UNIVERSITI PUTRA MALAYSIA

Macrobrachium rosenbergii DE MAN NODAVIRUS CAPSID DISPLAYING INFLUENZA A AND HEPATITIS B VIRUS IMMUNODOMINANT REGIONS INDUCE HUMORAL AND CELLULAR IMMUNE RESPONSES IN MICE

YONG CHEAN YEAH

FBSB 2015 25

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By

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

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

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June 2015

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Hepatitis B virus (HBV) has infected one-third of the world population, where more than 350 million people infected chronically serve as HBV reservoir. It is responsible for the death of 1 million people each year. To date, vaccination remains the most effective approach to combat HBV. However, the emergence of vaccine escape mutants justified the need for a versatile system which is capable of targeting these mutants. Influenza A virus (IAV) is a highly infectious virus transmittable through air-borne droplets, which was responsible for the two well-recorded pandamics, the Spanish flu and swine flu. Each year, approximately 3-5 million people are infected by IAV, of which 250,000-500,000 people die annually. Current influenza vaccines composed of inactivated influenza viruses, which grant protection through haemagglutinin (H) and neuraminidase (N) induced antibodies. Although highly immunogenic, H and N are prone to mutation, thereby reducing the efficacy of these glycoproteins as vaccines. *Macrobrachium rosenbergii* nodavirus (MrNV) is a non-zoonotic virus which infects *Macrobrachium rosenbergii*, commonly known as giant river prawn, causing white muscle disease (WMD). Recombinant MrNV capsid proteins produced in bacteria *Escherichia coli* self-assembled into non-infectious virus-like particles (VLPs). These VLPs were hypothesised to be able to display foreign epitopes for enhancing immune responses. Therefore, a common immunodominant region of HBV known as the ‘a’ determinant, and a highly conserved IAV epitope known as Matrix 2 ectodomain (M2e), were fused to the Carboxyl-terminal ends of MrNv capsid proteins. These fusion proteins harbouring polyhistidine tags at their C-terminal ends can be purified in a single-step through immobilised metal affinity chromatography (IMAC). The purified fusion proteins self-assembled into VLPs of approximately 30 nm in diameter, exposing the fused epitopes on the surface of the VLPs. Both the ‘a’ determinant and M2e displayed on the VLPs were antigenic towards antibodies specifically against HBV surface antigen (HBsAg) and Matrix 2 protein (M2) of IAV. When these fusion proteins were used for immunisation in BALB/c mice, they induced both humoral and cell-mediated immune responses specifically against the ‘a’ determinant and M2e displayed on the VLPs. Collectively, this study

demonstrated that MrNV capsid protein is a novel platform for displaying foreign epitopes, be it for diagnostic assay or vaccine development.

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

KAPSID NODAVIRUS *Macrobrachium rosenbergii* DE MAN YANG MEMAPARKAN BAHAGIAN IMUNODOMINAN VIRUS INFLUENZA A DAN HEPATITIS B MENDORONG TINDAK BALAS IMUNOLOGI SECARA HUMORAL DAN SELULAR DALAM TIKUS

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Virus hepatitis B (HBV) telah menjangkit satu pertiga daripada penduduk dunia, di mana lebih daripada 350 juta orang berjangkitan kronik berfungsi sebagai takungan HBV. Ia mengakibatkan kematian 1 juta orang setiap tahun. Setakat ini, vaksin masih merupakan penyelesaian yang paling berkesan terhadap penyebaran HBV. Walau bagaimanapun, kemunculan mutan-mutan yang imun terhadap vaksin sedia ada mencadangkan keperluan sistem baru untuk pembuatan vaksin yang berkesan terhadap mutan-mutan HBV. Virus influenza A (IAV) adalah virus yang senang berjangkit melalui titisan udara, di mana ia bertanggungjawab untuk beberapa pandemik, termasuk selsema Sepanyol dan selsema khinzir. Setiap tahun, kira-kira 3-5 juta orang dijangkiti oleh IAV, di mana 250,000-500,000 orang meninggal dunia. Vaksin influenza sedia ada terdiri daripada virus influenza terbunuh, di mana perlindungannya adalah bergantung kepada penghasilan antibodi terhadap hemagglutinin (H) dan neuraminidase (N). Walaupun H dan N adalah sangat imunogenik, tetapi ia adalah terdedah kepada mutasi, dengan itu mengurangkan keberkesanan vaksin ini. *Macrobrachium rosenbergii* nodavirus (MrNV) adalah virus bukan zoonotik yang menjangkit udang galah secara asli, menyebabkan "White Muscle Disease" (WMD). Protein kapsid MrNV yang dihasilkan dalam bakteria *Escherichia coli* secara rekombinan bercantum sendiri untuk membentuk partikel menyerupai virus (VLPs). Dari segi hipotesis, VLPs ini mampu memaparkan epitope-epitope asing dengan meningkatkan tindak balas imunologi perumah. Oleh itu, bahagian imunodominan daripada HBV yang dikenali sebagai penentu 'a', dan epitop IAV yang dikenali sebagai "Matrix 2 ectodomain" (M2e), telah digabungkan dengan protein kapsid MrNV pada hujung karboksil. Protein rekombinan ini boleh ditulenkan dalam satu langkah, iaitu melalui kromatografi afiniti penyekatan logam (IMAC), disebabkan kehadiran tag- polihistidina di hujung karboksil. Protein rekombinan ini bercantum sendiri kepada VLPs berdiameter lebih kurang 30 nm, mendedahkan epitop terpamer pada permukaan VLPs. Kedua-dua penentu 'a' dan M2e yang dipaparkan adalah antigenik, di mana antibodi spesifik

terhadap antigen permukaan HBV (HBsAg) dan Matrik 2 protein (M2) IAV boleh mengikat kepadanya. Apabila digunakan untuk imunisasi tikus BALB/c, protein rekombinan ini mendorong tindak balas imunologi secara humoral dan selular khusus terhadap penentu 'a' dan M2e yang dipamerkan. Secara keseluruhan, protein kapsid MrNV boleh berfungsi sebagai platform baru untuk paparan epitop-epitop asing, sama ada dalam bidang diagnostik ataupun penyelidikan vaksin.

