Analisis imunogenitas kandidat vaksin DNA prM-E virus dengue serotipe 2 strain Indonesia melalui galur sel U937 in vitro = Analysis of immunogenicity of DNA vaccine candidate prM-E dengue virus serotype 2 strain of indonesia through U937 cell line in vitro / Lenggo Geni

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Abstrak

[ABSTRAK
Demam berdarah dengue (DBD) merupakan penyakit yang disebabkan karena infeksi virus dengue (DENV), yang banyak ditemukan di Indonesia. Belum ada terapi yang spesifik dalam pengobatan DBD. Upaya pengembangan vaksin dengue yang efektif sangat diperlukan. Pada penelitian ini dilakukan analisa imunogenisitas kandidat vaksin DNA prM-E dengue serotipe 2 (pUMD2.kl.20) dengan menganalisis sel T CD4, sel T CD8, IFN-γ dan TNF-α. pada sel U937 dan PBMC secara in vitro, bertujuan untuk mengetahui respon imun ketika vaksin diinjeksikan ke dalam tubuh manusia. Dasar penelitian ini adalah sel U937 ditransfeksi dengan pUMD2.kl.20 menggunakan lipofectamin. Sel U937 akan berperan sebagai APCs yang akan mengekspresikan protein prM-E DENV-2 dan mempresentasikan protein tersebut melalui molekul MHC class I dan MHC class II kepada Peripheral Blood Mononuclear Cell (PBMC) manusia. Tahapan kerja yang dilakukan dalam penelitian ini terdiri dari: (a) kultur galur sel U937, (b) transfeksi sel U937 dengan pUMD2.kl.20, (c) transfeksi sel U937 dengan plasmid s(pUMVC), (d) infeksi sel U937 dengan DENV-2 strain DS.18/09, (e) pewarnaan dan pengamatan hasil pewarnaan menggunakan alat semi flowcytometri (TALI). pUMD2.kl.20 mengaktivasi sel T CD4 dan sel T CD8 untuk berploriferasi. Hasil penelitian ini menunjukkan bahwa konsentrasi sel T CD8 lebih tinggi dari konsentrasi sel T CD4 dan konsentrasi sel positif yang mensekresikan IFNγ lebih tinggi dari konsentrasi sel positif yang mensekresikan TNFα. Kondisi optimal dari aktivasi sel T CD4 oleh pUMD2.kl.20 adalah 24 jam setelah penambahan PBMC setelah transfeksi (8,53x10⁴sel/ml), untuk sel T CD8 adalah 24 jam setelah penambahan PBMC setelah transfeksi (49,4x10⁴ sel/ml). Kondisi optimal dari aktivasi sel+ yang mensekresikan IFNγ oleh pUMD2.kl.20 adalah 24 jam setelah penambahan PBMC 48 jam setelah transfeksi (9,86x10⁴ sel/ml), dan untuk sel+ yang mensekresikan TNFα adalah 2 jam setelah penambahan PBMC 48 jam setelah transfeksi (2,1x10⁴ sel/ml). Dari penelitian ini dapat disimpulkan bahwa pUMD2.kl.20 bersifat imunogenik.
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ABSTRACT
Dengue hemorrhagic fever (DHF) is a disease caused by infection with dengue

virus (DENV), which is found in Indonesia. There is no specific therapy in the treatment of DHF. An effort to develop an effective dengue vaccine is needed. In this research, analysis of the immunogenicity of the DNA vaccine candidate prME dengue serotype 2 (pUMD2.kl.20) by analyzing the CD4 T cells, CD8 T cells, IFN-γ and TNF-α in U937 cells and PBMC in vitro, aims to determine the immune response when the vaccine is injected into the human body. This research approach is based on transfection of U937 cells with pUMD2.kl.20 using lipofectamin. U937 cells acts as APCs which will express the protein prM-E DENV-2 and presenting these proteins through the MHC class I and MHC class II molecule to the Peripheral Blood Mononuclear Cell (PBMC) of human body. Stages of the work in this study consisted of: (a) culture cell line U937, (b) transfection of U937 cells with pUMD2.kl.20, (c) transfection of U937 cells with plasmid (pUMVC), (d) infection of U937 cells with DENV-2 strains DS.18/09, (e) staining and observation results of staining using a semi-flow cytometri (TALI). pUMD2.kl.20 activate CD4 T cells and CD8 T cells to proliferate. CD8 T cells concentration higher than the concentration of CD4 T cells and the secretion of INFγ-positive cells concentration higher than the concentration of the secretion of TNFα-positive cells. Optimal condition of CD4 T cells activation by pUMD2.kl.20 is 24 hours after the addition of PBMC after transfection (8,53x10⁴ cells/ml), for CD8 T cells was 24 hours after the addition of PBMC after transfection (49,4x10⁴ cells/ml). Optimal conditions secretion of IFNγ-positive cells were activated by pUMD2.kl.20 is 24 hours after the addition of PBMC 48 hours after transfection (9,86x10⁴ cells/ml), and for the secretion of TNFα-positive cells were activated by pUMD2.kl.20 is 2 hours after the addition of PBMC 48 hours after transfection (2,1x10⁴ cells/ml). From this study it can be concluded that the pUMD2.kl.20 immunogenic, Dengue hemorrhagic fever (DHF) is a disease caused by infection with dengue

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