UNIVERSITI PUTRA MALAYSIA

PROTECTIVE EFFICACY EVALUATION OF NPt-VP11-100 PROTEIN AS A CANDIDATE VACCINE AGAINST ENTEROVIRUS 71 INFECTIONS IN MOUSE MODEL

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By

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PROTECTIVE EFFICACY EVALUATION OF NPt-VP1$_{1-100}$ PROTEIN AS A CANDIDATE VACCINE AGAINST ENTEROVIRUS 71 INFECTIONS IN MOUSE MODEL

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June 2010

Chairman: **Norazizah Shafee, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Enterovirus 71 (EV71) is a type of human virus belonging to the *Enterovirus* genus within the *Picornaviridae* family. The virus mainly causes hand, foot and mouth disease in children which sometimes lead to severe neurological complications. Outbreaks of EV71 infections are serious health threats since effective antiviral drugs or vaccines are not currently available. Therefore, development of an effective vaccine is ideal for the prevention and control of EV71 disease outbreak. The use of recombinant EV71 viral protein offers an alternative to the more risky method of using whole live attenuated or inactivated virus. Our previous study using truncated VP1 protein (VP1$_{1-100}$) of EV71 virus fused to a carrier protein showed strong immune response in adult rabbits. The study however, did not address the issue of its effectiveness in young animals. This factor is important since EV71 mostly infected children younger than 5 year-old. In the present study, we investigated the protective

efficacy of NPt-VP1$_{1-100}$ protein against EV71 infection in a recently-developed newborn mouse model system. Prior to investigation in the newborn mouse model, we evaluated the type of immune responses developed by adult mice against NPt-VP1$_{1-100}$ protein. In adult mice, the protein induced high levels of anti-VP1 IgG production. Purified VP1 antigen stimulated activation, proliferation and differentiation of splenocytes harvested from the immunized mice. They also produced high levels of IFN-γ. Following determination of immune responses towards NPt-VP1$_{1-100}$ protein in adult mice, we performed immunization and virus challenge study in newborn mice model. Since the mice was only susceptible to EV71 infection before they are 14 day-old, only two doses of immunization were carried out. Even though the IgG produced lacked neutralization properties, immunized newborn mice were still partially protected from EV71 viral challenge. They showed high (47.4%) survival rate as compared to the control group and importantly, 50% of them fully recovered from paralysis symptoms at the end of the study. Histological analysis of all the surviving mice revealed a complete clearance of EV71 viral antigens from their brains and spinal cords. In hind limb muscles, the level of antigens detected correlated directly with tissue damage and their paralysis symptoms. We also initiated a similar study in a hamster model which had longer EV71 susceptibility period. In hamster, the NPt-VP1$_{1-100}$ protein was also found to be highly immunogenic. Findings from the study showed that immunization with NPt-VP1$_{1-100}$ protein in newborn mice model confer them a partial protection against EV71 infection. NPt-VP1$_{1-100}$ protein therefore offers a great promise towards finding a vaccine for EV71 infections.

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

PENILAIAN TERHADAP KEBERKESANAN PERLINDUNGAN PROTEIN NPt-VP1$_{1-100}$ SEBAGAI SATU CALON VAKSIN UNTUK JANGKITAN ENTEROVIRUS 71 DALAM MODEL MENCIT

Oleh

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Enterovirus 71 (EV71) adalah sejenis virus manusia yang tergolong dalam genus *Enterovirus* dan famili *Picornaviridae*. Virus ini merupakan penyebab penyakit tangan, kaki dan mulut di kalangan kanak-kanak dan ia boleh menyebabkan komplikasi neurologi yang teruk. Wabak jangkitan EV71 merupakan ancaman kesihatan yang serius kerana sehingga kini, tiada ubat atau vaksin antivirus yang berkesan untuk merawat dan mencegah penyakit ini. Oleh itu, penghasilan vaksin yang berkesan adalah penting untuk pencegahan dan kawalan wabak penyakit EV71. Penggunaan protein rekombinan daripada virus EV71 menawarkan satu alternatif kepada kaedah yang lebih berisiko seperti menggunakan virus yang telah dilemahkan atau tidak aktif. Dalam kajian sebelum ini, kami menggunakan protein VP1$_{1-100}$ yang merupakan sebahagian daripada protein VP1 virus EV71 yang digabungkan dengan protein pembawa. Protein tersebut menunjukkan tindak balas

imun yang kuat dalam arnab dewasa. Namun begitu, kajian berkenaan tidak mengemukakan persoalan tentang keberkesanannya dalam haiwan muda. Faktor ini adalah penting kerana kebanyakan pesakit yang dijangkiti EV71 adalah kanak-kanak berumur kurang daripada 5 tahun. Dalam kajian ini, kami mengkaji keberkesanan protein NPt-$VP1_{1-100}$ dalam memberi perlindungan terhadap jangkitan EV71 menggunakan model mencit muda yang dihasilkan baru-baru ini. Sebelum kajian dijalankan terhadap model mencit muda, kami menilai jenis tindak balas imun dalam mencit dewasa terhadap protein NPt-$VP1_{1-100}$. Protein itu menyebabkan penghasilan paras anti-IgG VP1 yang tinggi. Antigen VP1 yang telah ditulenkan merangsang pengaktifan, penggandaan dan pembezaan splenosit yang diperolehi daripada mencit yang diimunisasi. Mencit itu juga menghasilkan paras IFN-γ yang tinggi. Setelah penentuan tindak balas imun terhadap protein NPt-$VP1_{1-100}$ dalam mencit dewasa dilakukan, kami mengkaji pula imunisasi dan cabaran virus dalam model mencit muda. Oleh kerana mencit mudah dijangkiti oleh EV71 sebelum mereka berumur 14 hari, hanya dua dos imunisasi diberikan. Meskipun IgG yang dihasilkan tidak mempunyai sifat neutralisasi, mencit yang diimunisasi masih dapat dilindungi secara tidak sepenuhnya daripada cabaran virus EV71. Mereka menunjukkan kadar hidup yang tinggi (47.4%) berbanding dengan kumpulan kawalan, dan pemerhatian terpenting ialah 50% daripada mereka pulih sepenuhnya daripada simptom kelumpuhan pada akhir kajian. Analisis histologi mereka menunjukkan tiada antigen virus EV71 dijumpai di otak dan saraf tunjang. Aras antigen yang dikesan di otot-otot kaki belakang mempunyai hubung kait secara langsung dengan kerosakan tisu dan simptom kelumpuhan. Kami juga melakukan kajian yang sama menggunakan

model hamster. Hamster mempunyai tempoh kerentanan terhadap EV71 yang lebih panjang berbanding mencit. Protein NPt-VP1$_{1-100}$ yang disuntik ke dalam hamster juga mempunyai kadar imunogenik yang tinggi. Keputusan daripada kajian menunjukkan bahawa imunisasi dengan protein NPt-VP1$_{1-100}$ dalam model mencit memberikan perlindungan separa kepada mereka terhadap jangkitan EV71. Oleh itu, protein NPt-VP1$_{1-100}$ mempunyai potensi yang tinggi sebagai vaksin untuk jangkitan EV71.

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I certify that a Thesis Examination Committee has met on **28th JUNE 2010** to conduct the final examination of **Ch'ng Wei Choong** on his thesis entitled **"Protective Efficacy Evaluation of NPt-VP1$_{1-100}$ Protein as a Candidate Vaccine against Enterovirus 71 Infections in Mouse Model"** in accordance with 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 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 institutions.

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CH'NG WEI CHOONG

Date: 12 August 2010

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