UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF A UNIVERSAL INFLUENZA A VACCINE BASED ON VIRUS-LIKE PARTICLES OF MACROBRACHIUM ROSENBERGII NODAVIRUS

ONG HUI KIAN

FPSK(m) 2019 12

DEVELOPMENT OF A UNIVERSAL INFLUENZA A VACCINE BASED ON VIRUS-LIKE PARTICLES OF *Macrobrachium rosenbergii* NODAVIRUS

By

ONG HUI KIAN

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

November 2018

© COPYRIGHT UPM

COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia

© COPYRIGHT UPM

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DEVELOPMENT OF A UNIVERSAL INFLUENZA A VACCINE BASED ON VIRUS-LIKE PARTICLES OF *Macrobrachium rosenbergii* NODAVIRUS

By

ONG HUI KIAN

November 2018

Chairman : Ho Kok Lian, PhD
Faculty : Medicine and Health Sciences

Influenza A virus (IAV) claims approximately 290,000 to 650,000 lives annually across the globe. Current influenza vaccines are composed of haemagglutinin (HA) and neuraminidase (NA) of the IAV and were shown to be effective in inducing strong and long-lasting HA and NA specific immunities. However, annual reformulation of the seasonal influenza vaccine is required to catch up the rapid mutations of HA and NA. Effectiveness of a seasonal influenza vaccine could vary considerably from 60 to 90% depending on the similarities of HA and NA proteins of the circulating strains and that of the vaccine strains. Seasonal influenza vaccine effectiveness could be as low as 10% in the case of incorrect prediction of IAV mutations. Therefore, a universal influenza vaccine is in urgent need. Extracellular domain of matrix 2 protein (M2e) of IAV represents a potential candidate for the development of a universal influenza vaccine due to its highly conserved amino acid sequence among IAVs. Nevertheless, M2e is poorly immunogenic in nature and requires a larger carrier to enhance its immunogenicity. Multiple copies of M2e epitopes were previously fused to the C-terminal end of *Macrobrachium rosenbergii* nodavirus capsid protein (NvC), producing a chimeric protein with three copies of M2e (NvC-M2ex3) which self-assemblies into virus-like particles (VLPs). Although NvC-M2ex3 was demonstrated to be immunogenic in the presence of adjuvants, its protective efficacy has not been investigated *in vivo*. Therefore, as a continuation of the previous study, induced immune responses, protective efficacy and universality of NvC-M2ex3 against influenza A virus infections were elucidated in this project. BALB/c mice immunised subcutaneously with NvC-M2ex3 were shown to elicit strong M2e specific humoral immune responses even in the absence of adjuvant. When challenged with lethal mouse-adapted H1N1 or H3N2, NvC-M2ex3 immunised mice exhibited 100% survival with reduced morbidity and weight loss in addition to the reduced viral load and viral shedding compared to the control groups. In the histopathological aspect, NvC-M2ex3 immunised mice also experienced mitigated immunopathology in lungs upon influenza A infections. Cytokine

© COPYRIGHT UPM

responses of the mice immunised with NvC-M2ex3 were found to be different when challenged with different influenza A viruses. A higher level of IFN-γ and IL-12 but no significant difference of IL-6 was detected in the lungs of the NvC-M2ex3 immunised mice compared to the control group upon H1N1 infection. Contrarily, when challenged with H3N2, they exhibited lower level of IFN-γ and IL-6 although a higher level of IL-12 was observed in the lungs. Collectively, this study demonstrated the protective efficacy of NvC-M2ex3 against lethal H1N1 and H3N2 infections.

© COPYRIGHT UPM

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

VAKSIN INFLUENZA A UNIVERSAL BERDASARKAN PARTIKEL MENYERUPAI *Macrobrachium rosenbergii* NODAVIRUS

Oleh

ONG HUI KIAN

November 2018

Chairman : Ho Kok Lian, PhD
Fakulti : Perubatan dan Sains Kesihatan

Virus Influenza A (IAV) meragut kira-kira 290,000 hingga 650,000 nyawa setiap tahun di seluruh dunia. Vaksin influenza semasa terdiri daripada haemagglutinin (HA) dan neuraminidase (NA) daripada IAV dan ditunjukkan berkesan untuk mendorong tindak balas imunologi khusus terhadap HA dan NA yang berkekalan. Walau bagaimanapun, pembaharuan vaksin influenza bermusim diperlukan setiap tahun untuk mengambil kira mutasi pesat HA dan NA. Keberkesanan vaksin influenza bermusim boleh berbeza jauh dari 60 hingga 90% bergantung kepada persamaan protein HA dan NA antara strain yang sedang beredar dan strain yang digunakan dalam vaksin. Keberkesanan vaksin influenza bermusim boleh serendah 10% dalam kes ramalan mutasi IAV yang salah. Oleh kerana itu, vaksin influenza universal adalah sangat diperlukan. Domain ekstrasel matriks 2 protein (M2e) IAV merupakan satu calon yang berpotensi untuk pembangunan vaksin influenza universal kerana urutan asid amino yang sangat konservatif di kalangan IAVs. Walau bagaimanapun, M2e adalah kurang imunogenik dan memerlukan pembawa yang lebih besar untuk meningkatkan immunogenisitinya. Sebelum ini beberapa salinan epitop M2e telah bersandar ke hujung karboksil *Macrobrachium rosenbergii* nodavirus kapsid protein (NvC), menghasilkan fusi protein dengan tiga salinan M2e (NvC-M2ex3) yang bercantum sendiri untuk membentuk partikel menyerupai virus (VLPs). Walaupun kajian immunisasi telah menunjukkan bahawa NvC-M2ex3 adalah imunogenik di bawah kesan adjuvan, keberkesanannya terhadap pencegahan jangkitan virus influenza A tidak dikaji secara *in vivo*. Oleh itu, sebagai sambungan kajian terdahulu, tindak balas sistem imun, keberkesanan dan keuniversalan NvC-M2ex3 terhadap jangkitan virus influenza A telah dijalankan dalam projek ini. Tikus BALB/c yang diimmunisasi secara subkutan dengan NvC-M2ex3 telah mendorong tindak balas immunologi secara humoral yang spesifik terhadap M2e walaupun tanpa penggunaan adjuvan. Ketika tikus yang telah diimunisasi dengan NvC-M2ex3 dijangkiti dengan H1N1 atau H3N2 yang telah diadaptasi dalam tikus, 100% kadar kemandirian telah diperhatikan. Selain itu, bagi tikus yang telah diimunisasi dengan

© COPYRIGHT UPM

NvC-M2ex3, morbiditi dan penurunan berat badan tikus juga dikurangkan bersama dengan penurunan beban viral dan penumpahan virus berbanding dengan kumpulan kawalan. Dalam aspek histopatologi, tikus yang diimunisasi dengan NvC-M2ex3 juga mengalami pengurangan imunopatologi dalam paru-paru atas jangkitan influenza A. Tindak balas sitokin tikus yang diimunisasi dengan NvC-M2ex3 didapati berbeza apabila dicabar dengan virus influenza A yang berlainan. Tahap IFN-γ dan IL-12 yang lebih tinggi tetapi tiada perbezaan yang signifikan dalam paras IL-6 dikesan di paru-paru tikus yang diimunisasi dengan NvC-M2ex3 berbanding dengan kumpulan kawalan selepas jangkitan H1N1. Sebaliknya, apabila dicabar dengan H3N2, mereka menunjukkan tahap IFN-γ dan IL-6 yang lebih rendah walaupun paras IL-12 yang lebih tinggi telah diperhatikan di dalam paru-paru. Secara kolektif, kajian ini menunjukkan bahawa NvC-M2ex3 adalah efektif dalam pencegahan jangkitan H1N1 dan H3N2 maut.

© COPYRIGHT UPM

ACKNOWLEDGEMENTS

First and foremost, my deepest gratitude to my supervisor, Dr. Ho Kok Lian for his endless support and providing me with funding and materials essential for the project. I would also like to thank Prof. Dr. Tan Wen Siang for his invaluable advice and guidance throughout the study.

Many thanks to Dr. Yeap Swee Keong for his constructive comments and suggestions in *in vivo* experiment, and Dr. Yong Chean Yeah, who is a good friend and a good mentor that always enlighten me in many aspects. Special thanks to my lab-mates, Beh Poay Ling, Nursyaza Zulkifli and Chia Jyh Yea for their generous guidance in protein purifications.

Last but not least, million thanks to my family and friends, especially my parents who have supported and tolerated me with love and patience throughout the study.

© COPYRIGHT UPM

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follow:

Ho Kok Lian, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Tan Wen Siang, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

© COPYRIGHT UPM

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No: Ong Hui Kian, GS42219

© COPYRIGHT UPM

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Ho Kok Lian

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Tan Wen Siang

© COPYRIGHT UPM

TABLE OF CONTENTS

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF TABLES

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF FIGURES

© COPYRIGHT UPM

LIST OF ABBREVIATIONS

ρg	picogram (10^{-12} g)
µg	microgram (10^{-6} g)
µL	microlitre (10^{-6} L)
µm	micrometre (10^{-6} m)
µM	micromolar (10^{-6} M)
Abs	antibodies
ADCC	antibody dependent cell mediated cytotoxicity
ANOVA	one-way analysis of variance
APC	allophycocyanin
APCs	antigen presenting cells
AS03	adjuvant system 03
BCIP	5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt
BCR	B-cell receptor
bp	basepair
BSA	bovine serum albumin
BTV	bluetongue virus
℃	degree Celsius
CD	cluster of differentiation
cDNA	complimentary DNA
Ct	cycle threshold
C-terminal	carboxyl terminal
CTL	cytotoxic T-cells
CV	column volume

© COPYRIGHT UPM

cvRNA	complimentary viral RNA intermediate
DC	dendritic cells
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
Fc	fragment crystallizable
FITC	fluorescein isothiocyanate
FPLC	fast protein liquid chromatography
g	gram
G	gauge
p-NPP	p-nitrophenyl phosphate
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPEI	Global Polio Eradication Initiative
GTPase	guanosine triphosphatase
HA	haemagglutinin
HAU	haemagglutination units
HCV	hepatitis C virus
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HEV	hepatitis E virus
HPV	human papilloma virus
HRP	horseradish peroxidase
IAV	influenza A virus
iBALT	induced bronchus-associated lymphoid tissue
IFN	interferon