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

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LIST OF ABBREVIATIONS

µg	microgram (10^{-6} g)
µl	microlitre (10^{-6} l)
µm	micrometer (10^{-6} m)
µM	micromolar (10^{-6} M)
a.a.	amino acids
APC	allophycocyanin
APCs	antigen presenting cells
APS	ammonium persulphate
ATP	adenosine triphosphate
bp	basepair
BSA	bovine serum albumin
ccc	covalently closed circular
CD3	clusters of differentiation 3
CD4	clusters of differentiation 4
CD8	clusters of differentiation 8
C-terminal	carboxyl terminal
CTL	cytotoxic T lymphocyte
CV	column volume
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxy-ribonucleic acid
dNTP	deoxyribonucleotide phosphate
DTT	1, 4-dithiothreitol
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FITC	fluorescein isothiocyanate
g	gram
H	haemagglutinnin
h	hour

HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HEPES acid	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic
HIV	human immunodeficiency virus
HPV	human papilloma virus
IAV	influenza A virus
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
IMAC	immobilised metal affinity chromatography
IPTG	isopropyl-β-D-thiogalactopyranoside
kb	kilobase
kbp	kilo basepair
kDa	kilo Dalton
l	litre
LB	Luria-Bertani broth
LD_{50}	median lethal dose
L-HBsAg	large hepatitis B surface antigen
M	molar
M1	matrix 1 protein
M2	matrix 2 protein
M2e	matrix 2 protein ectodomain
mA	miliampere
mg	milligram (10^{-3} g)
M-HBsAg	medium hepatitis B surface antigen
min	minute
ml	mililitre (10^{-3} l)

mRNA	messenger RNA
MrNV	*Macrobrachium rosenbergii* nodavirus
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N	neuraminidase
NK	natural killer
nm	nanometer (10^{-9} m)
NP	nucleoprotein
NTCP	sodium taurocholate cotransporting polypeptide
N-terminal	amino terminal
^{o}C	degree Celsius
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	phycoerythrin
PEG	polyethylene glycol
pH	*Puissance hydrogen*
pmol	picomole (10^{-12} mol)
preS1	N-terminal region of L-HBsAg comprising 108 or 119 amino acids
preS2	region of L- and M-HBsAg comprising 55 amino acids
PvNV	*Penaeus vannamei* nodavirus
RdRP	RNA-dependent RNA polymerase
RNA	ribonucleic acid
RNP	ribonucleoprotein
rpm	revolutions per minute
RT	room temperature (approximately 25^{o}C)
s	second

SDS	sodium dodecyl sulphate
S-HBsAg	small hepatitis B surface antigen
T=	triangulation number of
TBS	tris-buffered saline
TE	tris-EDTA buffer
TEM	transmission electron microscope
TEMED	tetramethyl ethylenediamine
T_h1	type 1 T helper immune response
TNF	tumor necrosis factor
U	unit
V	volt
v/v	volume/volume
VLPs	virus-like particles
vRNA	viral RNA
w/v	weight/volume
WMD	white muscle disease
WTD	white tail disease
xg	centrifugal force
α	alpha
β	beta
γ	gamma
λ	lambda

AMINO ACIDS ABBREVIATIONS

Alanine	A
Arginine	R
Asparagine	N
Aspartic acid	D
Cysteine	C
Glutamic acid	E
Glycine	G
Histidine	H
Isoleucine	I
Leucine	L
Lysine	K
Methionine	M
Phenylalanine	F
Proline	P
Serine	S
Threonine	T
Tryptophan	W
Tyrosine	Y
Valine	V

CHAPTER 1

INTRODUCTION

Hepatitis B virus (HBV) has infected 2 billion people worldwide, which correspond to one third of the world population. Currently, there are approximately 370 million people chronically infected by HBV (Ward and Byrd, 2012), where their immune systems failed to remove the virus, resulting in persisting infection. About 60-80% of liver cancers were caused by a prolonged HBV infection (Lavanchy, 2005). Although treatments are available for chronic HBV patients, none was able to purge the virus from people chronically infected by HBV. Hence, HBV vaccines remained irreplaceable for protection against HBV before an infection takes place. Currently available yeast-derived HBV vaccines are considered highly effective. These vaccines consist of recombinant HBV surface antigen (HBsAg) produced in yeasts such as *Saccharomyces cerevisiae* (Engerix B; Recombivax HB), *P. pastoris* (Elovac-B; Shanvac-B), and *Hansenula polymorpha* (Gene Vac-B).

HBsAg-based vaccines provide cross-protection to vaccine recipients against HBV of various subtypes, due to the presence of a common immunodominant region known as the 'a' determinant (Howard and Allison, 1995), which is located at residues 121-149 of the small (S-) HBsAg (Tan et al., 2005). However, a prolonged usage of nucleoside analogs for the treatment of chronic HBV patients has caused mutations to the HBV 'a' determinant, rendering the commercial yeast-derived vaccines ineffective (Coleman, 2006; Pawlotsky, 2005; Zanetti et al., 1988; Zuckerman and Zuckerman, 2003). Therefore, a versatile vaccine platform which allows a rapid production of new vaccines specifically against these mutants is in demand.

Influenza A virus (IAV), being the most common influenza virus, infects approximately 3-5 million people worldwide, causing death of 250,000-500,000 people each year (World Health Organization, 2014a). IAV is responsible for several influenza pandemics in human history, which includes Spanish flu (1918), Asian flu (1957), Hong Kong flu (1968), and swine flu (2009), killing up to 50 million people worldwide. Currently available influenza vaccines are mostly composed of trivalent or quadrivalent inactivated influenza viruses, which include IAV subtypes H1N1 and H3N2, and 1 (in trivalent vaccines) or 2 (in quadrivalent vaccines) influenza B virus strains. The protection of these vaccines is mediated through induction of antibodies in vaccine recipients, against the haemagglutinin (H) and neuraminidase (N) glycoproteins of the inactivated viruses presence in the vaccines. Therefore, the efficiency of these vaccines depends heavily on the similarities of H and N between the outbreak strain and the vaccine strain (El Bakkouri et al., 2011; Hashemi et al., 2012).

IAV H and N proteins have high tendencies to mutate over time through a process known as antigenic drift, due to the absence of a proof read function on the viral

RNA polymerase. Influenza vaccines are revised yearly through worldwide monitoring of the northern and southern hemisphere by the World Health Organization. Even so, with vast avian gene pool serving as IAV reservoir, gene re-assortments between human IAV and IAV native to avian can take place within a common host, a phenomenon known as an antigenic shift. There is no telling when the sudden emergence of a new highly virulent IAV subtypes can occur, causing the 2nd influenza pandemic in the 21st century. To answer this problem, a universal influenza vaccine which is effective towards all IAV, disregard H and N subtypes is urgently needed. IAV matrix 2 ectodomain (M2e) was found to be highly conserved independent to H and N mutations, thus it is of great interest in the development of a universal vaccine.

