



UNIVERSITI PUTRA MALAYSIA

**DEVELOPMENT OF A NOVEL ORAL VACCINE AGAINST HUMAN
RESPIRATORY SYNCYTIAL VIRUS**

FARID AZIZI JALILIAN

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By

FARID AZIZI JALILIAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

March 2011



Dedication

I gratefully dedicate this work to the loving memory of my father who exemplified a passion for kindness, love and honesty.



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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FARID AZIZI JALILIAN

March 2011

Chairman: Professor Datin Paduka Dr. Khatijah Mohd. Yusoff, PhD

Faculty: Biotechnology and Biomolecular Sciences

Human respiratory syncytial virus (HRSV) is the leading cause of bronchiolitis and pneumonia in infants, children, the elderly and the immune-compromised. The goal of immunization is to provide sufficient protection to prevent serious lower respiratory tract diseases leading to hospitalization and reducing the frequency of complications such as otitis media. Prevention and treatment of HRSV infection using antiviral agents is challenging because it is a rapid acute infection and by the time the infection is recognized it may be too late to control the disease with any antiviral therapy alone. Thus, there is a worldwide need for an HRSV vaccine.

Studies have shown that the immunogenic domains of F and G proteins could confer protection against HRSV infection in vaccinated hosts. In the present study, firstly the



immunogenic domain of HRSV G domain was expressed in *Escherichia coli*. Then a rabbit was immunized using purified-recombinant G domain protein. The results of neutralization assay showed that G domain alone could raise active polyclonal antibodies against HRSV successfully. Secondly, the potential of G and F immunogenic domains as vaccine candidates were studied by using live bacterial vaccines. Both the G and F domains were separately initially cloned in pKMSInak plasmid before they were surface displayed on *Salmonella typhi Ty21a* used as the delivery system. The surface displayed G and F domains were detected using indirect immunofluorescence, sero-agglutination and outer membrane protein separation approaches suggesting that the Inak protein successfully carried the G and F domains to the surface of *Salmonella* cells. For *in vivo* evaluation of the designed vaccines, Balb/c mice were immunized orally with live *Salmonella* cells harboring pKMSInak-G or pKMSInak-F and challenged against HRSV.

The humoral (TH2), cellular (TH1) and mucosal immune (IgA) responses of the immunized mice were studied by measuring cytokines (IL-2, IL-4, IL-5, IL-9, IL-10, IL-12, IL-13, IL-17, IFN- γ and TNF- α), chemokines (RANTES and MIP- α) and immunoglobulins (IgG, IgG1, IgG2a, IgG2b and IgA) levels in their sera before and after challenging with HRSV. Lymphocyte proliferation assay was performed to evaluate the cell mediated immunity. Histopathological examinations were also carried out as confirmatory tests. The results showed that pKMSInak-G and pKMSInak-F vaccines could significantly enhance TH1 and TH2 responses as well as mucosal immunity in the immunized mice compared to the control group. Histopathological examinations indicated that the immunized mice had significantly lesser lung tissue



damage than the control. Moreover, the obtained ratios of TH1/TH2 were desirable (~1) suggesting that *Salmonella* cells carrying pKMSInak-G and pKMSInak-F are potent vaccine candidates against HRSV.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGEMBANGAN NOVEL VAKSIN ORAL MELAWAN VIRUS SINSITIUM
PERNAFASAN MANUSIA**

Oleh

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Pengerusi : Profesor Datin Paduka Dr.Khatijah Mohd. Yusoff, PhD

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Virus sinsitium pernafasan manusia (hRSV) merupakan penyebab utama kepada jangkitan bronchiolitis dan pneumonia di kalangan bayi, kanak-kanak, dewasa dan pesakit kurang daya tahan. Memandangkan jangkitan RSV tidak dapat dibendung sekaligus, matlamat pengimunan adalah untuk memberikan perlindungan secukupnya untuk menghalang penyakit bahagian bawah sistem pernafasan yang serius sehingga mengakibatkan kemasukkan ke hospital dan mengurangkan frekuensi komplikasi seperti otitis media. Pencegahan dan rawatan bagi jangkitan RSV menggunakan agen antivirus memberikan cabaran kerana ia merupakan satu jangkitan akut yang pesat dan mungkin terlambat untuk mengawal penyakit itu dengan mana-mana terapi antivirus apabila jangkitan dikesan. Oleh yang demikian, vaksin terhadap RSV adalah diperlukan di seluruh dunia.

Kajian telah menunjukkan bahawa domain imunogenik protein F dan G boleh memberikan perlindungan terhadap jangkitan RSV dalam hos yang divaksinakan. Untuk

menilai keimmunogenan gen yang terpilih, pertamanya domain G diekspreskan di dalam sistem pengekspresan bakteria (*E. coli*). Kemudian, arnab disuntik dengan domain G yang telah dituliskan. Keputusan daripada penetapan kadar peneutralan menunjukkan bahawa domain G sahaja berjaya mengaruhkan antibodi poli-klon. Keduanya, potensi domain imunogenik G dan F sebagai calon vaksin telah dikaji dengan menggunakan vaksin bakteria hidup. Kedua-dua domain G dan F pada mulanya diklonkan secara berasingan ke dalam plasmid pKMSInak sebelum ianya wujud pada bahagian permukaan *Salmonella typhimurium 21a*. Permukaan di sel yang mempamerkan domain G dan F telah dikesan dengan menggunakan imunopendarfluor secara tak langsung, sero- pengaglutinatan dan pendekatan pemisahan protein membran luar yang mana mencadangkan protein Inak dengan jayanya dapat membawa domain-domain ke permukaan *Salmonella*. Penilaian secara *in vivo* untuk penghasilan vaksin, tikus Balb/c telah disuntik secara oral dengan sel hidup *Salmonella* yang mempunyai pKMSInak-G or pKMSInak-F dan menentang RSV.

Respon bagi keimunan humoral (TH2), selular (TH1) dan mukosa (IgA) yang telah disuntik pada tikus dikaji dengan menganalisa sitokin (IL-2, IL-4, IL-5, IL-9, IL-10, IL-12, IL-13, IL-17, IFN- γ and TNF- α), chemokines (RANTES AND MIP- α) dan imunoglobulin (IgG, IgG1, IgG2a, IgG2b and IgA) secara berperingkat serta ujian percambahan limfosit. Ujian histopatologi turut dijalankan sebagai ujian-ujian pengesahan. Keputusan menunjukkan bahawa vaksin pKMSInak-G dan pKMSInak-F itu boleh meningkatkan respon-respon TH1 dan TH2 serta keimunan mukosa tikus yang disuntik berbanding dengan kumpulan kawalan. Ujian histopatologi juga menunjukkan bahawa tikus-tikus yang telah diimmunisasi mempunyai kerosakan tisu paru-paru yang

sedikit berbanding dengan kawalan. Tambahan pula, nisbah TH1/TH2 yang telah diperolehi (~ 1) menunjukkan sel-sel *Salmonella* yang membawa pKMSInak-G and pKMSInak-F merupakan vaksin yang berpotensi menentang jangkitan HRSV.



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I certify that an Examination Committee met on 2th March 2011 to conduct the final examination of Farid Azizi Jalilian on his Doctor of Philosophy thesis entitled “Development of A Novel Oral Vaccine Against Respiratory Syncytial Virus” in accordance with Universities and University Colleges Act 1971 and the constitution of the university Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy. Members of the Thesis Examination Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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Date: 2 March 2011



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