© COPYRIGHT UPM

Ig	immunoglobulin
IIV	inactivated influenza A vaccine
IL	interleukin
IMAC	immobilised metal affinity chromatography
IPTG	isopropyl β-D-1-thiogalactopyranoside
ISG	interferon stimulated genes
kDa	kilo Dalton
kg	kilogram
L	litre
LB	Luria-Bertani
LD_{50}	median lethal dose
LIAV	live attenuated influenza A vaccine
M1	matrix 1
M2	matrix 2
M2e	extracellular domain of matrix 2 protein
mA	milliampere
MA	mouse-adapted
MF59	immunologic adjuvant that uses squalene
mg	milligram (10^{-3} g)
MHC	major histocompatibility complex
mL	millilitre (10^{-3} L)
mm	millimetre (10^{-3} m)
mRNA	messenger RNA
MrNV	*Macrobrachium rosenbergii* nodavirus
Mx	*myxovirus*

© COPYRIGHT UPM

NA	neuraminidase
NEP/NS2	nuclear export protein
ng	nanogram (10^{-9} g)
NK	natural killer
NLRP3	NOD-like receptor family pryin domain containing 3
NLS	nuclear localisation signal
nm	nanometre (10^{-9} m)
NOD	nucleotide-binding oligomerisation domain
NP	nucleoprotein
NPT	nitro-blue tetrazolium chloride
NS1	non-structural
N-terminal	amino terminal
OD	optical density
PA	polymerase acidic
PAGE	polyacrylamide gel electrophoresis
PB1	polymerase basic 1
PB2	polymerase basic 2
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	phycoerythrin
pH	*Puissance hydrogen*
qPCR	real-time polymerase chain reaction
RAG	recombination-activating gene
RdRp	RNA dependent RNA polymerase
RIG-1	retinoic acid inducible gene-I

© COPYRIGHT UPM

RLRs	retinoic acid inducible gene-I-like receptors
RNA	ribonucleic acid
RNP	ribonucleoproteins
SDS	sodium dodecyl sulphate
SIV-HIV	hybrid VLP between simian immunodeficiency virus and human immunodeficiency virus
TAE	tris-acetate-EDTA
TBS	tris-buffered saline
TBST	tris-buffered saline supplemented with Tween-20
TCR	T-cells receptor
Th	T-helper cells
Th1	type 1 T-helper cells
Th2	type 2 T-helper cells
TLRs	toll-like receptors
TNF	tumour necrosis factor
Treg	regulatory T-cells
USD	United States dollar
V	volt
v/v	volume/volume
VLPs	virus-like particles
w/v	weight/volume
WHO	World Health Organization
α	alpha
β	beta
γ	gamma

© COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

Infectious diseases are the most menacing global killers in human history. They contribute to 90% of the world's health problems and have killed more people than famine, war, accidents and crimes. Annually, it accounts for the deaths of more than 14 million people worldwide (Stefansson, 2003). To date, the most effective countermeasure against infectious diseases is still early vaccination. The first vaccine designed by the "Father of Immunology", Edward Jenner has laid the foundation of vaccinology, and eventually, in 1979, World Health Organization (WHO) declared fatal smallpox as an eradicated disease (Strassburg, 1982). Remarkably, following the introduction of polio vaccines and the launch of Global Polio Eradication Initiative (GPEI), previously widespread poliovirus is now confined to just Afghanistan and Pakistan (Toole, 2016). These fascinating milestones imply that eradication of deadly viruses is plausible with intensive researches and education. Nevertheless, eradication of influenza remains extremely challenging to date, due to the large non-human reservoirs and rapid mutation of the influenza A virus (IAV) (Medicine, 2002). Despite the highly contagious nature of IAV is well known and investigated, a very recent study has confirmed that infectious IAV could now be detected even in the exhaled breath of the infected symptomatic patients, presenting a risk of airborne transmission (Yan et al., 2018).

Influenza A virus is a Class V virus belonging to the *Orthomyxoviridae* family. It was responsible for one of the deadliest pandemics in human history, "Spanish flu" occurred in 1918-1919 which claimed more than 50 million lives, and infected approximately one third of the world population (Taubenberger & Morens, 2006). Globally, 290,000 to 650,000 people still die from annual seasonal influenza epidemics according to the World Health Organization (WHO). Current licenced influenza A vaccines comprise inactivated vaccines and live attenuated vaccines which rely on the combination of immunogenic glycoproteins haemagglutinin (HA) and neuraminidase (NA) of the IAV (Sridhar, Brokstad, & Cox, 2015). These combinatorial vaccines are effective in eliciting strong, lasting, and specific immune responses but require annual reformulation due to overtime rapid mutation of HA and NA via a process known as antigenic drift (Houser & Subbarao, 2015). Vaccines effectiveness varies considerably from 60 to 90% depending on the similarities of HA and NA proteins of the circulating strains and that of the vaccine strains (Cox & Subbarao, 1999). Recently, seasonal influenza outbreak at Australia hit a record breaking hospitalisation and mortality rates, and the seasonal influenza vaccine effectiveness was reported to be as low as 10% due to incorrect prediction on IAV mutations (Sullivan et al., 2017). In addition, it was also previously showed that repeated annual influenza vaccinations could blunt the vaccine effectiveness in following seasons (Murray, 2015).

Seasonal influenza outbreaks due to antigenic drift often lead to mild to moderate illness with lower mortality but influenza pandemics are usually more pathogenic and cause more severe symptoms with higher fatality such as the Asian flu in 1957, Hong Kong flu in 1968, and Swine flu in 2009 (Cowling et al., 2010; Kilbourne, 2006). Influenza pandemics occur when a new highly virulent strain of IAV emerges from genetic reassortment between different strains in a common host through a process known as antigenic shift (de Silva, Tanaka, Nakamura, Goto, & Yasunaga, 2012). A large gene pool of IAV harboured by different avian species escalated the antigenic shift (Hoffmann et al., 2000). Unfortunately, in the event of influenza pandemic, seasonal influenza vaccines are often not protective and the occurrence of the next pandemic is usually unpredictable (Zhang et al., 2014). Therefore, a universal influenza vaccine which targets all IAV strains regardless of HA and NA subtypes is urgently needed.

A highly conserved viral protein, the extracellular domain of matrix 2 protein (M2e) of IAV was identified as a potential target for the development of universal influenza vaccine. It is the N-terminal segment of the matrix 2 protein and comprises 23 amino acid residues. M2e is non-immunogenic and present at a very low copy number on the virion surface compared to HA and NA, thus under natural IAV infection, M2e specific antibodies is normally undetectable (Cho et al., 2015; Feng et al., 2006). However, various approaches have been employed to enhance its immunogenicity including the use of virus-like particles (VLPs) (De Filette et al., 2006; Matic, Rinaldi, Masenga, & Noris, 2011; Petukhova et al., 2013).

VLPs are non-replicative and non-infectious nanoparticles morphologically resembling the native viruses. They are composed of repetitive protein subunits with inherent self-assembly properties but are devoid of infectious genetic materials. VLPs have been extensively used as vaccines and drug delivery platforms for decades. Many foreign epitopes or peptides that are unable to produce sufficient "danger signals" to prime the immune systems were inserted onto the larger VLPs to enhance the immunogenicity. Furthermore, VLPs were also demonstrated to be an extraordinary inducer in eliciting both B-cells and T-cells responses, even in the absence of adjuvants (Ong, Tan, & Ho, 2017). Previously, Goh, Tan, Bhassu, and Tan (2011) demonstrated that *Macrobrachium rosenbergii* nodavirus (MrNV) capsid protein (NvC) expressed in *Escherichia coli* self-assembled into VLPs of approximately 30 nm in diameter. Later, multiple copies of M2e epitopes of IAV were genetically fused to the C-terminal end of the NvC, producing a chimeric protein (NvC-M2ex3) which self-assembles into VLPs, forming a potential IAV vaccine. Immunisation studies in BALB/c mice in the presence of Freund's adjuvant suggested that NvC-M2ex3 was indeed immunogenic and a strong M2e specific antibody response was observed (Yong, Yeap, Ho, Omar, & Tan, 2015). Nevertheless, protective efficacy of NvC-M2ex3 has yet to be evaluated and the immune responses of the potential vaccine alone without the adjuvant have never been investigated. In this study, it is hypothesised that NvC-M2ex3 alone is immunogenic and protect immunised BALB/c mice from lethal mouse adapted (MA) A/PR/8/34 (H1N1) and A/HK/8/68 (H3N2) challenges in the absence of an adjuvant. H1N1 and H3N2 were selected in this study because they are the most common subtypes emerge in annual flu seasons according to WHO.

© COPYRIGHT UPM

Therefore, the objectives of this study were:

1. To investigate the immunogenicity of NvC-M2ex3 in the absence of adjuvants.
2. To evaluate the protective efficacy of NvC-M2ex3 against mouse adapted (MA) A/PR/8/34 (H1N1) and A/HK/8/68 (H3N2).
3. To study the immune responses of the vaccinated mice upon IAV challenges.

© COPYRIGHT UPM

REFERENCES

Alberts, R., Srivastava, B., Wu, H., Viegas, N., Geffers, R., Klawonn, F., et al. (2010). Gene expression changes in the host response between resistant and susceptible inbred mouse strains after influenza A infection. *Microbes Infect, 12*(4), 309-318. doi: 10.1016/j.micinf.2010.01.008

Alexopoulou, L., Holt, A. C., Medzhitov, R., & Flavell, R. A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature, 413*(6857), 732-738. doi: 10.1038/35099560

Altekruse, S. F., Henley, S. J., Cucinelli, J. E., & McGlynn, K. A. (2014). Changing hepatocellular carcinoma incidence and liver cancer mortality rates in the United States. *Am J Gastroenterol, 109*(4), 542-553. doi: 10.1038/ajg.2014.11

Alvarez, D., Vollmann, E. H., & von Andrian, U. H. (2008). Mechanisms and consequences of dendritic cell migration. *Immunity, 29*(3), 325-342. doi: 10.1016/j.immuni.2008.08.006

Andersson, A. M., Hakansson, K. O., Jensen, B. A., Christensen, D., Andersen, P., Thomsen, A. R., & Christensen, J. P. (2012). Increased immunogenicity and protective efficacy of influenza M2e fused to a tetramerizing protein. *PLoS One, 7*(10), e46395. doi: 10.1371/journal.pone.0046395

Andrewes, C. H. (1939). Immunity in Influenza: The Bearing of Recent Research Work: (Section of Epidemiology and State Medicine). *Proc R Soc Med, 32*(3), 145-152.

