UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF *Salmonella*-BASED DNA VACCINE AGAINST RESPIRATORY SYNCYTIAL VIRUS

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By

SITI SYAZANI SUHAIMI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

August 2011

DEDICATION

To my beloved family
for their endless love, concern and encouragement….

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for degree of Master Science.

DEVELOPMENT OF *Salmonella*-BASED DNA VACCINE AGAINST RESPIRATORY SYNCYTIAL VIRUS

By

SITI SYAZANI BINTI SUHAIMI

August 2011

Chairman : Fatemeh Jahanshiri, PhD

Faculty : Biotechnology and Biomolecular Sciences

Human respiratory syncytial virus (HRSV) remains a major respiratory pathogen responsible for severe pulmonary disease in young children, immunodeficient patients and the elderly. Although these complications are public health concern worldwide, an effective RSV vaccine is still unavailable. In 1960s the formalin-inactivated (FI) RSV vaccine failed due to an imbalanced Th2-biased immune response as enhanced diseases in vaccinated infants were induced upon infection with RSV. It is believed that an effective and safe vaccine needs to elicit a balanced immune response, including high levels of HRSV-specific neutralizing antibodies, Th1/Th2 CD4+ T-cells, CD8+ T-cells, and preferably strong mucosal IgA to provide complete protection against RSV.

In the present study a *Salmonella*-based DNA vaccine against HRSV was designed. The G glycoprotein which has been implicated as the attachment protein of RSV is a potentially important target for protective antiviral immune response. While, cholera toxin B subunit (*CTB*) gene which acts as genetic adjuvant is an effective strategy to enhance induction of both humoral and cellular immune responses. Therefore, firstly the RSV G epitope regions including residues 125-226 was cloned along with the *CTB* gene into pVAX1, a mammalian expression vector, resulting a DNA vaccine vector designated as pVAX1-CTB/G. *In vitro* expression of CTB-G was confirmed by transfection of the recombinant pVAX1-CTB/G into COS-7 cells as the expected protein band was observed in western blot analysis. Secondly, the pVAX1-CTB/G vector was transformed into *Salmonella typhi* Ty21a as a vehicle for DNA vaccine delivery. The immunogenicity and protective efficacy of recombinant *Salmonella* harbouring the pVAX1-CTB/G was assessed in BALB/c mice model before and after RSV challenge.

The capacity of pVAX1-CTB/G to enhance humoral (Th2), cellular (Th1), as well as mucosal responses were evaluated by measurement of cytokines and immunoglobulin levels in serum of immunized BALB/c mice before and after challenge with RSV. Results indicated that the developed vaccine could significantly enhance Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-10) cytokines response compared to control group. While, antibody isotype immunoglobulin analysis revealed that the DNA vaccine induced significant concentrations of systemic antibody (IgG1, IgG2a) as well as mucosal (IgA) in vaccinated mice compared to control group. Moreover, the obtained ratio of Th1/Th2 was desirable (~1) suggesting that *Salmonella* carrying pVAX1-CTB/G is potent

vaccine candidate against HRSV. Lymphocyte proliferation assay showed that cell mediated immunity was also significantly increased in response to this vaccine. Finally, significant reduction of HRSV titer and presence of less viral RNA in the lung tissues of vaccinated mice compared to control confirmed the efficacy of *Salmonella* vaccine harboring pVAX1-CTB/G.

Abstrak tesis yang telah dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

PEMBANGUNAN DNA VAKSIN BERASASKAN *Salmonella* MENENTANG VIRUS RESPIRASI SINSITIUM (RSV)

Oleh

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Virus Respirasi Sinsitium (RSV) masih merupakan pathogen respirasi utama yang bertanggungjawab kepada penyakit pulmonary yang teruk di kalangan kanak-kanak muda, pesakit imunodefisiensi and orang tua. Walaupun komplikasi-komplikasi ini merupakan keprihatinan kesihatan awam seluruh dunia, vaksin menentang RSV yang efektif masih tiada. Pada tahun 1960, vaksin Formalin-tidak aktif (FI) RSV gagal disebabkan ketidakseimbangan bias Th2 respon imun dimana penyakit meningkat di kalangan bayi yang divaksinkan setelah dijangkiti dengan RSV. Dipercayai bahawa vaksin yang efektif dan selamat memerlukan induksi respon immun yang seimbang, termasuk aras tinggi antibodi neutral RSV yang spesifik, sel-sel Th1/Th2 CD4+ T, sel-sel CD8+ T dan mukosal IgA yang kuat untuk menyediakan perlindungan yang lengkap terhadap RSV.

