Some epitopes conservation in non structural 3 protein dengue virus serotype 4

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Abstract

Background: Non Structural 3 (NS3) protein of dengue virus (DENV) is known to induce antibody, CD4⁺ and CD8⁺ T cell responses, and playing role in viral replication. NS3 protein has T and B cell epitopes, which has conservation difference between DENV-4 strains. This study aimed to identify conservation of T and B cell epitope in NS3 protein among DENV-4 strains and four serotypes DENV of Indonesia strains.

Methods: Research was held at the Department of Microbiology, Faculty of Medicine, Universitas Indonesia, June 2013 to April 2014. NS3 amino acid sequence of DENV-4 081 strain was obtained after the PCR products of NS3 strain 081 were sequenced. T and B cell epitopes of NS3 protein of DENV-4 081 strain were analysed and compared to NS3 proteins of 124 DENV-4 strains around the world and four serotypes of Indonesia strains. World strains were isolated from America (i.e. Venezuela, Colombia, etc.) and Asia (i.e. China, Singapore, etc.). For the comparison, T and B cell epitope positions of NS3 protein were obtained from published report.

Results: Eight positions of T cell epitopes and two positions of B cell epitopes of NS3 DENV-4 081 were identical and conserved to NS3 protein of DENV-4 081 strains around the world. B cell epitope of NS3 081 protein at aa 537-544 was found identical and conserved to four serotypes DENV of Indonesia strains.

Conclusion: This wide conservation of T and B epitopes in almost all DENV-4 strains around the world and all serotypes of Indonesia strains. (Health Science Journal of Indonesia 2015;6:126-31)

Keywords: dengue virus, NS3 protein, T cell epitope, B cell epitope
Dengue virus (DENV) infection remains a public health problem, either in the world or in Indonesia. According to data from World Health Organization (WHO), 50 to 100 million infections were estimated to occur each year, including 500,000 cases of dengue fever and 22,000 deaths, mostly among children.\(^1\) Based on data from Indonesian Ministry of Health, the number of patients with DHF increased annually, in 2013 there were 112,511 of DHF cases in 32 provinces (incidence rate of 45.85 per 100,000 population).\(^2\)

The virus has three structural proteins, the capsid protein (C), envelope (E) and membrane (M). These structural proteins along with seven non-structural proteins (NS) are encoded by the viral genome in the form of single-stranded RNA with positive polarity. DENV genome is a sequence of 5’UTR-C-PRM-E-NS3-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3’ UTR.\(^3\)

NS3 protein plays an important role in DENV life cycle.\(^4,5\) The functions of NS3 includes a viral protease that plays a role in cutting the DENV polyprotein after translation, and plays a role in viral RNA replication through the activities as nucleotide triphosphatase (NTPase), RNA 5’ triphosphatase (RTPase) and helicase.\(^6,7\)

Non-Structural (NS) 3 protein has the ability to induce antibody responses,\(^7\) CD4\(^+\) and CD8\(^+\) T cells. T cells activated by NS3 can cross-react between serotypes.\(^8\) Anti-NS3 antibodies were proven in vivo to protect and delay the time of death (survival time) of mice challenged with DENV at doses of 100 LD\(_{50}\).\(^9\) NS3 sized 618 amino acid/ 69 kD, is the second largest gene/protein of DENV and its primary amino acid sequence is the most conserved.

This study aimed to obtain information about the similarity of T cell and B cell epitopes of NS3 DENV-4 081 Indonesian strain in comparison to DENV-4 world strains, as well as the similarity to the NS3 of the other serotypes Indonesia strains.

**METHODS**

The research was held in Department of Microbiology, Faculty of Medicine, Universitas Indonesia from June 2013 to April 2014. DENV-4 strain 081 was isolated in year 2013, a collection of National Institute of Health Research and Development, Ministry of Health, Indonesia. To get DNA sequence from DENV-4 strain 081, first viral RNA was extracted from culture supernatants of infected-C6/36 cell using QIAamp viral RNA extraction kit according to the manufacturer’s instruction (Qiagen, Hilden, Germany). Complementary DNA (cDNA) strands were reverse-transcribed using Super Script II First Strand Synthesis System with Random hexanucleotide primer according to the manufacturer’s instructions (Invitrogen, Massachusetts, USA). The entire NS3 gene was then amplified by polymerase chain reaction (PCR) using Platinum Taq Polymerase (Invitrogen, Massachusetts, USA). After purification of the PCR products using QIAquick PCR purification Kits (Qiagen GmbH, Hilden, Germany), the samples were sent to PT Genetika Science Indonesia for direct sequencing. The sequencing processes used Sanger method with Taq Big Dye Deoxy Terminator Cycle sequencing kits (Applied Biosystems, Foster City, CA USA). Nucleotide and amino acid sequence analysis was performed using GENETIC BIOSYSTEMS and BLAST program. Codon positions described in this study were based on the data of DENV-4 Singapore strain (GenBank accession no.GQ398256.1). Primer sequences for DNA synthesis and sequence were designed using PRIMER program and obtained from the published data of DENV4 Singapore strain (GenBank accession no.GQ398256.1).