Antunes, I., & Kassiotis, G. (2010). Suppression of innate immune pathology by regulatory T cells during Influenza A virus infection of immunodeficient mice. *J Virol, 84*(24), 12564-12575. doi: 10.1128/JVI.01559-10

Arnon, T. I., Lev, M., Katz, G., Chernobrov, Y., Porgador, A., & Mandelboim, O. (2001). Recognition of viral hemagglutinins by NKp44 but not by NKp30. *Eur J Immunol, 31*(9), 2680-2689. doi: 10.1002/1521-4141(200109)31:9<2680::aid-immu2680>3.0.co;2-a

Badham, M. D., & Rossman, J. S. (2016). Filamentous influenza viruses. *Curr Clin Microbiol Rep, 3*(3), 155-161. doi: 10.1007/s40588-016-0041-7

Bancroft, C. T., & Parslow, T. G. (2002). Evidence for segment-nonspecific packaging of the influenza a virus genome. *J Virol, 76*(14), 7133-7139.

Belshe, R. B., Edwards, K. M., Vesikari, T., Black, S. V., Walker, R. E., Hultquist, M., et al. (2007). Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med, 356*(7), 685-696. doi: 10.1056/NEJMoa065368

© COPYRIGHT UPM

Bergmann-Leitner, E. S., & Leitner, W. W. (2014). Adjuvants in the driver's seat: How magnitude, type, fine specificity and longevity of immune responses are driven by distinct classes of immune potentiators. *Vaccines (Basel), 2*(2), 252-296. doi: 10.3390/vaccines2020252

Betts, R. J., Prabhu, N., Ho, A. W., Lew, F. C., Hutchinson, P. E., Rotzschke, O., et al. (2012). Influenza A virus infection results in a robust, antigen-responsive, and widely disseminated Foxp3+ regulatory T cell response. *J Virol, 86*(5), 2817-2825. doi: 10.1128/JVI.05685-11

Blok, D. C., van der Sluijs, K. F., Florquin, S., de Boer, O. J., van 't Veer, C., de Vos, A. F., & van der Poll, T. (2013). Limited anti-inflammatory role for interleukin-1 receptor like 1 (ST2) in the host response to murine postinfluenza pneumococcal pneumonia. *PLoS One, 8*(3), e58191. doi: 10.1371/journal.pone.0058191

Blum, J. S., Wearsch, P. A., & Cresswell, P. (2013). Pathways of Antigen Processing. *Annual Review of Immunol, 31*, 443-473. doi: 10.1146/annurev-immunol-032712-095910

Bodewes, R., Kreijtz, J. H., Baas, C., Geelhoed-Mieras, M. M., de Mutsert, G., van Amerongen, G., et al. (2009). Vaccination against human influenza A/H3N2 virus prevents the induction of heterosubtypic immunity against lethal infection with avian influenza A/H5N1 virus. *PLoS One, 4*(5), e5538. doi: 10.1371/journal.pone.0005538

Boivin, S., Cusack, S., Ruigrok, R. W., & Hart, D. J. (2010). Influenza A virus polymerase: structural insights into replication and host adaptation mechanisms. *J Biol Chem, 285*(37), 28411-28417. doi: 10.1074/jbc.R110.117531

Boulo, S., Akarsu, H., Ruigrok, R. W., & Baudin, F. (2007). Nuclear traffic of influenza virus proteins and ribonucleoprotein complexes. *Virus Res, 124*(1-2), 12-21. doi: 10.1016/j.virusres.2006.09.013

Bouvier, N. M., & Lowen, A. C. (2010). Animal models for influenza virus pathogenesis and transmission. *Viruses, 2*(8), 1530-1563. doi: 10.3390/v20801530

Bouvier, N. M., & Palese, P. (2008). The biology of influenza viruses. *Vaccine, 26 Suppl 4*, D49-53.

Braciale, T. J., Morrison, L. A., Sweetser, M. T., Sambrook, J., Gething, M. J., & Braciale, V. L. (1987). Antigen presentation pathways to class I and class II MHC-restricted T lymphocytes. *Immunol Rev, 98*, 95-114.

© COPYRIGHT UPM

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem, 72*, 248-254.

Brown, D. M., Roman, E., & Swain, S. L. (2004). CD4 T cell responses to influenza infection. *Semin Immunol, 16*(3), 171-177. doi: 10.1016/j.smim.2004.02.004

Cha, T. A., Kao, K., Zhao, J., Fast, P. E., Mendelman, P. M., & Arvin, A. (2000). Genotypic stability of cold-adapted influenza virus vaccine in an efficacy clinical trial. *J Clin Microbiol, 38*(2), 839-845.

Chen, B. J., Leser, G. P., Jackson, D., & Lamb, R. A. (2008). The influenza virus M2 protein cytoplasmic tail interacts with the M1 protein and influences virus assembly at the site of virus budding. *J Virol, 82*(20), 10059-10070. doi: 10.1128/jvi.01184-08

Chen, W., Calvo, P. A., Malide, D., Gibbs, J., Schubert, U., Bacik, I., et al. (2001). A novel influenza A virus mitochondrial protein that induces cell death. *Nat Med, 7*(12), 1306-1312. doi: 10.1038/nm1201-1306

Cheung, T. K., Guan, Y., Ng, S. S., Chen, H., Wong, C. H., Peiris, J. S., & Poon, L. L. (2005). Generation of recombinant influenza A virus without M2 ion-channel protein by introduction of a point mutation at the 5' end of the viral intron. *J Gen Virol, 86*(Pt 5), 1447-1454. doi: 10.1099/vir.0.80727-0

Cho, K. J., Schepens, B., Seok, J. H., Kim, S., Roose, K., Lee, J. H., et al. (2015). Structure of the extracellular domain of matrix protein 2 of influenza A virus in complex with a protective monoclonal antibody. *J Virol, 89*(7), 3700-3711. doi: 10.1128/JVI.02576-14

Choi, K. H. (2012). Viral polymerases. *Adv Exp Med Biol, 726*, 267-304. doi: 10.1007/978-1-4614-0980-9_12

Choppin, P. W., Murphy, J. S., & Tamm, I. (1960). Studies of two kinds of virus particles which comprise influenza A2 virus strains. III. Morphological characteristics: independence to morphological and functional traits. *J Exp Med, 112*, 945-952.

Cline, T. D., Beck, D., & Bianchini, E. (2017). Influenza virus replication in macrophages: balancing protection and pathogenesis. *J Gen Virol, 98*(10), 2401-2412. doi: 10.1099/jgv.0.000922

Coelingh, K. L., Luke, C. J., Jin, H., & Talaat, K. R. (2014). Development of live attenuated influenza vaccines against pandemic influenza strains. *Expert Rev Vaccines, 13*(7), 855-871. doi: 10.1586/14760584.2014.922417

© COPYRIGHT UPM

Cohen, M., Zhang, X. Q., Senaati, H. P., Chen, H. W., Varki, N. M., Schooley, R. T., & Gagneux, P. (2013). Influenza A penetrates host mucus by cleaving sialic acids with neuraminidase. *Virol J, 10*, 321. doi: 10.1186/1743-422X-10-321

Compans, R. W., Content, J., & Duesberg, P. H. (1972). Structure of the ribonucleoprotein of influenza virus. *J Virol, 10*(4), 795-800.

Cottey, R., Rowe, C. A., & Bender, B. S. (2001). Influenza Virus. *Current Protocols in Immunology, 42*(1), 19.11.11-19.11.32. doi: 10.1002/0471142735.im1911s42

Couceiro, J. N., Paulson, J. C., & Baum, L. G. (1993). Influenza virus strains selectively recognize sialyloligosaccharides on human respiratory epithelium; the role of the host cell in selection of hemagglutinin receptor specificity. *Virus Res, 29*(2), 155-165.

Cowling, B. J., Chan, K. H., Fang, V. J., Lau, L. L. H., So, H. C., Fung, R. O. P., et al. (2010). Comparative epidemiology of pandemic and seasonal influenza A in households. *N Engl J Med, 362*(23), 2175-2184. doi: 10.1056/NEJMoa0911530

Cox, N. J., & Subbarao, K. (1999). Influenza. *Lancet, 354*(9186), 1277-1282. doi: 10.1016/S0140-6736(99)01241-6

Cox, R. J., Brokstad, K. A., Zuckerman, M. A., Wood, J. M., Haaheim, L. R., & Oxford, J. S. (1994). An early humoral immune response in peripheral blood following parenteral inactivated influenza vaccination. *Vaccine, 12*(11), 993-999.

Crisci, E., Barcena, J., & Montoya, M. (2012). Virus-like particles: the new frontier of vaccines for animal viral infections. *Vet Immunol Immunopathol, 148*(3-4), 211-225. doi: 10.1016/j.vetimm.2012.04.026

Cros, J. F., & Palese, P. (2003). Trafficking of viral genomic RNA into and out of the nucleus: influenza, Thogoto and Borna disease viruses. *Virus Res, 95*(1-2), 3-12.