Virus-like particles (VLPs) are non-infectious particles which resemble viruses in their native forms. These VLPs have been utilized for various purposes, which include delivery of molecules (Lee et al., 2012a; Petry et al., 2003), and display of foreign epitopes on VLPs derived from human papilloma virus (HPV; Matic et al., 2011), HBV (Ibanez et al., 2013; Murray and Shiau, 1999; Yap et al., 2012), as well as the bacteriophages (Hashemi et al., 2012; Kok et al., 2002; Tan et al., 2005; Wan et al., 2001). Some small antigens are often not immunogenic due to poor visibility to the immune cells. The immunogenicity of these small antigens however, can be enhanced through display on a larger structure such as the VLPs (Murata et al., 2003; Quan et al., 2008), which facilitates uptake of the displayed antigen by antigen presenting cells (APCs).

Macrobrachium rosenbergii nodavirus (MrNV) is a non-zoonotic virus which infects prawns. It was first isolated by Arcier et al. (1999) from *M. rosenbergii*. The gene encoding MrNV capsid protein was cloned and expressed in *Escherichia coli* (Goh et al., 2011). This recombinant MrNV capsid protein was found to self-assemble into VLPs of approximately 30 nm in diameter. When this study started, the recombinant MrNV VLPs was not employed to display any foreign epitopes. The immunogenicity of MrNV VLPs and epitopes to be displayed on it thus remain unknown. In this study, it is hypothesised that the MrNV VLPs can be used for the display of 'a' determinant and M2e, thereby induce specific antibodies against the displayed epitopes as humoral immune responses, as well as some cell-mediated immune responses mediated by cytotoxic T and natural killer (NK) cells.

Therefore, the objectives of this study were:

1. To display HBV 'a' determinant on the surface of the MrNV VLPs.
2. To display multiple copies of IAV M2e on the surface of MrNV VLPs.
3. To characterise the 'a' determinant and M2e displayed on MrNV VLPs.
4. To study the immunogenicities of the 'a' determinant and M2e displayed on MrNV VLPs.

REFERENCES

Aguilar, J. C., Lobaina, Y., Muzio, V., Garcia, D., Penton, E., Iglesias, E., et al. (2004). Development of a nasal vaccine for chronic hepatitis B infection that uses the ability of hepatitis B core antigen to stimulate a strong Th1 response against hepatitis B surface antigen. *Immunology and Cell Biology, 82*(5), 539-546.

Akbar, S. M., Al-Mahtab, M., Uddin, M. H., & Khan, M. S. (2013). HBsAg, HBcAg, and combined HBsAg/HBcAg-based therapeutic vaccines in treating chronic hepatitis B virus infection. *Hepatobiliary & Pancreatic Diseases International, 12*(4), 363-369.

Akbar, S. M., Chen, S., Al-Mahtab, M., Abe, M., Hiasa, Y., & Onji, M. (2012). Strong and multi-antigen specific immunity by hepatitis B core antigen (HBcAg)-based vaccines in a murine model of chronic hepatitis B: HBcAg is a candidate for a therapeutic vaccine against hepatitis B virus. *Antiviral Research, 96*(1), 59-64.

Arase, H., Arase, N., & Saito, T. (1996). Interferon gamma production by natural killer (NK) cells and NK1.1+ T cells upon NKR-P1 cross-linking. *The Journal of Experimental Medicine, 183*(5), 2391-2396.

Arcier, J. M., Herman, F., Lightner, D. V., Redman, R. M., Mari, J., & Bonami, J. R. (1999). A viral disease associated with mortalities in hatchery-reared postlarvae of the giant freshwater prawn macrobrachium rosenbergii. *Diseases of Aquatic Organisms, 38*(3), 177-181.

Ball, L. A., & Johnson, K. L. (1998). Nodaviruses of insects. In L. K. Miller, & L. A. Ball (Eds.), *The insect viruses* (pp. 225-267) Plenum Publishing Corporation.

Baumgarth, N., & Kelso, A. (1996). In vivo blockade of gamma interferon affects the influenza virus-induced humoral and the local cellular immune response in lung tissue. *Journal of Virology, 70*(7), 4411-4418.

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. *The New England Journal of Medicine, 356*(7), 685-696.

Bertoletti, A., Maini, M., & Williams, R. (2003). Role of hepatitis B virus specific cytotoxic T cells in liver damage and viral control. *Antiviral Research, 60*(2), 61-66.

Birnboim, H. C., & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research, 7*(6), 1513-1523.

Black, R. A., Rota, P. A., Gorodkova, N., Klenk, H. D., & Kendal, A. P. (1993). Antibody response to the M2 protein of influenza A virus expressed in insect cells. *The Journal of General Virology, 74 (Pt 1)*(Pt 1), 143-146.

Bordignon, V., Cordiali-Fei, P., Rinaldi, M., Signori, E., Cottarelli, A., Zonfrillo, M., et al. (2012). Evaluation of antigen specific recognition and cell mediated cytotoxicity by a modified lysispot assay in a rat colon carcinoma model. *Journal of Experimental & Clinical Cancer Research : CR, 31*, 9-9966-31-9.

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

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

Bruss, V., & Ganem, D. (1991). The role of envelope proteins in hepatitis B virus assembly. *Proceedings of the National Academy of Sciences of the United States of America, 88*(3), 1059-1063.

Burrell, C. J., Mackay, P., Greenaway, P. J., Hofschneider, P. H., & Murray, K. (1979). Expression in *Escherichia coli* of hepatitis B virus DNA sequences cloned in plasmid pBR322. *Nature, 279*(5708), 43-47.

Cai, D., Qiu, Y., Qi, N., Yan, R., Lin, M., Nie, D., et al. (2010). Characterization of wuhan nodavirus subgenomic RNA3 and the RNAi inhibition property of its encoded protein B2. *Virus Research, 151*(2), 153-161.

Cao, T., Meuleman, P., Desombere, I., Sallberg, M., & Leroux-Roels, G. (2001). In vivo inhibition of anti-hepatitis B virus core antigen (HBcAg) immunoglobulin G production by HBcAg-specific CD4(+) Th1-type T-cell clones in a hu-PBL-NOD/SCID mouse model. *Journal of Virology, 75*(23), 11449-11456.

Celesia, G. G. (2012). Alcmaeon of croton's observations on health, brain, mind, and soul. *Journal of the History of the Neurosciences, 21*(4), 409-426.