Analysis of T and B cell epitope on NS3 protein were done by comparing the amino acid sequence of NS3 protein DENV4 081 to the NS3 protein of DENV-4 world strains and serotypes of Indonesia strains which obtained from the Genbank. Total world strains were 124 strains which 25 strains were from Asia (China, Singapore, India, Malaysia, Cambodia, India, Thailand, Philippines, Pakistan, and Taiwan), 99 strains were from America (Venezuela, Dominica, Colombia, US, Haiti, and Brazil) isolated between 1961 and 2011; and twelve Indonesian strains. The references for analyzing NS3 T\(^8\),\(^10\)-\(^17\) and B cell epitopes\(^18-20\) were listed in Table 1. NS3 protein epitopes of DENV-4 081, 124 DENV-4 world strains and other serotypes of Indonesia strains were analysed using Bioedit version 7.0.5.3 year 2005 (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

**RESULTS**

Homology analysis of T cell epitopes of NS3 protein of DENV-4 081 to the NS3 protein of 124 DENV-4 world strains (25 strains from Asia and 99 strains from America) was shown in Table 2.

From fourteen T cell epitopes of the NS3 protein of DENV-4 081 compared to NS3 protein of 124 DENV-4 world strains, different amino acid at six epitopes were found in nine strains (Table 2), while the other eight epitopes were well conserved in all 124 world strains. T cell epitopes that have different amino acids were at aa 45-59, aa 145-159, aa 231-245, aa 495-509, aa 526-540 and aa 596-610 (Table 2).
### Table 1. B and T cell epitopes in NS3 DENV-4 protein

| No | NS3 epitope position (AA) | Reference strains | AA sequence | Reference |
|----|--------------------------|-------------------|-------------|-----------|
|    | **T cell epitopes**      |                   |             |           |
| 1  | 45-59                    | DENV2 16681 Vietnam strain | TFHTMWHVTRGAVLM | 10 |
| 2  | 145-159                  | DENV2 16681 strain | KVVLGNYGNVVTRSG | 10 |
| 3  | 221-235                  | DENV3 CH53489 strain | LAPTRVAAEMEAL | 11, 12 |
| 4  | 231-245                  | DENV2 16681 strain | MEEALRGLPIRYQTP | 10 |
| 5  | 250-264                  | DENV2 16681 strain | EHTGREIVDLMCHAT | 10 |
| 6  | 251-265                  | DENV4 814669 strain | HTGREIVDLMCHATF | 13, 14 |
| 7  | 255-269                  | DENV2 16681 strain | EIVDLMCHATFTMLR | 10 |
| 8  | 258-272                  | DENV4 814669 strain | DLMCHATFTTRLLSS | 14 |
| 9  | 275-289                  | DENV2 16681 strain | VPYNFLIMDEAHFT | 10 |
| 10 | 296-310                  | DENV4 814669 strain | ARGYISTRVEMGEAA | 4, 15 |
| 11 | 336-350                  | DENV2 2005 Singapore strain | EREIPERSWNSGHED | 16 |
| 12 | 495-509                  | DENV2 16681 Vietnam strain | LDNINTPEGIIPSMF | 10 |
| 13 | 526-540                  | - | RGEQRKTFVELMRRG | 17 |
| 14 | 596-610                  | DENV22005 Singapore strain | LDARIYSDPLALKEF | 16 |
|    | **B cell epitopes**       |                   |             |           |
| 1  | 425-432                  | DENV3 | PRRCLKPV | 18 |
| 2  | 460-469                  | DENV2 Tr1751 strain | RVGRPNK1NEN | 19 |
| 3  | 521-532                  | DENV2 Eden 3295 strain | DETPMRGETRKV | 20 |
| 4  | 537-544                  | DENV3 | MRRGDLPV | 18 |