Dadonaite, B., Vijayakrishnan, S., Fodor, E., Bhella, D., & Hutchinson, E. C. (2016). Filamentous influenza viruses. *J Gen Virol, 97*(8), 1755-1764. doi: 10.1099/jgv.0.000535

Dawood, F. S., Iuliano, A. D., Reed, C., Meltzer, M. I., Shay, D. K., Cheng, P. Y., et al. (2012). Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infect Dis, 12*(9), 687-695. doi: 10.1016/S1473-3099(12)70121-4

© COPYRIGHT UPM

De Filette, M., Fiers, W., Martens, W., Birkett, A., Ramne, A., Lowenadler, B., et al. (2006). Improved design and intranasal delivery of an M2e-based human influenza A vaccine. *Vaccine, 24*(44-46), 6597-6601. doi: 10.1016/j.vaccine.2006.05.082

De Filette, M., Min Jou, W., Birkett, A., Lyons, K., Schultz, B., Tonkyro, A., et al. (2005). Universal influenza A vaccine: optimization of M2-based constructs. *Virology, 337*(1), 149-161. doi: 10.1016/j.virol.2005.04.004

De Filette, M., Ramne, A., Birkett, A., Lycke, N., Lowenadler, B., Min Jou, W., et al. (2006). The universal influenza vaccine M2e-HBc administered intranasally in combination with the adjuvant CTA1-DD provides complete protection. *Vaccine, 24*(5), 544-551. doi: 10.1016/j.vaccine.2005.08.061

de Silva, U. C., Tanaka, H., Nakamura, S., Goto, N., & Yasunaga, T. (2012). A comprehensive analysis of reassortment in influenza A virus. *Biol Open, 1*(4), 385-390. doi: 10.1242/bio.2012281

Deng, L., Cho, K. J., Fiers, W., & Saelens, X. (2015). M2e-Based Universal Influenza A Vaccines. *Vaccines (Basel), 3*(1), 105-136. doi: 10.3390/vaccines3010105

Denis, J., Acosta-Ramirez, E., Zhao, Y., Hamelin, M. E., Koukavica, I., Baz, M., et al. (2008). Development of a universal influenza A vaccine based on the M2e peptide fused to the papaya mosaic virus (PapMV) vaccine platform. *Vaccine, 26*(27-28), 3395-3403. doi: 10.1016/j.vaccine.2008.04.052

DiazGranados, C. A., Dunning, A. J., Kimmel, M., Kirby, D., Treanor, J., Collins, A., et al. (2014). Efficacy of high-dose versus standard-dose influenza vaccine in older adults. *N Engl J Med, 371*(7), 635-645. doi: 10.1056/NEJMoa1315727

Dienz, O., Rud, J. G., Eaton, S. M., Lanthier, P. A., Burg, E., Drew, A., et al. (2012). Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung. *Mucosal Immunol, 5*(3), 258-266. doi: 10.1038/mi.2012.2

Dittmann, J., Stertz, S., Grimm, D., Steel, J., Garcia-Sastre, A., Haller, O., & Kochs, G. (2008). Influenza A virus strains differ in sensitivity to the antiviral action of Mx-GTPase. *J Virol, 82*(7), 3624-3631. doi: 10.1128/JVI.01753-07

Edenborough, K. M., Gilbertson, B. P., & Brown, L. E. (2012). A mouse model for the study of contact-dependent transmission of influenza A virus and the factors that govern transmissibility. *J Virol, 86*(23), 12544-12551. doi: 10.1128/JVI.00859-12

Ekiert, D. C., Bhabha, G., Elsliger, M. A., Friesen, R. H., Jongeneelen, M., Throsby, M., et al. (2009). Antibody recognition of a highly conserved influenza virus epitope. *Science, 324*(5924), 246-251. doi: 10.1126/science.1171491

© COPYRIGHT UPM

El Bakkouri, K., Descamps, F., De Filette, M., Smet, A., Festjens, E., Birkett, A., et al. (2011). Universal vaccine based on ectodomain of matrix protein 2 of influenza A: Fc receptors and alveolar macrophages mediate protection. *J Immunol, 186*(2), 1022-1031. doi: 10.4049/jimmunol.0902147

Enami, M., Sharma, G., Benham, C., & Palese, P. (1991). An influenza virus containing nine different RNA segments. *Virology, 185*(1), 291-298.

Evans, A. S. (1977). Influenza: The Last Great Plague. *The Yale Journal of Biology and Medicine, 50*(5), 595-595.

Feng, J., Zhang, M., Mozdzanowska, K., Zharikova, D., Hoff, H., Wunner, W., et al. (2006). Influenza A virus infection engenders a poor antibody response against the ectodomain of matrix protein 2. *Virol J, 3*, 102. doi: 10.1186/1743-422X-3-102

Fujii, Y., Goto, H., Watanabe, T., Yoshida, T., & Kawaoka, Y. (2003). Selective incorporation of influenza virus RNA segments into virions. *Proceedings of the National Academy of Sciences, 100*(4), 2002.

Gamblin, S. J., & Skehel, J. J. (2010). Influenza hemagglutinin and neuraminidase membrane glycoproteins. *J Biol Chem, 285*(37), 28403-28409. doi: 10.1074/jbc.R110.129809

Gambotto, A., Barratt-Boyes, S. M., de Jong, M. D., Neumann, G., & Kawaoka, Y. (2008). Human infection with highly pathogenic H5N1 influenza virus. *Lancet, 371*(9622), 1464-1475. doi: 10.1016/S0140-6736(08)60627-3

Gasparini, R., Amicizia, D., Lai, P. L., & Panatto, D. (2011). Live attenuated influenza vaccine--a review. *J Prev Med Hyg, 52*(3), 95-101.

Gazit, R., Gruda, R., Elboim, M., Arnon, T. I., Katz, G., Achdout, H., et al. (2006). Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nat Immunol, 7*(5), 517-523. doi: 10.1038/ni1322

Gerhard, W. (2001). The role of the antibody response in influenza virus infection. *Curr Top Microbiol Immunol, 260*, 171-190.

Gerhard, W., Mozdzanowska, K., Furchner, M., Washko, G., & Maiese, K. (1997). Role of the B-cell response in recovery of mice from primary influenza virus infection. *Immunol Rev, 159*, 95-103.

Gerhard, W., Mozdzanowska, K., & Zharikova, D. (2006). Prospects for universal influenza virus vaccine. *Emerg Infect Dis, 12*(4), 569-574. doi: 10.3201/eid1204.051020

© COPYRIGHT UPM

GeurtsvanKessel, C. H., Bergen, I. M., Muskens, F., Boon, L., Hoogsteden, H. C., Osterhaus, A. D. M. E., et al. (2009). Both Conventional and Interferon Killer Dendritic Cells Have Antigen-Presenting Capacity during Influenza Virus Infection. *PLoS One, 4*(9), e7187. doi: 10.1371/journal.pone.0007187

GeurtsvanKessel, C. H., & Lambrecht, B. N. (2008). Division of labor between dendritic cell subsets of the lung. *Mucosal Immunol, 1*(6), 442-450. doi: 10.1038/mi.2008.39

Ghoneim, H. E., Thomas, P. G., & McCullers, J. A. (2013). Depletion of alveolar macrophages during influenza infection facilitates bacterial superinfections. *J Immunol, 191*(3), 1250-1259. doi: 10.4049/jimmunol.1300014

Goh, Z. H., Tan, S. G., Bhassu, S., & Tan, W. S. (2011). Virus-like particles of *Macrobrachium rosenbergii* nodavirus produced in bacteria. *J Virol Methods, 175*(1), 74-79. doi: 10.1016/j.jviromet.2011.04.021

Gold, E. (1987). Pandemic influenza 1700-1900: A study in historical epidemiology. *JAMA, 257*(19), 2656-2656. doi: 10.1001/jama.1987.03390190134045

Grohskopf, L. A., Olsen, S. J., Sokolow, L. Z., Bresee, J. S., Cox, N. J., Broder, K. R., et al. (2014). Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP) -- United States, 2014-15 influenza season. *MMWR Morb Mortal Wkly Rep, 63*(32), 691-697.

Haller, O., & Kochs, G. (2002). Interferon-induced mx proteins: dynamin-like GTPases with antiviral activity. *Traffic, 3*(10), 710-717.

Hasan, N. H., & Ebrahimie, E. (2016). Epitope Mapping of Avian Influenza M2e Protein: Different Species Recognise Various Epitopes. *11*(6), e0156418. doi: 10.1371/journal.pone.0156418

Hashemi, H., Pouyanfard, S., Bandehpour, M., Noroozbabaei, Z., Kazemi, B., Saelens, X., & Mokhtari-Azad, T. (2012). Immunization with M2e-displaying T7 bacteriophage nanoparticles protects against influenza A virus challenge. *PLoS One, 7*(9), e45765. doi: 10.1371/journal.pone.0045765

Hay, A. J., Gregory, V., Douglas, A. R., & Lin, Y. P. (2001). The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci, 356*(1416), 1861-1870. doi: 10.1098/rstb.2001.0999

Haynes, J. R. (2009). Influenza virus-like particle vaccines. *Expert Rev Vaccines, 8*(4), 435-445. doi: 10.1586/erv.09.8

© COPYRIGHT UPM

He, X. S., Draghi, M., Mahmood, K., Holmes, T. H., Kemble, G. W., Dekker, C. L., et al. (2004). T cell-dependent production of IFN-gamma by NK cells in response to influenza A virus. *J Clin Invest, 114*(12), 1812-1819. doi: 10.1172/JCI22797

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., et al. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science, 303*(5663), 1526-1529. doi: 10.1126/science.1093620

Herzenberg, L. A., & Tokuhisa, T. (1982). Epitope-specific regulation. I. Carrier-specific induction of suppression for IgG anti-hapten antibody responses. *J Exp Med, 155*(6), 1730-1740.

Hirst, G. K. (1947). Studies on the Mechanism of Adaptation of Influenza Virus to Mice. *J Exp Med, 86*(5), 357-366.

Hoffman, W., Lakkis, F. G., & Chalasani, G. (2016). B Cells, Antibodies, and More. *Clin J Am Soc Nephrol, 11*(1), 137-154. doi: 10.2215/CJN.09430915

Hoffmann, E., Mahmood, K., Chen, Z., Yang, C. F., Spaete, J., Greenberg, H. B., et al. (2005). Multiple gene segments control the temperature sensitivity and attenuation phenotypes of ca B/Ann Arbor/1/66. *J Virol, 79*(17), 11014-11021. doi: 10.1128/JVI.79.17.11014-11021.2005

Hoffmann, E., Stech, J., Leneva, I., Krauss, S., Scholtissek, C., Chin, P. S., et al. (2000). Characterization of the influenza A virus gene pool in avian species in southern China: was H6N1 a derivative or a precursor of H5N1? *J Virol, 74*(14), 6309-6315.