Centers for Disease Control and Prevention. (2009). *Hepatitis B information for the public*. Retrieved 2/4, 2015, from http://www.cdc.gov/hepatitis/B/bFAQ.htm

Centers for Disease Control and Prevention. (2014a). *Flublok seasonal influenza (flu) vaccine*. Retrieved 2/7, 2015, from http://www.cdc.gov/flu/protect/vaccine/qa_flublok-vaccine.htm

Centers for Disease Control and Prevention. (2014b). *Influenza (Flu)*. Retrieved 2/4, 2015, from http://www.cdc.gov/flu/protect/vaccine/vaccines.htm

Centers for Disease Control and Prevention. (2014c). *Live attenuated influenza vaccine [LAIV] (the nasal spray flu vaccine).* Retrieved 2/7, 2015, from http://www.cdc.gov/flu/about/qa/nasalspray.htm

Centers for Disease Control and Prevention. (2015). *How influenza (flu) vaccines are made.* Retrieved 2/7, 2015, from http://www.cdc.gov/flu/protect/vaccine/how-fluvaccine-made.htm#egg-based

Chackerian, B., Caldeira Jdo, C., Peabody, J., & Peabody, D. S. (2011). Peptide epitope identification by affinity selection on bacteriophage MS2 virus-like particles. *Journal of Molecular Biology, 409*(2), 225-237.

Chaipan, C., Kobasa, D., Bertram, S., Glowacka, I., Steffen, I., Tsegaye, T. S., et al. (2009). Proteolytic activation of the 1918 influenza virus hemagglutinin. *Journal of Virology, 83*(7), 3200-3211.

Chen, G. L., Lamirande, E. W., Jin, H., Kemble, G., & Subbarao, K. (2010). Safety, immunogencity, and efficacy of a cold-adapted A/Ann Arbor/6/60 (H2N2) vaccine in mice and ferrets. *Virology, 398*(1), 109-114.

Chen, W., Shi, M., Shi, F., Mao, Y., Tang, Z., Zhang, B., et al. (2009). HBcAg-pulsed dendritic cell vaccine induces Th1 polarization and production of hepatitis B virus-specific cytotoxic T lymphocytes. *Hepatology Research : The Official Journal of the Japan Society of Hepatology, 39*(4), 355-365.

Chisari, F. V., & Ferrari, C. (1995). Hepatitis B virus immunopathogenesis. *Annual Review of Immunology, 13*, 29-60.

Clark, J. R., & March, J. B. (2004). Bacteriophage-mediated nucleic acid immunisation. *FEMS Immunology and Medical Microbiology, 40*(1), 21-26.

Clark, J. R., Bartley, K., Jepson, C. D., Craik, V., & March, J. B. (2011). Comparison of a bacteriophage-delivered DNA vaccine and a commercially available recombinant protein vaccine against hepatitis B. *FEMS Immunology and Medical Microbiology, 61*(2), 197-204.

Coleman, P. F. (2006). Detecting hepatitis B surface antigen mutants. *Emerging Infectious Diseases, 12*(2), 198-203.

Cooper, A., Tal, G., Lider, O., & Shaul, Y. (2005). Cytokine induction by the hepatitis B virus capsid in macrophages is facilitated by membrane heparan sulfate and involves TLR2. *Journal of Immunology (Baltimore, Md.: 1950), 175*(5), 3165-3176.

Cox, N. J., & Subbarao, K. (1999). Influenza. *Lancet, 354*(9186), 1277-1282.

Cros, J. F., Garcia-Sastre, A., & Palese, P. (2005). An unconventional NLS is critical for the nuclear import of the influenza A virus nucleoprotein and ribonucleoprotein. *Traffic (Copenhagen, Denmark), 6*(3), 205-213.

Dane, D. S., Cameron, C. H., & Briggs, M. (1970). Virus-like particles in serum of patients with australia-antigen-associated hepatitis. *Lancet, 1*(7649), 695-698.

Davidson, M. K., Lindsey, J. R., & Davis, J. K. (1987). Requirements and selection of an animal model. *Israel Journal of Medical Sciences, 23*(6), 551-555.

Davis, C. J., Dunbrasky, D., Oonk, M., Taishi, P., Opp, M. R., & Krueger, J. M. (2014). The neuron-specific interleukin-1 receptor accessory protein is required for homeostatic sleep and sleep responses to influenza viral challenge in mice. *Brain, Behavior, and Immunity,* ePub ahead of print.

Davis, H. L., Michel, M. L., & Whalen, R. G. (1993). DNA-based immunization induces continuous secretion of hepatitis B surface antigen and high levels of circulating antibody. *Human Molecular Genetics, 2*(11), 1847-1851.

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. *The Lancet.Infectious Diseases, 12*(9), 687-695.

De Filette, M., Martens, W., Smet, A., Schotsaert, M., Birkett, A., Londono-Arcila, P., et al. (2008). Universal influenza A M2e-HBc vaccine protects against disease even in the presence of pre-existing anti-HBc antibodies. *Vaccine, 26*(51), 6503-6507.

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.

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.

Dehoux, P., Ribes, V., Sobczak, E., & Streeck, R. E. (1986). Expression of the hepatitis B virus large envelope protein in *Saccharomyces cerevisiae*. *Gene, 48*(1), 155-163.

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.

Dias, A., Bouvier, D., Crepin, T., McCarthy, A. A., Hart, D. J., Baudin, F., et al. (2009). The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. *Nature, 458*(7240), 914-918.

Diminsky, D., Schirmbeck, R., Reimann, J., & Barenholz, Y. (1997). Comparison between hepatitis B surface antigen (HBsAg) particles derived from mammalian cells (CHO) and yeast cells (*Hansenula polymorpha*): Composition, structure and immunogenicity. *Vaccine, 15*(6-7), 637-647.

Dong, L., Mori, I., Hossain, M. J., & Kimura, Y. (2000). The senescence-accelerated mouse shows aging-related defects in cellular but not humoral immunity against influenza virus infection. *The Journal of Infectious Diseases, 182*(2), 391-396.

Edman, J. C., Hallewell, R. A., Valenzuela, P., Goodman, H. M., & Rutter, W. J. (1981). Synthesis of hepatitis B surface and core antigens in E. coli. *Nature, 291*(5815), 503-506.

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. *Journal of Immunology (Baltimore, Md.: 1950), 186*(2), 1022-1031.

Elghanam, M. S., Attia, A. S., Shoeb, H. A., & Hashem, A. E. (2012). Expression and purification of hepatitis B surface antigen S from *Escherichia coli*; a new simple method. *BMC Research Notes, 5*, 125-0500-5-125.

Ericsson, A. C., Crim, M. J., & Franklin, C. L. (2013). A brief history of animal modeling. *Missouri Medicine, 110*(3), 201-205.

