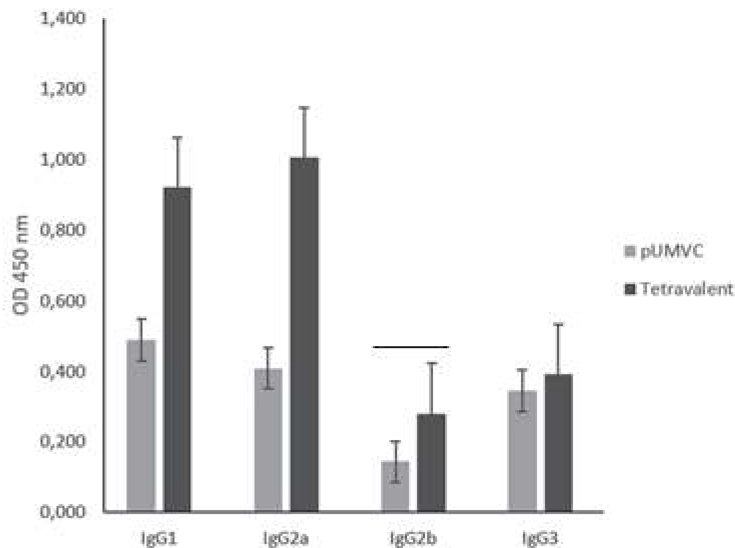

Fig 5 Comparison of mean groups titer of each group for all IgG subclasses from termination sera.

stimulated by a Th2 immune response (Wang *et al*. 2019). IgG2a response in homologous mice to human IgG1, where the levels are the most abundant in human serum. Both subclasses have the equivalent responses to the same FcγR class (Mehlhop *et al.* 2007). In humans, the presence of IgG subclasses strongly triggers complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP)) (Brezski and Georgiou 2016). IgG2a antibodies in the serum that are usually associated with the Th1 subset of CD4+ T cells secrete gamma interferon (IFN-γ) and tumor necrosis factor (TNF) which induce the induction of cell-mediated immune responses. The IgG2a response was reported to be correlated with neutralizing antibody titres. The IgG1 antibody in mice (mIgG1) has a binding pattern comparable to human IgG2 (hIgG2). This was proven by Overdijk *et al* (2012), that hIgG2 was able to compete with mIgG1 in binding mFcγRIIb and mFcγRIV (Overdijk *et al*. 2012). Yamanaka *et al* (2013) in their study mention that the binding affinity of dengue antibodies to FcγR affects the activity of neutralizing and enhancing antibodies. IgG2 antibodies in mice are known to have stronger binding affinity to complements than IgG1 (Yamanaka, Kotaki and Konishi 2013). Whereas in humans, it is known that IgG1 and IgG3 can bind complements the most effectively, compared to IgG2 and IgG4 (Rodrigo *et al*. 2009).

In this study, the profiling of IgG subclasses levels were only carried out against DENV-2 as an antigen by ELISA. Therefore, in further research it is necessary to evaluate other serotypes. In addition, there has not been an evaluation of the potential antibody response to vaccine candidates in inducing ADE activity. This is recommended to determine the safety of the vaccine candidate.

ACKNOWLEDGMENTS

This research was funded by Indonesian Dengue Vaccine Consortium.

REFERENCES

Abbas AK, Lichtman AH, Pillai S, Baker DL. 2010. B cell activation and antibody production. Cellular and Molecular Immunology. Elsevier: 243–268. doi: 10.1016/B978-1-4160-3123-9.50017-6.

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI. 2013. The global distribution and burden of dengue. Nature, 496(7446):504-7. doi: 10.1038/nature12060.

Brezski, R. J. and Georgiou, G. 2016. Immunoglobulin isotype knowledge and application to Fc engineering. Current Opinion in Immunology. Elsevier Ltd, 40(May 2015): 62–69. doi: 10.1016/j.coi.2016.03.002.

Fahimi H, Mohammadipour M, Haddad Kashani H, Parvini F, Sadeghizadeh M. 2018. Dengue viruses and promising envelope protein domain III-based vaccines. Appl Microbiol Biotechnol. 102(7): 2977–2996. doi: 10.1007/s00253-018-8822-y.

Gallichotte EN, Widman DG, Yount BL, Wahala WM, Durbin A, Whitehead S, Sariol CA, Crowe JE Jr, de

Silva AM, Baric RS. 2015. A New Quaternary Structure Epitope on Dengue Virus Serotype 2 Is the Target of Durable Type-Specific Neutralizing Antibodies. mBio. Edited by W. I. Lipkin, 6(5): 1–8. doi: 10.1128/mBio.01461-15.