Hoft, D. F., Babusis, E., Worku, S., Spencer, C. T., Lottenbach, K., Truscott, S. M., et al. (2011). Live and inactivated influenza vaccines induce similar humoral responses, but only live vaccines induce diverse T-cell responses in young children. *J Infect Dis, 204*(6), 845-853. doi: 10.1093/infdis/jir436

Hogner, K., Wolff, T., Pleschka, S., Plog, S., Gruber, A. D., Kalinke, U., et al. (2013). Macrophage-expressed IFN-beta contributes to apoptotic alveolar epithelial cell injury in severe influenza virus pneumonia. *PLoS Pathog, 9*(2), e1003188. doi: 10.1371/journal.ppat.1003188

Holsinger, L. J., & Lamb, R. A. (1991). Influenza virus M2 integral membrane protein is a homotetramer stabilized by formation of disulfide bonds. *Virology, 183*(1), 32-43.

Houser, K., & Subbarao, K. (2015). Influenza Vaccines: Challenges and Solutions. *Cell host & microbe, 17*(3), 295-300. doi: 10.1016/j.chom.2015.02.012

© COPYRIGHT UPM

Huang, F. F., Barnes, P. F., Feng, Y., Donis, R., Chroneos, Z. C., Idell, S., et al. (2011). GM-CSF in the lung protects against lethal influenza infection. *Am J Respir Crit Care Med, 184*(2), 259-268. doi: 10.1164/rccm.201012-2036OC

Huang, Q., Sivaramakrishna, R. P., Ludwig, K., Korte, T., Bottcher, C., & Herrmann, A. (2003). Early steps of the conformational change of influenza virus hemagglutinin to a fusion active state: stability and energetics of the hemagglutinin. *Biochim Biophys Acta, 1614*(1), 3-13.

Hufford, M. M., Kim, T. S., Sun, J., & Braciale, T. J. (2011). Antiviral CD8+ T cell effector activities in situ are regulated by target cell type. *J Exp Med, 208*(1), 167-180. doi: 10.1084/jem.20101850

Hwang, I., Scott, J. M., Kakarla, T., Duriancik, D. M., Choi, S., Cho, C., et al. (2012). Activation mechanisms of natural killer cells during influenza virus infection. *PLoS One, 7*(12), e51858. doi: 10.1371/journal.pone.0051858

Ibanez, L. I., Roose, K., De Filette, M., Schotsaert, M., De Sloovere, J., Roels, et al. (2013). M2e-displaying virus-like particles with associated RNA promote T helper 1 type adaptive immunity against influenza A. *PLoS One, 8*(3), e59081. doi: 10.1371/journal.pone.0059081

Ichinohe, T., Pang, I. K., & Iwasaki, A. (2010). Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat Immunol, 11*(5), 404-410. doi: 10.1038/ni.1861

Imai, Y., Kuba, K., Neely, G. G., Yaghubian-Malhami, R., Perkmann, T., van Loo, G., et al. (2008). Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell, 133*(2), 235-249. doi: 10.1016/j.cell.2008.02.043

Iwatsuki-Horimoto, K., Horimoto, T., Noda, T., Kiso, M., Maeda, J., Watanabe, et al. (2006). The cytoplasmic tail of the influenza A virus M2 protein plays a role in viral assembly. *J Virol, 80*(11), 5233-5240. doi: 10.1128/jvi.00049-06

Jefferson, T., Di Pietrantonj, C., Al-Ansary, L. A., Ferroni, E., Thorning, S., & Thomas, R. E. (2010). Vaccines for preventing influenza in the elderly. *Cochrane Database Syst Rev*(2), CD004876. doi: 10.1002/14651858.CD004876.pub3

Jefferson, T., Di Pietrantonj, C., Rivetti, A., Bawazeer, G. A., Al-Ansary, L. A., & Ferroni, E. (2010). Vaccines for preventing influenza in healthy adults. *Cochrane Database Syst Rev*(7), CD001269. doi: 10.1002/14651858.CD001269.pub4

© COPYRIGHT UPM

Jefferson, T., Rivetti, A., Di Pietrantonj, C., Demicheli, V., & Ferroni, E. (2012). Vaccines for preventing influenza in healthy children. *Cochrane Database Syst Rev*(8), CD004879. doi: 10.1002/14651858.CD004879.pub4

Jin, H., Lu, B., Zhou, H., Ma, C., Zhao, J., Yang, C. F., et al. (2003). Multiple amino acid residues confer temperature sensitivity to human influenza virus vaccine strains (FluMist) derived from cold-adapted A/Ann Arbor/6/60. *Virology, 306*(1), 18-24.

Kalish, R. S. (1995). Antigen processing: the gateway to the immune response. *J Am Acad Dermatol, 32*(4), 640-652.

Kilbourne, E. D. (2006). Influenza pandemics of the 20th century. *Emerg Infect Dis, 12*(1), 9-14. doi: 10.3201/eid1201.051254

Killian, M. L. (2014). Hemagglutination assay for influenza virus. *Methods Mol Biol, 1161*, 3-9. doi: 10.1007/978-1-4939-0758-8_1

Killingley, B., & Nguyen-Van-Tam, J. (2013). Routes of influenza transmission. *Influenza Other Respir Viruses, 7 Suppl 2*, 42-51. doi: 10.1111/irv.12080

Kim, M. C., Song, J. M., O, E., Kwon, Y. M., Lee, Y. J., Compans, R. W., & Kang, S. M. (2013). Virus-like particles containing multiple M2 extracellular domains confer improved cross-protection against various subtypes of influenza virus. *Mol Ther, 21*(2), 485-492. doi: 10.1038/mt.2012.246

Kobasa, D., Jones, S. M., Shinya, K., Kash, J. C., Copps, J., Ebihara, H., et al. (2007). Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature, 445*(7125), 319-323. doi: 10.1038/nature05495

Kochs, G., Garcia-Sastre, A., & Martinez-Sobrido, L. (2007). Multiple anti-interferon actions of the influenza A virus NS1 protein. *J Virol, 81*(13), 7011-7021. doi: 10.1128/JVI.02581-06

Kool, M., Fierens, K., & Lambrecht, B. N. (2012). Alum adjuvant: some of the tricks of the oldest adjuvant. *J Med Microbiol, 61*(Pt 7), 927-934. doi: 10.1099/jmm.0.038943-0

Kreijtz, J. H., Fouchier, R. A., & Rimmelzwaan, G. F. (2011). Immune responses to influenza virus infection. *Virus Res, 162*(1-2), 19-30. doi: 10.1016/j.virusres.2011.09.022

Kuiken, T., Rimmelzwaan, G. F., Van Amerongen, G., & Osterhaus, A. D. (2003). Pathology of human influenza A (H5N1) virus infection in cynomolgus macaques (Macaca fascicularis). *Vet Pathol, 40*(3), 304-310. doi: 10.1354/vp.40-3-304

© COPYRIGHT UPM

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*(5259), 680-685.

Lai, S., Qin, Y., Cowling, B. J., Ren, X., Wardrop, N. A., Gilbert, M., et al. (2016). Global epidemiology of avian influenza A H5N1 virus infection in humans, 1997-2015: a systematic review of individual case data. *Lancet Infect Dis, 16*(7), e108-e118. doi: 10.1016/S1473-3099(16)00153-5

Lakspere, T., Tynell, J., Kaloinen, M., Vanlede, M., Parsons, A., Ikonen, N., et al. (2014). Full-Genome Sequences of Influenza A(H1N1)pdm09 Viruses Isolated from Finnish Patients from 2009 to 2013. *Genome Announcements, 2*(1), e01004-01013. doi: 10.1128/genomeA.01004-13

Lamb, R. A., Choppin, P. W., Chanock, R. M., & Lai, C. J. (1980). Mapping of the two overlapping genes for polypeptides NS1 and NS2 on RNA segment 8 of influenza virus genome. *Proc Natl Acad Sci U S A, 77*(4), 1857-1861.

Lamb, R. A., Lai, C. J., & Choppin, P. W. (1981). Sequences of mRNAs derived from genome RNA segment 7 of influenza virus: colinear and interrupted mRNAs code for overlapping proteins. *Proc Natl Acad Sci U S A, 78*(7), 4170-4174.

Lanier, L. L., & Sun, J. C. (2009). Do the terms innate and adaptive immunity create artificial conceptual barriers? *Nature reviews. Immunology, 9*(5), 302-303. doi: 10.1038/nri2547

Lauder, S. N., Jones, E., Smart, K., Bloom, A., Williams, A. S., Hindley, J. P., et al. (2013). Interleukin-6 limits influenza-induced inflammation and protects against fatal lung pathology. *Eur J Immunol, 43*(10), 2613-2625. doi: 10.1002/eji.201243018

Le Goffic, R., Balloy, V., Lagranderie, M., Alexopoulou, L., Escriou, N., Flavell, R., et al. (2006). Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathog, 2*(6), e53. doi: 10.1371/journal.ppat.0020053

Lee, J. S., Song, C. H., Kim, C. H., Kong, S. J., Shon, M. H., Kim, H. J., et al. (2002). Profiles of IFN-γ and its regulatory cytokines (IL-12, IL-18 and IL-10) in peripheral blood mononuclear cells from patients with multidrug-resistant tuberculosis. *Clinical and Experimental Immunology, 128*(3), 516-524. doi: 10.1046/j.1365-2249.2002.01858.x

Lee, N., Chan, P. K., Hui, D. S., Rainer, T. H., Wong, E., Choi, K. W., et al. (2009). Viral loads and duration of viral shedding in adult patients hospitalized with influenza. *J Infect Dis, 200*(4), 492-500. doi: 10.1086/600383

© COPYRIGHT IPM

Lee, S. M., Gardy, J. L., Cheung, C. Y., Cheung, T. K., Hui, K. P., Ip, N. Y., et al. (2009). Systems-level comparison of host-responses elicited by avian H5N1 and seasonal H1N1 influenza viruses in primary human macrophages. *PLoS One, 4*(12), e8072. doi: 10.1371/journal.pone.0008072

Lee, V. J., Chen, M. I., Chan, S. P., Wong, C. S., Cutter, J., Goh, K. T., & Tambyah, P. A. (2007). Influenza pandemics in Singapore, a tropical, globally connected city. *Emerg Infect Dis, 13*(7), 1052-1057. doi: 10.3201/eid1307.061313

Leekha, S., Zitterkopf, N. L., Espy, M. J., Smith, T. F., Thompson, R. L., & Sampathkumar, P. (2007). Duration of influenza A virus shedding in hospitalized patients and implications for infection control. *Infect Control Hosp Epidemiol, 28*(9), 1071-1076. doi: 10.1086/520101

Lemaitre, M., & Carrat, F. (2010). Comparative age distribution of influenza morbidity and mortality during seasonal influenza epidemics and the 2009 H1N1 pandemic. *BMC Infect Dis, 10*, 162. doi: 10.1186/1471-2334-10-162

Leser, G. P., & Lamb, R. A. (2005). Influenza virus assembly and budding in raft-derived microdomains: a quantitative analysis of the surface distribution of HA, NA and M2 proteins. *Virology, 342*(2), 215-227. doi: 10.1016/j.virol.2005.09.049

Lietzen, N., Ohman, T., Rintahaka, J., Julkunen, I., Aittokallio, T., Matikainen, S., & Nyman, T. A. (2011). Quantitative subcellular proteome and secretome profiling of influenza A virus-infected human primary macrophages. *PLoS Pathog, 7*(5), e1001340. doi: 10.1371/journal.ppat.1001340

Liu, B., Mori, I., Hossain, M. J., Dong, L., Chen, Z., & Kimura, Y. (2003). Local immune responses to influenza virus infection in mice with a targeted disruption of perforin gene. *Microb Pathog, 34*(4), 161-167.