### Table 2. T and B cell epitopes analysis of NS3 DENV4 protein. Bold letters show amino acid mutation

| No | NS3 Epitope position | World strains | Amino acid sequence |
|----|----------------------|---------------|---------------------|
|    | **T cell epitopes**  |               |                     |                     |
| 1  | 45-59                | DENV4 Cina 2010 (JF741967) | VFHTVGHVTRGSVIC | VFHTMWHVTRGAVLM |
| 2  | 145-159              | DENV4 India 1961 (JF262783) | RVIGLYGNIVTKSG | KVLHGMGWGVVTKS |
| 3  | 231-245              | DENV4 Venezuela 2007 (QG199876) | MEEALRGLPIRYQTP | MEEALRGLPIRYQTP |
| 4  | 495-509              | DENV4 Pakistan 2009 (KF041260) | LDNIHTPEGHPTLF | LDNIYTPEGHPTLF |
| 5  | 526-540              | DENV4 Singapore 2005 (QG398256) | RGEQRKTFVELMRRG | RGEQRKTFVELMRRG |
| 6  | 596-610              | DENV4 Colombia 2004 (QG868583) | LDARYADPMALQDF | LDARYADPMALKDF |
|    | **B cell epitopes**  |               |                     |                     |
| 1  | 460-469              | DENV4 Venezuela 2007 (EU854300) | RGRNLAQED | RGRNPQED |
|    | DENV4 Puerto Rico 1998 (FJ024424) | RGRNPTQED | RGRNPQED |
|    | DENV4 Venezuela 2007 (FJ882581) | RGRNLAQED | RGRNPQED |
|    | DENV4 Venezuelan 2007 (FJ882582) | RGRNLAQED | RGRNPQED |
|    | DENV4 Venezuela 2007 (FJ882588) | RGRNLAQED | RGRNPQED |
|    | DENV4 Puerto Rico 1999 (FJ882599) | RGRNPTQED | RGRNPQED |
|    | DENV4 Haiti 1994 (FJ262782) | RGRNLAQED | RGRNPQED |
| 4  | 537-544              | DENV4 Singapore 2005 (QG398256) | MKRGDLPV | MKRGDLPV |
|    | DENV4 China 2012 (KC33651) | MKRGDLPV | MKRGDLPV |
|    | DENV4 Thailand 1997 (AY618988) | MKRGDLPV | MKRGDLPV |
|    | DENV4 Thailand 1997 (AY618989) | MKRGDLPV | MKRGDLPV |
From analysis of four B cell epitopes of the NS3 protein of DENV-4 081 and 124 DENV-4 world strains, different amino acid at two epitopes were found in several strains of 124 DENV-4 world strains, i.e. aa 460 to 469 and aa 537 to 544 (Table 2). The other two B cell epitopes were well conserved in all 124 DENV-4 world strains. However, B cell epitope at aa 537 to 544 was well conserved in all four serotypes of Indonesia strains.

The T and B cell epitopes in the NS3 DENV-4 081 protein were compared to NS3 protein of DENV 1, 2, 3, and 4 Indonesia strains to identified similarity between T and B cell epitopes of NS3 protein DENV-4 and four DENV serotypes. The T cell epitopes of NS3 protein DENV-4 081 at aa 221-235, aa 231-245 and aa 296-310 were found 100% identical to the other NS3 protein DENV-4 Indonesia strains and DENV-2 Indonesia strains. The B cell epitope of NS3 protein DENV-4 081 at aa 425-432 was found 100% identical to the NS3 protein of DENV 1, 3 and 4 Indonesia strains. From all B cell epitopes, the epitope at position 537-544 was found conserved and identical in all serotypes of DENV Indonesia strains.

The characteristics of different amino acids in the six T cell epitopes which showed variability in DENV-4 081 and several 124 DENV-4 strains (Table 2) were identified by their hydrophobicity. The result showed that only at aa 145-159, aa 526-540 and aa 596-610 of NS3 protein different property of amino acid were found (Table 3), i.e. T cell epitopes of NS3 DENV-4 081 at aa 526-540 have arginine which is hydrophilic, compared to the same epitope positions of several DENV-4 strains (Table 2) have lysine which is hydrophobic. On the other T cell epitope of NS3 DENV-4 081 at aa 596-610 compared to several DENV-4 world strains there is amino acid changing from lysine (hydrophobic) to glutamine (hydrophilic).