Dalam kajian ini, DNA vaksin berasaskan *Salmonella* menentang RSV telah direkacipta. Glikoprotein G yang telah diimplikasikan sebagai protein perlekatan RSV merupakan sasaran utama yang berpotensi untuk perlindungan respon imun antiviral. Sementara itu, gen cholera toxin B subunit (CTB) yang bertindak sebagai adjuvan genetik merupakan strategi yang effektif untuk meningkatkan induksi kedua-dua respon imun humoral dan selular. Maka, pertama kawasan epitop RSV G termasuk residu 125-226 amino asid telah diklon bersama dengan gen *CTB* ke dalam pVAX1, ekspresi vector mamalia menghasilkan vektor vaksin DNA digelar pVAX1-CTB/G. Expresi in vitro oleh G-CTB telah disahkan oleh transfeksi pVAX1-CTB/G rekombinan ke dalam sel COS-7 dimana jalur protein yang dijangkakan telah diperhatikan menerusi analisis western blot. Kedua, vektor pVAX1-CTB/G telah ditransfomasi ke dalam *Salmonella typhi* Ty21a sebagai kenderaan untuk penghantaran DNA vaksin. Keimunan dan keefisyenan perlindungan oleh rekombinan *Salmonella* yang membawa pVAX1-CTB/G telah dinilai dalam model mencit BALB/c sebelum dan selepas dicabar RSV.

Kapasiti pVAX1-CTB/G untuk meningkatkan respon imun humoral (Th2), selular (Th1), dan mukosal telah dinilai dengan menggunakan pengukuran aras sitokin dan imunoglobolin dalam serum mencit BALB/c yang diimunkan sebelum dan selepas dicabar dengan RSV. Keputusan menunjukkan pembangunan vaksin boleh meningkatkan Th1 (IL-2, IFN-γ) dan Th2 (IL-4, IL10) respon sitokin dengan signifikan berbanding dengan kumpulan kawalan dengan signifikan. Sementara itu, analisis isotype immunoglobulin menunjukkan bahawa DNA vaksin menginduksi kosentrasi antibodi sistemik (IgG1, IgG2a) dan mukosal (IgA) dengan signifikan pada mencit yang

divaksinkan berbanding kumpulan kawalan. Tambahan pula, nisbah Th1/Th2 yang diperolehi adalah patut (~1), mencadangkan bahawa *Salmonella* yang membawa pVAX1-CTB/G merupakan calon vaksin yang kuat menetang RSV. Ujian proliferasi limfosit menunjukkan keimunan mengantarai sel juga meningkat dengan signifikan dalam respon vaksin ini. Akhirnya, penurunan titer RSV yang signifikan dan pengurangan kehadiran virus-virus RNA pada tisu hati paru-paru dalam respon terhadap vaksin ini berbanding dengan kawalan mengesahkan kekuatan vaksin *Salmonella* yang membawa pVAX1-CTB/G.

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I certify that a Thesis Examination Committee has met on 3 August 2011 to conduct the final examination of Siti Syazani Suhaimi on her thesis entitled "Development of Salmonella-Based DNA Vaccine Against Respiratory Syncytial Virus (RSV)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998 The Committee recommends that the student be awarded the Master of Science.

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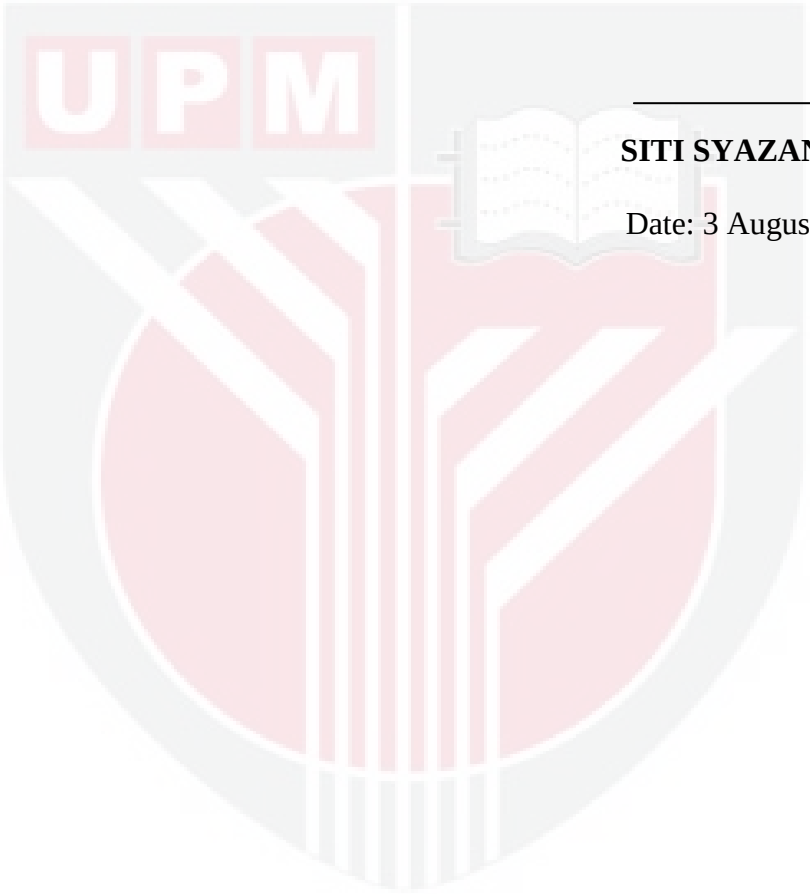
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



SITI SYAZANI SUHAIMI

Date: 3 August 2011

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