Fang, R., Min Jou, W., Huylebroeck, D., Devos, R., & Fiers, W. (1981). Complete structure of A/duck/Ukraine/63 influenza hemagglutinin gene: Animal virus as progenitor of human H3 hong kong 1968 influenza hemagglutinin. *Cell, 25*(2), 315-323.

Favoreel, H. W., Van de Walle, G. R., Nauwynck, H. J., & Pensaert, M. B. (2003). Virus complement evasion strategies. *The Journal of General Virology, 84*(Pt 1), 1-15.

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. *Virology Journal, 3*, 102.

Ferrari, C., Penna, A., Bertoletti, A., Cavalli, A., Valli, A., Schianchi, C., et al. (1989). The preS1 antigen of hepatitis B virus is highly immunogenic at the T cell level in man. *The Journal of Clinical Investigation, 84*(4), 1314-1319.

Fiers, W., De Filette, M., Birkett, A., Neirynck, S., & Min Jou, W. (2004). A "universal" human influenza A vaccine. *Virus Research, 103*(1-2), 173-176.

Fiers, W., De Filette, M., El Bakkouri, K., Schepens, B., Roose, K., Schotsaert, M., et al. (2009). M2e-based universal influenza A vaccine. *Vaccine, 27*(45), 6280-6283.

Fluarix. (2014). *Drug description.* Retrieved 2/7, 2015, from http://www.rxlist.com/fluarix-drug.htm

Franco, A., Paroli, M., Testa, U., Benvenuto, R., Peschle, C., Balsano, F., et al. (1992). Transferrin receptor mediates uptake and presentation of hepatitis B envelope antigen by T lymphocytes. *The Journal of Experimental Medicine, 175*(5), 1195-1205.

Fujisawa, Y., Ito, Y., Sasada, R., Ono, Y., Igarashi, K., Marumoto, R., et al. (1983). Direct expression of hepatitis B surface antigen gene in *Escherichia coli*. *Nucleic Acids Research, 11*(11), 3581-3591.

Gagliardi, M. C., Nisini, R., Benvenuto, R., De Petrillo, G., Michel, M. L., & Barnaba, V. (1994). Soluble transferrin mediates targeting of hepatitis B envelope antigen to transferrin receptor and its presentation by activated T cells. *European Journal of Immunology, 24*(6), 1372-1376.

Gao, X., Wang, W., Li, Y., Zhang, S., Duan, Y., Xing, L., et al. (2013). Enhanced influenza VLP vaccines comprising matrix-2 ectodomain and nucleoprotein epitopes protects mice from lethal challenge. *Antiviral Research, 98*(1), 4-11.

Goh, Z. H., Mohd, N. A., Tan, S. G., Bhassu, S., & Tan, W. S. (2014). RNA-binding region of *Macrobrachium rosenbergii* nodavirus capsid protein. *The Journal of General Virology, 95*(Pt 9), 1919-1928.

Goh, Z. H., Tan, S. G., Bhassu, S., & Tan, W. S. (2011). Virus-like particles of *Macrobrachium rosenbergii* nodavirus produced in bacteria. *Journal of Virological Methods, 175*(1), 74-79.

Gomez-Puertas, P., Albo, C., Perez-Pastrana, E., Vivo, A., & Portela, A. (2000). Influenza virus matrix protein is the major driving force in virus budding. *Journal of Virology, 74*(24), 11538-11547.

Graef, K. M., Vreede, F. T., Lau, Y. F., McCall, A. W., Carr, S. M., Subbarao, K., et al. (2010). The PB2 subunit of the influenza virus RNA polymerase affects virulence by interacting with the mitochondrial antiviral signaling protein and inhibiting expression of beta interferon. *Journal of Virology, 84*(17), 8433-8445.

Grotmol, S., Totland, G.K., Thorud, K., & Hjeltnes, B. K. (1997). Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: A probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Diseases of Aquatic Organisms, 29*(2), 85-97.

Gui, X., Li, R., Zhang, X., Shen, C., Yu, H., Guo, X., et al. (2014). An important amino acid in nucleoprotein contributes to influenza A virus replication by interacting with polymerase PB2. *Virology, 464-465*, 11-20.

Guidotti, L. G., Ishikawa, T., Hobbs, M. V., Matzke, B., Schreiber, R., & Chisari, F. V. (1996). Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity, 4*(1), 25-36.

Guidotti, L. G., Matzke, B., Schaller, H., & Chisari, F. V. (1995). High-level hepatitis B virus replication in transgenic mice. *Journal of Virology, 69*(10), 6158-6169.

Han, X., Ye, L. B., Li, B. Z., Bo, G., Cai, W. J., Hong, Z., et al. (2006). Expression, purification and characterization of the hepatitis B virus entire envelope large protein in *Pichia pastoris*. *Protein Expression and Purification, 49*(2), 168-175.

Hashemi, H., Pouyanfard, S., Bandehpour, M., Noroozbabaei, Z., Kazemi, B., Saelens, X., et al. (2012). Immunization with M2e-displaying T7 bacteriophage nanoparticles protects against influenza A virus challenge. *PloS One, 7*(9), e45765.

Hayakijkosol, O., & Owens, L. (2012). B2 or not B2: RNA interference reduces *Macrobrachium rosenbergii* nodavirus replication in redclaw crayfish (*Cherax quadricarinatus*). *Aquaculture, 326–329*(0), 40-45.

He, F., Tang, H., Liu, L., Zhao, L. S., & Liu, C. (2007). Immunoprotection role of a hepatitis B virus DNA vaccine responsible to BAlb/c mice. *Sichuan Da Xue Xue Bao.Yi Xue Ban = Journal of Sichuan University. Medical Science Edition, 38*(4), 613-616.

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. *The Journal of Clinical Investigation, 114*(12), 1812-1819.

Hepatitis B Foundation. (2014). *Statistics*. Retrieved 2/4, 2015, from http://www.hepb.org/hepb/statistics.htm

Hitzeman, R. A., Chen, C. Y., Hagie, F. E., Patzer, E. J., Liu, C. C., Estell, D. A., et al. (1983). Expression of hepatitis B virus surface antigen in yeast. *Nucleic Acids Research, 11*(9), 2745-2763.

Howard, C. R., & Allison, L. M. (1995). Hepatitis B surface antigen variation and protective immunity. *Intervirology, 38*(1-2), 35-40.

Hutchings, C. L., Gilbert, S. C., Hill, A. V., & Moore, A. C. (2005). Novel protein and poxvirus-based vaccine combinations for simultaneous induction of humoral and cell-mediated immunity. *Journal of Immunology (Baltimore, Md.: 1950), 175*(1), 599-606.