Kemenkes RI (2017) Profil Kesehatan Indonesia. 351.077 In. Edited by T. Kurniawan, Rudy; Yudianto; Hardhana, Boga; Soenardi. Jakarta: Kementerian Kesehatan Republik Indonesia. Available at: https://pusdatin.kemkes.go.id/download.php?file=download/pusdatin/profil-kesehatan-indonesia/Profil-Kesehatan-Indonesia-2016.pdf.

Konishi E, Kosugi S, Imoto J. 2006. Dengue tetravalent DNA vaccine inducing neutralizing antibody and anamnestic responses to four serotypes in mice. Vaccine, 24(12): 2200–2207. doi: 10.1016/j.vaccine.2005.11.002.

Mehlhop E, Ansarah-Sobrinho C, Johnson S, Engle M, Fremont DH, Pierson TC, Diamond MS. 2007. Complement Protein C1q Inhibits Antibody-Dependent Enhancement of Flavivirus Infection in an IgG Subclass-Specific Manner. Cell Host Microbe, 2(6): 417–426. doi: 10.1016/j.chom.2007.09.015.

Overdijk MB, Verploegen S, Ortiz Buijsse A, Vink T, Leusen JH, Bleeker WK, Parren PW. 2012. Crosstalk between Human IgG Isotypes and Murine Effector Cells. J Immunol, 189(7): 3430–3438. doi: 10.4049/jimmunol.1200356.

Putri DH. 2015. Pengembangan vaksin DNA tetravalen dengue berbasis gen prM-E DENV strain Indonesia : uji imunogenesitas vaksin dalam menginduksi antibodi netralisasi dan respon anamnestik untuk semua serotipe DENV di mencit. Universitas Indonesia.

Rachmayanti N. 2013 Konstruksi dan ekspresi gen pre-membrane dan envelope virus dengue serotype 4 strain indonesia sebagai kandidat vaksin DNA. Universitas Indonesia.

Rodrigo WW, Block OK, Lane C, Sukupolvi-Petty S, Goncalvez AP, Johnson S, Diamond MS, Lai CJ, Rose RC, Jin X, Schlesinger JJ. 2009. Dengue virus neutralization is modulated by IgG antibody subclass and Fcγ receptor subtype. Virology. Elsevier Inc., 394(2): 175–182. doi: 10.1016/j.virol.2009.09.024.

Sjatha F, Kuwahara M, Sudiro TM, Kameoka M, Konishi E. 2014. Evaluation of chimeric DNA vaccines consisting of premembrane and envelope genes of Japanese encephalitis and dengue viruses as a strategy for reducing induction of dengue virus infection-enhancing antibody response. Microbiol Immunol. 58(2): 126–134. doi: 10.1111/1348-0421.12125.

Stachyra, A., Góra-Sochacka, A. and Sirko, A. 2014. DNA vaccines against influenza. Acta Biochimica Polonica, 61(3): 515–522. doi: 10.18388/abp.2014_1873.

Wang R, Zheng X, Sun J, Feng K, Gao N, Fan D, Chen H, Jin X, An J. 2019. Vaccination with a single consensus envelope protein ectodomain sequence administered in a heterologous regimen induces tetravalent immune responses and protection against dengue viruses in mice. Front Microbiol. 10(MAY): 1–14. doi: 10.3389/fmicb.2019.01113.

WHO. 2017. Epidemiology.

Yam-Puc JC, Cedillo-Barrón L, Aguilar-Medina EM, Ramos-Payán R, Escobar-Gutiérrez A, Flores-Romo L. 2016. The cellular bases of antibody responses during dengue virus infection. Front Immunol, 7(June): 1–12. doi: 10.3389/fimmu.2016.00218.

Yamanaka, A., Kotaki, T. and Konishi, E. 2013. A Mouse Monoclonal Antibody against Dengue Virus Type 1 Mochizuki Strain Targeting Envelope Protein Domain II and Displaying Strongly Neutralizing but Not Enhancing Activity. J Virol, 87(23): 12828–12837. doi: 10.1128/jvi.01874-13.

Yunita R. 2012. Imunogenisitas kandidat vaksin DNA rekombinan dengan gen insersi premembran dan envelope virus dengue tipe 2 pada mencit. Universitas Indonesia.