Evaluasi Kemampuan Netralisasi Beberapa Isolat Virus Dengue 1 oleh Antibodi Anti-membran dan Envelop DENV-1 yang Dihasilkan dari Kandidat Vaksin Dengue Indonesia pUMVC RDS 59/09 = Neutralization Ability Evaluation of DENV-1 Isolates by Anti-DENV-1 Membrane and Envelope Antibody Produced by Indonesia Dengue Vaccine Candidate pUMVC RDS 59/09

Abstrak

[Latar belakang:

Infeksi virus dengue masih menjadi masalah di negara tropis seperti Indonesia. Infeksi virus dengue menyebabkan mortalitas dan morbiditas yang tinggi. Belum ada obat ataupun vaksin yang telah disetujui penggunaannya dan tersedia di dunia untuk penyakit infeksi dengue. Pencegahan infeksi dengue masih terbatas pada pengendalian vektor nyamuk Aedes aegypti. Keempat serotipe virus dengue beredar di Indonesia. Dua genotipe dari virus dengue tipe 1 (DENV-1) yaitu genotipe I dan IV lebih dominan bersirkulasi di Indonesia. Pada tahun 2012, kami mengembangkan kandidat vaksin DNA DENV-1 pUMVC RDS 59/09. Tujuan penelitian ini untuk mengetahui kadar antibodi anti-membran dan envelop DENV-1 yang dihasilkan mencit ddY setelah imunisasi sebanyak tiga kali dengan pUMVC RDS 59/09 dan untuk mengetahui kemampuan antibodi tersebut dalam menetralkan beberapa genotipe DENV-1 yang diisolasi di Indonesia.

Metode:

Penelitian eksperimental ini dimulai dari perbanyakan pUMVC RDS 59/09 di sel E.coli untuk mendapatkan konsentrasi yang tinggi. Imunisasi dilakukan pada 12 mencit strain ddY dengan pUMVC RDS 59/09 25 µg/100 µl menggunakan needle-free injector, sebanyak tiga kali dengan interval waktu 3 minggu. Sebanyak 12 mencit disediakan sebagai kontrol yang tidak diimunisasi. Antibodi anti-membran dan envelop DENV-1 pada masing-masing serum mencit diperiksa dengan ELISA dan dibaca pada panjang gelombang 450 nm. Berikutnya, pooled serum mencit pasca imunisasi ke 3, digunakan untuk netralisasi 13 isolat DENV-1 dengan metode focus reduction neutralization test (FRNT). Fokus yang didapatkan dari FRNT diwarnai dengan tehnik imunoperoksidase dan dihitung secara manual.

Hasil:

Rerata nilai OD ELISA antibodi anti-membran dan envelop DENV-1 dari serum mencit kelompok imunisasi yang diambil sebelum imunisasi, pasca imunisasi 1, pasca imunisasi 2 dan pasca imunisasi 3 adalah 0,329;0,843;1,524 dan 1,598, secara berurutan. Terdapat peningkatan nilai OD ELISA antibodi anti-membran dan envelop DENV-1 dari pooled serum mencit kelompok imunisasi pasca imunisasi pertama, kedua dan ketiga dibandingkan dengan baseline. Titer FRNT antibodi anti-membran dan envelop DENV-1

dari pooled serum mencit pasca imunisasi 3 dan pooled serum mencit kontrol terhadap strain DV-1 RDS 59/09 adalah 1/320 dan < 1/10. Titer FRNT 13 isolat DENV-1 oleh antibodi anti-membran dan envelop DENV-1 dari pooled serum mencit pasca imunisasi 3 berkisar dari 1/320 sampai lebih dari 1/1280.

Kesimpulan:

Variasi genotipe DENV-1 tidak menyebabkan perbedaan titer antibodi netralisasi yang bermakna (p = 0,222), sehingga dapat diuraikan bahwa antibodi anti-membran dan envelop DENV-1 dapat menetralkan 13 isolat DENV-1 Indonesia yang diuji.
, Introduction:

Dengue virus infection is still a burden in tropical country such as Indonesia. Dengue virus infection causes high mortality and morbidity. No drugs or vaccines are approved and available in the world for this disease. Dengue prevention is still limited to vector control. Four dengue serotypes are circulated in Indonesia. Two genotypes of Dengue virus type 1 (DENV-1), namely genotypes I and IV are found predominantly in Indonesia. Previously in 2012, we constructed pUMVC RDS 59/09, the DENV-1 DNA vaccine candidate. The objective of this study is to assess antibody level produced in ddY strain mice after three times immunization with pUMVC RDS 59/09 and to assess the antibody ability to neutralize genotypes of DENV-1 isolated in Indonesia.

Methods:

This experimental study was started with propagation of pUMVC RDS 59/09 in E. coli cells to produce high concentration of the DNA. Immunization was carried out with 25 µg/ 100 µl pUMVC RDS 59/09 by needle-free injector, three times in 3 weeks interval. Twelve mice were provided for control without immunization. Anti-DENV-1 membrane and envelope antibody of individual sera were examined by ELISA and absorbance value was measured by ELISA reader in 450 nm wave length. Further, pooled sera of 3rd immunization were used to neutralize 13 DENV-1 isolates by focus reduction neutralization test (FRNT) method. The focus obtained in FRNT was stained by immune-peroxides technique and counted manually.

Results:

ELISA OD value mean of anti-DENV-1 membrane and envelope antibody in individual ddY mice sera of immunized group before immunization, post first immunization, after second immunization and post third immunization were 0.329; 0.843; 1.524 and 1.598, respectively. An increase in ELISA OD value of anti-DENV-1 membrane and envelope antibody in ddY mice pooled sera of immunized group after first, second and third immunization compared to baseline was observed. FRNT titre of anti-DENV-1 membrane and envelope antibody from third immunization pooled sera compared to control mice pooled sera in RDS 59/09 isolate neutralization was 1/320 compared to < 1/10. Neutralization titre of 13 DENV-1 isolates by anti-DENV-1 membrane and envelope antibody from third immunization pooled sera ranged from 1/320 to more than 1/1280.

Conclussions:

DENV-1 genotype variation did not lead to significant neutralization antibody titre difference ($p = 0,222$), so it can be explained that anti-DENV-1 membrane and envelope antibody was able to neutralize 13 strains of Indonesia DENV-1 isolates examined.
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