Ibanez, L. I., Roose, K., De Filette, M., Schotsaert, M., De Sloovere, J., Roels, S., 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.

Jackwood, D. J. (2013). Multivalent virus-like-particle vaccine protects against classic and variant infectious bursal disease viruses. *Avian Diseases, 57*(1), 41-50.

Kakimi, K., Guidotti, L. G., Koezuka, Y., & Chisari, F. V. (2000). Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *The Journal of Experimental Medicine, 192*(7), 921-930.

Kan, Q., Li, D., & Yu, Z. (2012). Specific expression of human interferon-gamma controls hepatitis B virus replication in vitro in secreting hepatitis B surface antigen hepatocytes. *Journal of Virological Methods, 180*(1-2), 84-90.

Kanai, Y., Athmaram, T. N., Stewart, M., & Roy, P. (2013). Multiple large foreign protein expression by a single recombinant baculovirus: A system for production of multivalent vaccines. *Protein Expression and Purification, 91*(1), 77-84.

Karulin, A. Y., Hesse, M. D., Tary-Lehmann, M., & Lehmann, P. V. (2000). Single-cytokine-producing CD4 memory cells predominate in type 1 and type 2 immunity. *Journal of Immunology (Baltimore, Md.: 1950), 164*(4), 1862-1872.

Karupiah, G., Xie, Q. W., Buller, R. M., Nathan, C., Duarte, C., & MacMicking, J. D. (1993). Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. *Science (New York, N.Y.), 261*(5127), 1445-1448.

Kirnbauer, R., Booy, F., Cheng, N., Lowy, D. R., & Schiller, J. T. (1992). Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proceedings of the National Academy of Sciences of the United States of America, 89*(24), 12180-12184.

Kitikoon, P., Vincent, A. L., Jones, K. R., Nilubol, D., Yu, S., Janke, B. H., et al. (2009). Vaccine efficacy and immune response to swine influenza virus challenge in pigs infected with porcine reproductive and respiratory syndrome virus at the time of SIV vaccination. *Veterinary Microbiology, 139*(3-4), 235-244.

Kok, W. L., Yusoff, K., Nathan, S., & Tan, W. S. (2002). Cloning, expression and display of the PreS domain of hepatitis B virus on filamentous bacteriophage M13. *Journal of Biochemistry, Molecular Biology, and Biophysics, 6*(1), 55-58.

Kuroda, S., Otaka, S., Miyazaki, T., Nakao, M., & Fujisawa, Y. (1992). Hepatitis B virus envelope L protein particles. synthesis and assembly in *Saccharomyces cerevisiae*, purification and characterization. *The Journal of Biological Chemistry, 267*(3), 1953-1961.

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), e01004-13.

Lamb, R. A., & Choppin, P. W. (1983). The gene structure and replication of influenza virus. *Annual Review of Biochemistry, 52*, 467-506.

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. *Proceedings of the National Academy of Sciences of the United States of America, 78*(7), 4170-4174.

Lamb, R. A., Zebedee, S. L., & Richardson, C. D. (1985). Influenza virus M2 protein is an integral membrane protein expressed on the infected-cell surface. *Cell, 40*(3), 627-633.

Lanford, R. E., Luckow, V., Kennedy, R. C., Dreesman, G. R., Notvall, L., & Summers, M. D. (1989). Expression and characterization of hepatitis B virus surface antigen polypeptides in insect cells with a baculovirus expression system. *Journal of Virology, 63*(4), 1549-1557.

Lapinski, T. W., Pogorzelska, J., & Flisiak, R. (2012). HBV mutations and their clinical significance. *Advances in Medical Sciences, 57*(1), 18-22.

Laurence, J. C. (2005). Hepatitis A and B immunizations of individuals infected with human immunodeficiency virus. *The American Journal of Medicine, 118 Suppl 10A*, 75S-83S.

Lavanchy, D. (2005). Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *Journal of Clinical Virology : The Official Publication of the Pan American Society for Clinical Virology, 34 Suppl 1*, S1-3.

Lee, A. M., Tai, L. H., Zador, A., & Wilbrecht, L. (2015). Between the primate and 'reptilian' brain: Rodent models demonstrate the role of corticostriatal circuits in decision making. *Neuroscience*, ePub ahead of print.

Lee, K. W., Tey, B. T., Ho, K. L., & Tan, W. S. (2012a). Delivery of chimeric hepatitis B core particles into liver cells. *Journal of Applied Microbiology, 112*(1), 119-131.

Lee, K. W., Tey, B. T., Ho, K. L., Tejo, B. A., & Tan, W. S. (2012b). Nanoglue: An alternative way to display cell-internalizing peptide at the spikes of hepatitis B virus core nanoparticles for cell-targeting delivery. *Molecular Pharmaceutics, 9*(9), 2415-2423.

Lefeber, D. J., Benaissa-Trouw, B., Vliegenthart, J. F., Kamerling, J. P., Jansen, W. T., Kraaijeveld, K., et al. (2003). Th1-directing adjuvants increase the immunogenicity of oligosaccharide-protein conjugate vaccines related to *Streptococcus pneumoniae* type 3. *Infection and Immunity, 71*(12), 6915-6920.

Lim, D. G., Bieganowska Bourcier, K., Freeman, G. J., & Hafler, D. A. (2000). Examination of CD8+ T cell function in humans using MHC class I tetramers: Similar cytotoxicity but variable proliferation and cytokine production among different clonal CD8+ T cells specific to a single viral epitope. *Journal of Immunology (Baltimore, Md.: 1950), 165*(11), 6214-6220.

Lingel, A., Simon, B., Izaurralde, E., & Sattler, M. (2005). The structure of the flock house virus B2 protein, a viral suppressor of RNA interference, shows a novel mode of double-stranded RNA recognition. *EMBO Reports, 6*(12), 1149-1155.

Liu, B., Mori, I., Hossain, M. J., Dong, L., Takeda, K., & Kimura, Y. (2004). Interleukin-18 improves the early defence system against influenza virus infection by augmenting natural killer cell-mediated cytotoxicity. *The Journal of General Virology, 85*(Pt 2), 423-428.

Liu, W., Li, H., & Chen, Y. H. (2003). N-terminus of M2 protein could induce antibodies with inhibitory activity against influenza virus replication. *FEMS Immunology and Medical Microbiology, 35*(2), 141-146.

Lou, X. M., Yao, Q. H., Zhang, Z., Peng, R. H., Xiong, A. S., & Wang, H. K. (2007). Expression of the human hepatitis B virus large surface antigen gene in transgenic tomato plants. *Clinical and Vaccine Immunology, 14*(4), 464-469.