Liu, W., Zou, P., Ding, J., Lu, Y., & Chen, Y. H. (2005). Sequence comparison between the extracellular domain of M2 protein human and avian influenza A virus provides new information for bivalent influenza vaccine design. *Microbes Infect, 7*(2), 171-177. doi: 10.1016/j.micinf.2004.10.006

Lund, J. M., Alexopoulou, L., Sato, A., Karow, M., Adams, N. C., Gale, N. W., et al. (2004). Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci U S A, 101*(15), 5598-5603. doi: 10.1073/pnas.0400937101

Maines, T. R., Jayaraman, A., Belser, J. A., Wadford, D. A., Pappas, C., Zeng, H., et al. (2009). Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. *Science, 325*(5939), 484-487. doi: 10.1126/science.1177238

© COPYRIGHT UPM

Mandelboim, O., Lieberman, N., Lev, M., Paul, L., Arnon, T. I., Bushkin, Y., et al. (2001). Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature, 409*(6823), 1055-1060. doi: 10.1038/35059110

Manini, I., Domnich, A., Amicizia, D., Rossi, S., Pozzi, T., Gasparini, R., et al. (2015). Flucelvax (Optaflu) for seasonal influenza. *Expert Rev Vaccines, 14*(6), 789-804. doi: 10.1586/14760584.2015.1039520

Matic, S., Rinaldi, R., Masenga, V., & Noris, E. (2011). Efficient production of chimeric human papillomavirus 16 L1 protein bearing the M2e influenza epitope in Nicotiana benthamiana plants. *BMC Biotechnol, 11*, 106. doi: 10.1186/1472-6750-11-106

Matrosovich, M. N., Matrosovich, T. Y., Gray, T., Roberts, N. A., & Klenk, H. D. (2004). Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proc Natl Acad Sci U S A, 101*(13), 4620-4624. doi: 10.1073/pnas.0308001101

McCown, M. F., & Pekosz, A. (2005). The influenza A virus M2 cytoplasmic tail is required for infectious virus production and efficient genome packaging. *J Virol, 79*(6), 3595-3605. doi: 10.1128/jvi.79.6.3595-3605.2005

McGill, J., Heusel, J. W., & Legge, K. L. (2009). Innate immune control and regulation of influenza virus infections. *J Leukoc Biol, 86*(4), 803-812. doi: 10.1189/jlb.0509368

McKee, S. J., Young, V. L., Clow, F., Hayman, C. M., Baird, M. A., Hermans, I. F., et al. (2012). Virus-like particles and alpha-galactosylceramide form a self-adjuvanting composite particle that elicits anti-tumor responses. *J Control Release, 159*(3), 338-345. doi: 10.1016/j.jconrel.2012.02.015

Medicine, I. o. (2002). *Considerations for Viral Disease Eradication: Lessons Learned and Future Strategies: Workshop Summary*. Washington, DC: The National Academies Press.

Mohan, T., Berman, Z., Luo, Y., Wang, C., Wang, S., Compans, R. W., & Wang, B. Z. (2017). Chimeric virus-like particles containing influenza HA antigen and GPI-CCL28 induce long-lasting mucosal immunity against H3N2 viruses. *Sci Rep, 7*, 40226. doi: 10.1038/srep40226

Mohn, K. G., Bredholt, G., Brokstad, K. A., Pathirana, R. D., Aarstad, H. J., Tondel, C., & Cox, R. J. (2015). Longevity of B-cell and T-cell responses after live attenuated influenza vaccination in children. *J Infect Dis, 211*(10), 1541-1549. doi: 10.1093/infdis/jiu654

© COPYRIGHT UPM

Molinari, N. A., Ortega-Sanchez, I. R., Messonnier, M. L., Thompson, W. W., Wortley, P. M., Weintraub, E., & Bridges, C. B. (2007). The annual impact of seasonal influenza in the US: measuring disease burden and costs. *Vaccine, 25*(27), 5086-5096. doi: 10.1016/j.vaccine.2007.03.046

Monteiro, J. M., Harvey, C., & Trinchieri, G. (1998). Role of interleukin-12 in primary influenza virus infection. *J Virol, 72*(6), 4825-4831.

Mozdzanowska, K., Maiese, K., Furchner, M., & Gerhard, W. (1999). Treatment of influenza virus-infected SCID mice with nonneutralizing antibodies specific for the transmembrane proteins matrix 2 and neuraminidase reduces the pulmonary virus titer but fails to clear the infection. *Virology, 254*(1), 138-146. doi: 10.1006/viro.1998.9534

Mozdzanowska, K., Zharikova, D., Cudic, M., Otvos, L., & Gerhard, W. (2007). Roles of adjuvant and route of vaccination in antibody response and protection engendered by a synthetic matrix protein 2-based influenza A virus vaccine in the mouse. *Virol J, 4*, 118. doi: 10.1186/1743-422x-4-118

Murray, T. (2015). Repeated flu shots may blunt effectiveness. *CMAJ, 187*(6), E180. doi: 10.1503/cmaj.109-5000

Nayak, D. P., Hui, E. K., & Barman, S. (2004). Assembly and budding of influenza virus. *Virus Res, 106*(2), 147-165. doi: 10.1016/j.virusres.2004.08.012

Neirynck, S., Deroo, T., Saelens, X., Vanlandschoot, P., Jou, W. M., & Fiers, W. (1999). A universal influenza A vaccine based on the extracellular domain of the M2 protein. *Nat Med, 5*(10), 1157-1163. doi: 10.1038/13484

Nicholson, K. G., Wood, J. M., & Zambon, M. (2003). Influenza. *Lancet, 362*(9397), 1733-1745. doi: 10.1016/s0140-6736(03)14854-4

Noad, R., & Roy, P. (2003). Virus-like particles as immunogens. *Trends Microbiol, 11*(9), 438-444.

Oliphant, S., Lines, J. L., Hollifield, M. L., & Garvy, B. A. (2015). Regulatory T Cells Are Critical for Clearing Influenza A Virus in Neonatal Mice. *Viral Immunol, 28*(10), 580-589. doi: 10.1089/vim.2015.0039

Ong, H. K., Tan, W. S., & Ho, K. L. (2017). Virus like particles as a platform for cancer vaccine development. *PeerJ, 5*, e4053. doi: 10.7717/peerj.4053

Pang, I. K., & Iwasaki, A. (2011). Inflammasomes as mediators of immunity against influenza virus. *Trends Immunol, 32*(1), 34-41. doi: 10.1016/j.it.2010.11.004

© COPYRIGHT UPM

Paquette, S. G., Banner, D., Zhao, Z., Fang, Y., Huang, S. S., Leomicronn, A. J., et al. (2012). Interleukin-6 is a potential biomarker for severe pandemic H1N1 influenza A infection. *PLoS One, 7*(6), e38214. doi: 10.1371/journal.pone.0038214

Pasman, L. (2012). The complication of coinfection. *Yale J Biol Med, 85*(1), 127-132.

Paust, S., & von Andrian, U. H. (2011). Natural killer cell memory. *Nat Immunol, 12*(6), 500-508.

Peiris, J. S., de Jong, M. D., & Guan, Y. (2007). Avian influenza virus (H5N1): a threat to human health. *Clin Microbiol Rev, 20*(2), 243-267. doi: 10.1128/CMR.00037-06

Pennock, N. D., White, J. T., Cross, E. W., Cheney, E. E., Tamburini, B. A., & Kedl, R. M. (2013). T cell responses: naïve to memory and everything in between. *Advances in Physiology Education, 37*(4), 273-283. doi: 10.1152/advan.00066.2013

Petrovsky, N. (2015). Comparative Safety of Vaccine Adjuvants: A Summary of Current Evidence and Future Needs. *Drug Saf, 38*(11), 1059-1074. doi: 10.1007/s40264-015-0350-4

Petukhova, N. V., Gasanova, T. V., Stepanova, L. A., Rusova, O. A., Potapchuk, M. V., Korotkov, A. V., et al. (2013). Immunogenicity and protective efficacy of candidate universal influenza A nanovaccines produced in plants by Tobacco mosaic virus-based vectors. *Curr Pharm Des, 19*(31), 5587-5600.

Phipps, K. L., Marshall, N., Tao, H., Danzy, S., Onuoha, N., Steel, J., & Lowen, A. C. (2017). Seasonal H3N2 and 2009 Pandemic H1N1 Influenza A Viruses Reassort Efficiently but Produce Attenuated Progeny. *J Virol, 91*(17). doi: 10.1128/JVI.00830-17

Pinto, L. H., Holsinger, L. J., & Lamb, R. A. (1992). Influenza virus M2 protein has ion channel activity. *Cell, 69*(3), 517-528.

