UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF A NOVEL ORAL VACCINE AGAINST HUMAN RESIPRATORY 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

DEVELOPMENT OF A NOVEL ORAL VACCINE AGAINST RESPIRATORY SYNCYTIAL VIRUS

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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

FARID AZIZI JALILIAN

March 2011

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

Fakulti : Bioteknologi dan Sains Biomolekul

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 divaksinkan. Untuk

menilai keimunogenan gen yang terpilih, pertamanya domain G diekspreskan di dalam sistem pengekspresan bakteria (*E. coli*). Kemudian, arnab disuntik dengan domain G yang telah ditulenkan. 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 diimunisasi 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.

ACKNOWLEDGEMENTS

I express my sincere gratitude to my advisor, Prof.Khatijah Yusoff for her guidance and encouragement throughout the project. Her patience and understanding have helped me immensely in lab as well as outside.

I thank my committee members, Dr.Fatemeh Jahanshiri, Prof.Zamberi sekawi, Prof.Rahman Omar and Prof.Richard Surgue for their constructive suggestions and directions.

My special thanks to Dr.Jahanshiri who provided everything for me, a sympathetic ear and helping hand when it was needed which was often. I cannot thank her enough, and am forever grateful.

Thanks Dr.Zamberi for taking me into your Lab, letting me go about things on my own, and also allowing me create a comfortable lab environment in which to conduct research.

Members of the Molecular and clinical virology lab, Sobhan, Mehdi, Mohsen, Reza, Rand, Salmiah, Hermalizah, Syani, Hoon koon and Syaz have been a great bunch to work with who shared both their friendship and knowledge over the years. Special thanks to Syani for giving me a hand in a lot of my experiments and also my special thanks to Mehdi who helped me a lot to submit my thesis.

I cannot describe in words how important the love and support of my mother, my brother and sister and also my kind uncle has been in my achievements.

And last but not least, Razieh I don't even know where to start with you. Thank you for numerous supports stuff you have given me to cheer me up. Thank you for your technical help in facilitating the doing of this project. Thank you for your patience, understanding and comfort when I complain, whine and yell. Thank you for so much all of which I will never fully realize. You have been the one thing that kept me and still keeps me sane when things are rough and unbearable, you bring me smile on my face. When things are good, you make them better. Without your faith, your love, your encouragement, and you, I don't think I would have gotten this far so quickly or at all.

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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.The members of supervisory committee were as follows:

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Date:

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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FARID AZIZI JALILIAN

Date: 2 March 2011

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