March, J. B., Clark, J. R., & Jepson, C. D. (2004). Genetic immunisation against hepatitis B using whole bacteriophage lambda particles. *Vaccine, 22*(13-14), 1666-1671.

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 Biotechnology, 11*, 106-6750-11-106.

McAleer, W. J., Buynak, E. B., Maigetter, R. Z., Wampler, D. E., Miller, W. J., & Hilleman, M. R. (1984). Human hepatitis B vaccine from recombinant yeast. *Nature, 307*(5947), 178-180.

Milich, D. R., McNamara, M. K., McLachlan, A., Thornton, G. B., & Chisari, F. V. (1985). Distinct H-2-linked regulation of T-cell responses to the pre-S and S regions of the same hepatitis B surface antigen polypeptide allows circumvention of nonresponsiveness to the S region. *Proceedings of the National Academy of Sciences of the United States of America, 82*(23), 8168-8172.

Miyanohara, A., Toh-e, A., Nozaki, C., Hamada, F., Ohtomo, N., & Matsubara, K. (1983). Expression of hepatitis B surface antigen gene in yeast. *Proceedings of the National Academy of Sciences of the United States of America, 80*(1), 1-5.

Modarresi, M., Hosseinzadeh, L., Nematy, N., Siavash-Haghighi, Z. M., & Ghanbari, K. (2014). Acute and subchronic toxicological evaluation of *Stachys lavandulifolia* aqueous extract in wistar rats. *Research in Pharmaceutical Sciences, 9*(3), 165-172.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1-2), 55-63.

Mountford, A. P., Fisher, A., & Wilson, R. A. (1994). The profile of IgG1 and IgG2a antibody responses in mice exposed to *Schistosoma mansoni*. *Parasite Immunology, 16*(10), 521-527.

Mouse Genome Sequencing Consortium, Waterston, R. H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J. F., et al. (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature, 420*(6915), 520-562.

Murata, K., Lechmann, M., Qiao, M., Gunji, T., Alter, H. J., & Liang, T. J. (2003). Immunization with hepatitis C virus-like particles protects mice from recombinant hepatitis C virus-vaccinia infection. *Proceedings of the National Academy of Sciences of the United States of America, 100*(11), 6753-6758.

Murray, K., & Shiau, A. L. (1999). The core antigen of hepatitis B virus as a carrier for immunogenic peptides. *Biological Chemistry, 380*(3), 277-283.

Murray, K., Bruce, S. A., Hinnen, A., Wingfield, P., van Erd, P. M., de Reus, A., et al. (1984). Hepatitis B virus antigens made in microbial cells immunise against viral infection. *The EMBO Journal, 3*(3), 645-650.

National Institute of Allergy and Infectious Diseases. (2011). *New vaccine technologies: Overview*. Retrieved 2/7, 2015, from http://www.niaid.nih.gov/topics/Flu/Research/vaccineResearch/Pages/Technologies.aspx

NaveenKumar, S., Shekar, M., Karunasagar, I., & Karunasagar, I. (2013). Genetic analysis of RNA1 and RNA2 of *Macrobrachium rosenbergii* nodavirus (MrNV) isolated from India. *Virus Research, 173*(2), 377-385.

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. *Nature Medicine, 5*(10), 1157-1163.

Neurath, A. R., Kent, S. B., Parker, K., Prince, A. M., Strick, N., Brotman, B., et al. (1986). Antibodies to a synthetic peptide from the preS 120-145 region of the hepatitis B virus envelope are virus neutralizing. *Vaccine, 4*(1), 35-37.

Neurath, A. R., Kent, S. B., Strick, N., Stark, D., & Sproul, P. (1985). Genetic restriction of immune responsiveness to synthetic peptides corresponding to sequences in the pre-S region of the hepatitis B virus (HBV) envelope gene. *Journal of Medical Virology, 17*(2), 119-125.

Neurath, A. R., Strick, N., & Sproul, P. (1992). Search for hepatitis B virus cell receptors reveals binding sites for interleukin 6 on the virus envelope protein. *The Journal of Experimental Medicine, 175*(2), 461-469.

Nguyen, D. N., Mahon, K. P., Chikh, G., Kim, P., Chung, H., Vicari, A. P., et al. (2012). Lipid-derived nanoparticles for immunostimulatory RNA adjuvant delivery. *Proceedings of the National Academy of Sciences of the United States of America, 109*(14), E797-803.

Ni, Y., Lempp, F. A., Mehrle, S., Nkongolo, S., Kaufman, C., Falth, M., et al. (2014). Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology, 146*(4), 1070-1083.

Nishimura, A., Morita, M., Nishimura, Y., & Sugino, Y. (1990). A rapid and highly efficient method for preparation of competent *Escherichia coli* cells. *Nucleic Acids Research, 18*(20), 6169.

Ochsenbein, A. F., Pinschewer, D. D., Sierro, S., Horvath, E., Hengartner, H., & Zinkernagel, R. M. (2000). Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *Proceedings of the National Academy of Sciences of the United States of America, 97*(24), 13263-13268.

O'Neill, R. E., Talon, J., & Palese, P. (1998). The influenza virus NEP (NS2 protein) mediates the nuclear export of viral ribonucleoproteins. *The EMBO Journal, 17*(1), 288-296.

Ottone, S., Nguyen, X., Bazin, J., Berard, C., Jimenez, S., & Letourneur, O. (2007). Expression of hepatitis B surface antigen major subtypes in *Pichia pastoris* and purification for in vitro diagnosis. *Protein Expression and Purification, 56*(2), 177-188.

Park, S. G., Ryu, H. M., Lim, S. O., Kim, Y. I., Hwang, S. B., & Jung, G. (2005). Interferon-gamma inhibits hepatitis B virus-induced NF-kappaB activation through nuclear localization of NF-kappaB-inducing kinase. *Gastroenterology, 128*(7), 2042-2053.

Pasek, M., Goto, T., Gilbert, W., Zink, B., Schaller, H., MacKay, P., et al. (1979). Hepatitis B virus genes and their expression in *E. coli*. *Nature, 282*(5739), 575-579.

Pasquetto, V., Wieland, S. F., Uprichard, S. L., Tripodi, M., & Chisari, F. V. (2002). Cytokine-sensitive replication of hepatitis B virus in immortalized mouse hepatocyte cultures. *Journal of Virology, 76*(11), 5646-5653.