Pleschka, S. (2013). Overview of Influenza Viruses. In J. A. Richt & R. J. Webby (Eds.), *Swine Influenza* (pp. 1-20). Berlin, Heidelberg: Springer Berlin Heidelberg.

Plotch, S. J., Bouloy, M., Ulmanen, I., & Krug, R. M. (1981). A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell, 23*(3), 847-858.

Potter, C. W. (2001). A history of influenza. *J Appl Microbiol, 91*(4), 572-579.

© COPYRIGHT UPM

Qi, M., Zhang, X. E., Sun, X., Zhang, X., Yao, Y., Liu, S., et al. (2018). Intranasal Nanovaccine Confers Homo- and Hetero-Subtypic Influenza Protection. *Small, 14*(13), e1703207. doi: 10.1002/smll.201703207

Quan, F. S., Huang, C., Compans, R. W., & Kang, S. M. (2007). Virus-like particle vaccine induces protective immunity against homologous and heterologous strains of influenza virus. *J Virol, 81*(7), 3514-3524. doi: 10.1128/JVI.02052-06

Ramakrishnan, M. A. (2016). Determination of 50% endpoint titer using a simple formula. *World Journal of Virology, 5*(2), 85-86. doi: 10.5501/wjv.v5.i2.85

Reed, L. J., & Muench, H. (1938). A SIMPLE METHOD OF ESTIMATING FIFTY PER CENT ENDPOINTS12. *American Journal of Epidemiology, 27*(3), 493-497. doi: 10.1093/oxfordjournals.aje.a118408

Romagnani, S. (1999). Th1/Th2 cells. *Inflamm Bowel Dis, 5*(4), 285-294.

Roohani, K., Tan, S. W., Yeap, S. K., Ideris, A., Bejo, M. H., & Omar, A. R. (2015). Characterisation of genotype VII Newcastle disease virus (NDV) isolated from NDV vaccinated chickens, and the efficacy of LaSota and recombinant genotype VII vaccines against challenge with velogenic NDV. *J Vet Sci, 16*(4), 447-457. doi: 10.4142/jvs.2015.16.4.447

Roose, K., Schotsaert, M., Bakkouri, K. E., Schepens, B., Fiers, W., & Saelens, X. (2012). Cutting Edge Approaches Toward Novel and Cross-Protective Influenza Vaccines. In A. von Gabain & C. Klade (Eds.), *Development of Novel Vaccines: Skills, Knowledge and Translational Technologies* (pp. 205-232). Vienna: Springer Vienna.

Rossman, J. S., & Lamb, R. A. (2011). Influenza virus assembly and budding. *Virology, 411*(2), 229-236. doi: 10.1016/j.virol.2010.12.003

Rouse, B. T., & Sehrawat, S. (2010). IMMUNITY AND IMMUNOPATHOLOGY TO VIRUSES: what decides the outcome? *Nature reviews. Immunology, 10*(7), 514-526. doi: 10.1038/nri2802

Ruigrok, R. W., Calder, L. J., & Wharton, S. A. (1989). Electron microscopy of the influenza virus submembranal structure. *Virology, 173*(1), 311-316.

Rumke, H. C., Richardus, J. H., Rombo, L., Pauksens, K., Plassmann, G., Durand, C., et al. (2013). Selection of an adjuvant for seasonal influenza vaccine in elderly people: modelling immunogenicity from a randomized trial. *BMC Infect Dis, 13*, 348. doi: 10.1186/1471-2334-13-348

© COPYRIGHT UPM

Rynda-Apple, A., Patterson, D. P., & Douglas, T. (2014). Virus-like particles as antigenic nanomaterials for inducing protective immune responses in the lung. *Nanomedicine (Lond), 9*(12), 1857-1868. doi: 10.2217/nnm.14.107

Sakaguchi, T., Leser, G. P., & Lamb, R. A. (1996). The ion channel activity of the influenza virus M2 protein affects transport through the Golgi apparatus. *J Cell Biol, 133*(4), 733-747.

Sambhara, S., Kurichh, A., Miranda, R., Tumpey, T., Rowe, T., Renshaw, M., et al. (2001). Heterosubtypic immunity against human influenza A viruses, including recently emerged avian H5 and H9 viruses, induced by FLU-ISCOM vaccine in mice requires both cytotoxic T-lymphocyte and macrophage function. *Cell Immunol, 211*(2), 143-153. doi: 10.1006/cimm.2001.1835

Samji, T. (2009). Influenza A: understanding the viral life cycle. *Yale J Biol Med, 82*(4), 153-159.

Schneider, C., Nobs, S. P., Heer, A. K., Kurrer, M., Klinke, G., van Rooijen, N., et al. (2014). Alveolar macrophages are essential for protection from respiratory failure and associated morbidity following influenza virus infection. *PLoS Pathog, 10*(4), e1004053. doi: 10.1371/journal.ppat.1004053

Schotsaert, M., Ysenbaert, T., Neyt, K., Ibanez, L. I., Bogaert, P., Schepens, B., et al. (2013). Natural and long-lasting cellular immune responses against influenza in the M2e-immune host. *Mucosal Immunol, 6*(2), 276-287. doi: 10.1038/mi.2012.69

Scott, M. E., Kubin, M., & Kohl, S. (1997). High level interleukin-12 production, but diminished interferon-gamma production, by cord blood mononuclear cells. *Pediatr Res, 41*(4 Pt 1), 547-553. doi: 10.1203/00006450-199704000-00015

Seladi-Schulman, J., Campbell, P. J., Suppiah, S., Steel, J., & Lowen, A. C. (2014). Filament-producing mutants of influenza A/Puerto Rico/8/1934 (H1N1) virus have higher neuraminidase activities than the spherical wild-type. *PLoS One, 9*(11), e112462. doi: 10.1371/journal.pone.0112462

Seladi-Schulman, J., Steel, J., & Lowen, A. C. (2013). Spherical influenza viruses have a fitness advantage in embryonated eggs, while filament-producing strains are selected in vivo. *J Virol, 87*(24), 13343-13353. doi: 10.1128/JVI.02004-13

Shibata, Y., Berclaz, P. Y., Chroneos, Z. C., Yoshida, M., Whitsett, J. A., & Trapnell, B. C. (2001). GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. *Immunity, 15*(4), 557-567.

© COPYRIGHT UPM

Skehel, J. J., & Wiley, D. C. (2000). Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. *Annu Rev Biochem, 69*, 531-569. doi: 10.1146/annurev.biochem.69.1.531

Song, J. M., Wang, B. Z., Park, K. M., Van Rooijen, N., Quan, F. S., Kim, M. C., et al. (2011). Influenza virus-like particles containing M2 induce broadly cross protective immunity. *PLoS One, 6*(1), e14538. doi: 10.1371/journal.pone.0014538

Sridhar, S., Brokstad, K. A., & Cox, R. J. (2015). Influenza Vaccination Strategies: Comparing Inactivated and Live Attenuated Influenza Vaccines. *Vaccines (Basel), 3*(2), 373-389. doi: 10.3390/vaccines3020373

Starr, I. (1976). Influenza in 1918: recollections of the epidemic in Philadelphia. *Ann Intern Med, 85*(4), 516-518.

Stefansson, H. (2003). Infectious diseases and bioweapons. Science and political economics of affliction. *EMBO Rep, 4 Spec No*, S1-3. doi: 10.1038/sj.embor.embor843

Stein-Streilein, J., & Guffee, J. (1986). In vivo treatment of mice and hamsters with antibodies to asialo GM1 increases morbidity and mortality to pulmonary influenza infection. *J Immunol, 136*(4), 1435-1441.

Steinhauer, D. A., Domingo, E., & Holland, J. J. (1992). Lack of evidence for proofreading mechanisms associated with an RNA virus polymerase. *Gene, 122*(2), 281-288.

Stepanova, L. A., Kotlyarov, R. Y., Kovaleva, A. A., Potapchuk, M. V., Korotkov, A. V., Sergeeva, M. V., et al. (2015). Protection against multiple influenza A virus strains induced by candidate recombinant vaccine based on heterologous M2e peptides linked to flagellin. *PLoS One, 10*(3), e0119520. doi: 10.1371/journal.pone.0119520

Stills, H. F., Jr. (2005). Adjuvants and antibody production: dispelling the myths associated with Freund's complete and other adjuvants. *ILAR J, 46*(3), 280-293.

Strassburg, M. A. (1982). The global eradication of smallpox. *Am J Infect Control, 10*(2), 53-59.

Sugrue, R. J., & Hay, A. J. (1991). Structural characteristics of the M2 protein of influenza A viruses: evidence that it forms a tetrameric channel. *Virology, 180*(2), 617-624.

© COPYRIGHT UPM

Sullivan, S. G., Chilver, M. B., Carville, K. S., Deng, Y.-M., Grant, K. A., Higgins, G., et al. (2017). Low interim influenza vaccine effectiveness, Australia, 1 May to 24 September 2017. *Eurosurveillance, 22*(43), 17-00707. doi: 10.2807/1560-7917.ES.2017.22.43.17-00707

Sun, J., Madan, R., Karp, C. L., & Braciale, T. J. (2009). Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. *Nat Med, 15*(3), 277-284. doi: 10.1038/nm.1929

Suzuki, M., Kato, C., & Kato, A. (2015). Therapeutic antibodies: their mechanisms of action and the pathological findings they induce in toxicity studies. *Journal of Toxicologic Pathology, 28*(3), 133-139. doi: 10.1293/tox.2015-0031

Takeda, M., Leser, G. P., Russell, C. J., & Lamb, R. A. (2003). Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. *Proc Natl Acad Sci U S A, 100*(25), 14610-14617. doi: 10.1073/pnas.2235620100

Tang, Y., Zaitseva, F., Lamb, R. A., & Pinto, L. H. (2002). The gate of the influenza virus M2 proton channel is formed by a single tryptophan residue. *J Biol Chem, 277*(42), 39880-39886. doi: 10.1074/jbc.M206582200

Tao, P., Luo, M., Zhu, D., Qu, S., Yang, Z., Gao, M., et al. (2009). Virus-like particle vaccine comprised of the HA, NA, and M1 proteins of an avian isolated H5N1 influenza virus induces protective immunity against homologous and heterologous strains in mice. *Viral Immunol, 22*(4), 273-281. doi: 10.1089/vim.2009.0017

Tao, W., & Gill, H. S. (2015). M2e-immobilized gold nanoparticles as influenza A vaccine: role of soluble M2e and longevity of protection. *Vaccine, 33*(20), 2307-2315. doi: 10.1016/j.vaccine.2015.03.063

Tao, W., Hurst, B. L., Shakya, A. K., Uddin, M. J., Ingrole, R. S. J., Hernandez-Sanabria, M., et al. (2017). Consensus M2e peptide conjugated to gold nanoparticles confers protection against H1N1, H3N2 and H5N1 influenza A viruses. *Antiviral research, 141*, 62-72. doi: 10.1016/j.antiviral.2017.01.021

Taubenberger, J. K., & Morens, D. M. (2006). 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis, 12*(1), 15-22. doi: 10.3201/eid1201.050979

Taubenberger, J. K., & Morens, D. M. (2010). Influenza: The Once and Future Pandemic. *Public Health Reports, 125*(Suppl 3), 16-26.