Analysis of B cell epitopes of NS3 DENV-4 081 at aa 460-469 and aa 537-544 and several DENV-4 strains (Table 2) showed different properties of amino acids as shown in Table 3.

The B cell epitope at aa 460-469 of NS3 protein DENV-4 081 have proline which is hydrophilic, compared to the same epitope position of several DENV-4 world strains (Table 2) have leucine which is hydrophobic. The B cell epitope of NS3 protein DENV-4 081 at aa 537-544 have arginine (hydrophilic) compared to several 124 DENV-4 world strains, there is amino acid changing from arginine (hydrophilic) to lysine (hydrophobic).

**DISCUSSION**

From total fourteen T cell epitopes analysed, eight T cell epitopes were 100% identic and conserved to NS3 protein of 124 DENV-4 world strains (Table 2). Two of four B cell epitopes were well also conserved in 124 DENV-4 strains around the world (Table 2). This analysis also showed that from total 25 strains from Asia and 99 strains from America, 68% of Asia strains and 98% of America strains have similarity in all fourteen T cell epitopes of NS3 protein DENV-4. In total, 84% of Asia strains and 93% of America

| No | NS3 epitope position | AA change | Characteristics |
|----|----------------------|-----------|-----------------|
| T cell epitopes | | | |
| 1 | 45-59 | Met → Val | No Change, both are hydrophobic |
| | | Trp → Gly | No Change, both are hydrophobic |
| 2 | 145-159 | Lys → Arg | Lys → hydrophobic, Arg → hydrophilic |
| | | Val → Ile | No Change, both are hydrophobic |
| 3 | 231-245 | Ile → Val | No Change, both are hydrophobic |
| 4 | 495-509 | Tyr → His | No Change, both are hydrophobic |
| 5 | 526-540 | Arg → Lys | Lys → hydrophobic, Arg → hydrophilic |
| 6 | 596-610 | Met → Val | No Change, both are hydrophobic |
| | | Lys → Gln | Lys → hydrophobic, Gln → hydrophilic |
| B cell epitopes | | | |
| 1 | 460-469 | Pro → Leu | Pro → hydrophilic, Leu → hydrophobic |
| | | Ala → Thr | No Change, both are hydrophobic |
| 2 | 537-544 | Arg → Lys | Arg → hydrophilic, Lys → hydrophobic |
strains showed similarity in all four B cell epitopes of NS3 protein DENV-4. From total 25 DENV-4 strains from Asia, there were eight strains showed genetic variation in the amino acid sequence of NS3 protein. The 68% similarity in all fourteen epitopes analysed in DENV-4 strains from Asia needs further study, because only 25 DENV-4 strains were obtained of total 124 world strains. The variations in the amino acid sequence of T cell epitopes of NS3 protein DENV-4 were about one or two amino acid difference (Table 2 and Table 3).

It is known that the amino acid changes would not alter the preservation area epitope recognition by antibodies and lymphocytes as long as it still have same tertiary structure. Tertiary structure of a protein is determined by various factors, one of it is hydrophobic interaction of the protein-building amino acids. Amino acid changes can cause conformational change of the protein, which may change epitope recognition by antibodies. Mutated amino acids of T cell and B cell epitopes affect hydrophobicity of epitopes so further analysis is required to get three-dimensional structure of NS3 protein.

Table 4. T dan B cell epitopes position of NS3 DENV4 081 protein which is identical to NS3 DENV 1, 2, 3 dan 4 protein Indonesia strain

| No | T cell epitope position | B cell epitope position | AA epitope sequence of DENV4 081 strain | DENV1 | DENV2 | DENV3 |
|----|--------------------------|-------------------------|----------------------------------------|-------|-------|-------|
| 1  | 221-235                  | LAPTRVVAEMEEAL          | Identical                              |       |       |       |
| 2  | 231-245                  | MEEALRGLPIRYQTP         | Identical                              |       |       |       |
| 3  | 296-310                  | ARGYISTRVEMGEAA         | Identical                              |       |       |       |
| 4  | 425-432                  | PRRCLKPV                | Identical                              | Identical |      |       |
| 5  | 537-544                  | MRRGDLPV                | Identical                              | Identical | Identical |       |

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