Toapanta, F. R., & Ross, T. M. (2009). Impaired immune responses in the lungs of aged mice following influenza infection. *Respir Res, 10*, 112. doi: 10.1186/1465-9921-10-112

© COPYRIGHT UPM

Tong, S., Zhu, X., Li, Y., Shi, M., Zhang, J., Bourgeois, M., et al. (2013). New world bats harbor diverse influenza A viruses. *PLoS Pathog, 9*(10), e1003657. doi: 10.1371/journal.ppat.1003657

Toole, M. J. (2016). So close: remaining challenges to eradicating polio. *BMC Med, 14*, 43. doi: 10.1186/s12916-016-0594-6

Tosh, P. K., Jacobson, R. M., & Poland, G. A. (2010). Influenza Vaccines: From Surveillance Through Production to Protection. *Mayo Clinic Proceedings, 85*(3), 257-273. doi: 10.4065/mcp.2009.0615

Tripathi, S., White, M. R., & Hartshorn, K. L. (2015). The amazing innate immune response to influenza A virus infection. *Innate Immun, 21*(1), 73-98. doi: 10.1177/1753425913508992

Tumpey, T. M., Basler, C. F., Aguilar, P. V., Zeng, H., Solorzano, A., Swayne, D. E., et al. (2005). Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science, 310*(5745), 77-80. doi: 10.1126/science.1119392

van der Meer, F. J., Orsel, K., & Barkema, H. W. (2010). The new influenza A H1N1 virus: balancing on the interface of humans and animals. *Can Vet J, 51*(1), 56-62.

Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., et al. (2011). Innate or adaptive immunity? The example of natural killer cells. *Science, 331*(6013), 44-49. doi: 10.1126/science.1198687

Waithman, J., & Mintern, J. D. (2012). Dendritic cells and influenza A virus infection. *Virulence, 3*(7), 603-608. doi: 10.4161/viru.21864

Wang, B. Z., Gill, H. S., Kang, S. M., Wang, L., Wang, Y. C., Vassilieva, E. V., & Compans, R. W. (2012). Enhanced influenza virus-like particle vaccines containing the extracellular domain of matrix protein 2 and a Toll-like receptor ligand. *Clin Vaccine Immunol, 19*(8), 1119-1125. doi: 10.1128/CVI.00153-12

Wang, C., Lamb, R. A., & Pinto, L. H. (1995). Activation of the M2 ion channel of influenza virus: a role for the transmembrane domain histidine residue. *Biophys J, 69*(4), 1363-1371. doi: 10.1016/S0006-3495(95)80003-2

Wang, R., Song, A., Levin, J., Dennis, D., Zhang, N. J., Yoshida, H., et al. (2008). Therapeutic potential of a fully human monoclonal antibody against influenza A virus M2 protein. *Antiviral Res, 80*(2), 168-177. doi: 10.1016/j.antiviral.2008.06.002

Webster, R. G., Guan, Y., Poon, L., Krauss, S., Webby, R., Govorkovai, E., & Peiris, M. (2005). The spread of the H5N1 bird flu epidemic in Asia in 2004. *Arch Virol Suppl*(19), 117-129.

© COPYRIGHT UPM

Weiss, I. D., Wald, O., Wald, H., Beider, K., Abraham, M., Galun, E., et al. (2010). IFN-gamma treatment at early stages of influenza virus infection protects mice from death in a NK cell-dependent manner. *J Interferon Cytokine Res, 30*(6), 439-449. doi: 10.1089/jir.2009.0084

Wong, S. S., & Yuen, K. Y. (2006). Avian influenza virus infections in humans. *Chest, 129*(1), 156-168. doi: 10.1378/chest.129.1.156

Wrammert, J., Smith, K., Miller, J., Langley, W. A., Kokko, K., Larsen, C., et al. (2008). Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature, 453*(7195), 667-671. doi: 10.1038/nature06890

Wu, W., & Air, G. M. (2004). Binding of influenza viruses to sialic acids: reassortant viruses with A/NWS/33 hemagglutinin bind to alpha2,8-linked sialic acid. *Virology, 325*(2), 340-350. doi: 10.1016/j.virol.2004.05.013

Yan, J., Grantham, M., Pantelic, J., Bueno de Mesquita, P. J., Albert, B., Liu, F., et al. (2018). Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. *Proceedings of the National Academy of Sciences*.

Yang, X., Steukers, L., Forier, K., Xiong, R., Braeckmans, K., Van Reeth, K., & Nauwynck, H. (2014). A beneficiary role for neuraminidase in influenza virus penetration through the respiratory mucus. *PLoS One, 9*(10), e110026. doi: 10.1371/journal.pone.0110026

Ye, Z., Liu, T., Offringa, D. P., McInnis, J., & Levandowski, R. A. (1999). Association of influenza virus matrix protein with ribonucleoproteins. *J Virol, 73*(9), 7467-7473.

Yewdell, J. W., Frank, E., & Gerhard, W. (1981). Expression of influenza A virus internal antigens on the surface of infected P815 cells. *J Immunol, 126*(5), 1814-1819.

Yong, C. Y., Yeap, S. K., Ho, K. L., Omar, A. R., & Tan, W. S. (2015). Potential recombinant vaccine against influenza A virus based on M2e displayed on nodaviral capsid nanoparticles. *Int J Nanomedicine, 10*, 2751-2763. doi: 10.2147/ijn.s77405

Zebedee, S. L., & Lamb, R. A. (1988). Influenza A virus M2 protein: monoclonal antibody restriction of virus growth and detection of M2 in virions. *J Virol, 62*(8), 2762-2772.

Zhang, X. S., Pebody, R., De Angelis, D., White, P. J., Charlett, A., & McCauley, J. W. (2014). The Possible Impact of Vaccination for Seasonal Influenza on Emergence of Pandemic Influenza via Reassortment. *PLoS One, 9*(12), e114637. doi: 10.1371/journal.pone.0114637

© COPYRIGHT UPM

Zhao, G., Lin, Y., Du, L., Guan, J., Sun, S., Sui, H., et al. (2010). An M2e-based multiple antigenic peptide vaccine protects mice from lethal challenge with divergent H5N1 influenza viruses. *Virol J, 7*, 9. doi: 10.1186/1743-422x-7-9

Zheng, B., Zhang, Y., He, H., Marinova, E., Switzer, K., Wansley, D.,et al. (2007). Rectification of age-associated deficiency in cytotoxic T cell response to influenza A virus by immunization with immune complexes. *J Immunol, 179*(9), 6153-6159.

Zhou, B., Meliopoulos, V. A., Wang, W., Lin, X., Stucker, K. M., Halpin, R. A.,et al. (2016). Reversion of Cold-Adapted Live Attenuated Influenza Vaccine into a Pathogenic Virus. *J Virol, 90*(19), 8454-8463. doi: 10.1128/jvi.00163-16

Zhou, N. N., Senne, D. A., Landgraf, J. S., Swenson, S. L., Erickson, G., Rossow, K., . . . Webster, R. G. (1999). Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. *J Virol, 73*(10), 8851-8856.

Zhu, W., Wang, C., & Wang, B. Z. (2017). From Variation of Influenza Viral Proteins to Vaccine Development. *Int J Mol Sci, 18*(7). doi: 10.3390/ijms18071554

© COPYRIGHT UPM

BIODATA OF STUDENT

The student was born in Mentakab, Pahang on 25th November 1991. He received his primary and secondary educations at SJK(C) Mentakab 2 and SMJK Hwa Lian respectively. After completion of Fifth Form, Ong Hui Kian was engaged in National Service Training Programme for approximately 2 months and enrolled at Tunku Abdul Rahman College in May 2009. He graduated from tertiary education with a degree in Advance Diploma in Science Cum Bachelor of Science (Magna Cum Laude)-Chemistry and Biology in 2013. Prior to persuading his Master Degree in Universiti Putra Malaysia in 2015, Ong Hui Kian worked as a chemist in ALS Technichem Sdn. Bhd. in 2013 and became a medical representative in Kromh Sdn. Bhd. in 2014.

© COPYRIGHT UPM

LIST OF PUBLICATIONS

Tan, W. S., Omar, A. R., Yong, C. Y., Ho, K. L., Yeap, S. K., & Ong, H. K. 2018. An influenza A vaccine. (Patent Application Number: PI 2018702779)

Ong, H. K., Tan, W. S., and Ho, K. L. 2017. Virus like particles as a platform for cancer vaccine development. PeerJ 5:e4053. 10.7717/peerj.4053

Ong, H. K., Tan, W. S., Omar, A. R., Yeap, S. K., & Ho, K. L. Immunization study of extracellular domain of influenza A virus matrix protein using nodavirus capsid as nanocarrier. 9th International Conference on Fiber and Polymer Biotechnology, Osaka, Japan. 7-9 September 2016.

© COPYRIGHT UPM

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

DEVELOPMENT OF A UNIVERSAL INFLUENZA A VACCINE BASED ON VIRUS-LIKE PARTICLES OF *Macrobrachium rosenbergii* NODAVIRUS

NAME OF STUDENT: ONG HUI KIAN

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐ **PATENT** Embargo from _____ until _____
(date) (date)

Approved by:

_____ _____

(Signature of Student)
New IC No/ Passport No.:

Date :

(Signature of Chairman of Supervisory Committee)
Name:

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]