Patient, R., Hourioux, C., Sizaret, P. Y., Trassard, S., Sureau, C., & Roingeard, P. (2007). Hepatitis B virus subviral envelope particle morphogenesis and intracellular trafficking. *Journal of Virology, 81*(8), 3842-3851.

Paust, S., & Andrian, U. H. V. (2011). Natural killer cell memory. *Nature Immunology, 12*(6), 500-508.

Pawlotsky, J. M. (2005). The concept of hepatitis B virus mutant escape. *Journal of Clinical Virology : The Official Publication of the Pan American Society for Clinical Virology, 34 Suppl 1*, S125-9.

Pedersen, N. C., Liu, H., Gandolfi, B., & Lyons, L. A. (2014). The influence of age and genetics on natural resistance to experimentally induced feline infectious peritonitis. *Veterinary Immunology and Immunopathology, 162*(1-2), 33-40.

Petry, H., Goldmann, C., Ast, O., & Luke, W. (2003). The use of virus-like particles for gene transfer. *Current Opinion in Molecular Therapeutics, 5*(5), 524-528.

Phillips, S., Chokshi, S., Riva, A., Evans, A., Williams, R., & Naoumov, N. V. (2010). CD8(+) T cell control of hepatitis B virus replication: Direct comparison between cytolytic and noncytolytic functions. *Journal of Immunology (Baltimore, Md.: 1950), 184*(1), 287-295.

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

Pontisso, P., Ruvoletto, M. G., Gerlich, W. H., Heermann, K. H., Bardini, R., & Alberti, A. (1989). Identification of an attachment site for human liver plasma membranes on hepatitis B virus particles. *Virology, 173*(2), 522-530.

Portela, A., & Digard, P. (2002). The influenza virus nucleoprotein: A multifunctional RNA-binding protein pivotal to virus replication. *The Journal of General Virology, 83*(Pt 4), 723-734.

Price, G. E., Soboleski, M. R., Lo, C. Y., Misplon, J. A., Quirion, M. R., Houser, K. V., et al. (2010). Single-dose mucosal immunization with a candidate universal influenza vaccine provides rapid protection from virulent H5N1, H3N2 and H1N1 viruses. *PloS One, 5*(10), e13162.

Pride, M. W., Shi, H., Anchin, J. M., Linthicum, D. S., LoVerde, P. T., Thakur, A., et al. (1992). Molecular mimicry of hepatitis B surface antigen by an anti-idiotype-derived synthetic peptide. *Proceedings of the National Academy of Sciences of the United States of America, 89*(24), 11900-11904.

Qian, D., Shi, Z., Zhang, S., Cao, Z., Liu, W., Li, L., et al. (2003). Extra small virus-like particles (XSV) and nodavirus associated with whitish muscle disease in the giant freshwater prawn, *Macrobrachium rosenbergii*. *Journal of Fish Diseases, 26*(9), 521-527.

Quan, F. S., Compans, R. W., Nguyen, H. H., & Kang, S. M. (2008). Induction of heterosubtypic immunity to influenza virus by intranasal immunization. *Journal of Virology, 82*(3), 1350-1359.

Ravi, M., Nazeer Basha, A., Sarathi, M., Rosa Idalia, H. H., Sri Widada, J., Bonami, J. R., et al. (2009). Studies on the occurrence of white tail disease (WTD) caused by MrNV and XSV in hatchery-reared post-larvae of *Penaeus indicus* and *P. monodon*. *Aquaculture, 292*(1–2), 117-120.

Reed, C., Meltzer, M. I., Finelli, L., & Fiore, A. (2012). Public health impact of including two lineages of influenza B in a quadrivalent seasonal influenza vaccine. *Vaccine, 30*(11), 1993-1998.

Reefman, E., Kay, J. G., Wood, S. M., Offenhauser, C., Brown, D. L., Roy, S., et al. (2010). Cytokine secretion is distinct from secretion of cytotoxic granules in NK cells. *Journal of Immunology (Baltimore, Md.: 1950), 184*(9), 4852-4862.

Reisinger, K. S., Block, S. L., Izu, A., Groth, N., & Holmes, S. J. (2009). Subunit influenza vaccines produced from cell culture or in embryonated chicken eggs: Comparison of safety, reactogenicity, and immunogenicity. *The Journal of Infectious Diseases, 200*(6), 849-857.

Robek, M. D., Wieland, S. F., & Chisari, F. V. (2002). Inhibition of hepatitis B virus replication by interferon requires proteasome activity. *Journal of Virology, 76*(7), 3570-3574.

Rossman, J. S., & Lamb, R. A. (2011). Influenza virus assembly and budding. *Virology, 411*(2), 229-236.

Rossman, J. S., Jing, X., Leser, G. P., & Lamb, R. A. (2010). Influenza virus M2 protein mediates ESCRT-independent membrane scission. *Cell, 142*(6), 902-913.

Ruby, J., Bluethmann, H., & Peschon, J. J. (1997). Antiviral activity of tumor necrosis factor (TNF) is mediated via p55 and p75 TNF receptors. *The Journal of Experimental Medicine, 186*(9), 1591-1596.

Ryu, C. J., Gripon, P., Park, H. R., Park, S. S., Kim, Y. K., Guguen-Guillouzo, C., et al. (1997). In vitro neutralization of hepatitis B virus by monoclonal antibodies against the viral surface antigen. *Journal of Medical Virology, 52*(2), 226-233.

Sahul Hameed, A. S., Yoganandhan, K., Sri Widada, J., & Bonami, J. R. (2004). Experimental transmission and tissue tropism of *Macrobrachium rosenbergii* nodavirus (MrNV) and its associated extra small virus (XSV). *Diseases of Aquatic Organisms, 62*(3), 191-196.

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. *The Journal of Cell Biology, 133*(4), 733-747.

Salvatore, M., Basler, C. F., Parisien, J. P., Horvath, C. M., Bourmakina, S., Zheng, H., et al. (2002). Effects of influenza A virus NS1 protein on protein expression: The NS1 protein enhances translation and is not required for shutoff of host protein synthesis. *Journal of Virology, 76*(3), 1206-1212.

Sambrook, J., Russell, D.W., Irwin, N., & Janssen, K.A. (2001). Molecular Cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory.

Schirmbeck, R., Melber, K., Mertens, T., & Reimann, J. (1994). Antibody and cytotoxic T-cell responses to soluble hepatitis B virus (HBV) S antigen in mice: Implication for the pathogenesis of HBV-induced hepatitis. *Journal of Virology, 68*(3), 1418-1425.

Schnell, J. R., & Chou, J. J. (2008). Structure and mechanism of the M2 proton channel of influenza A virus. *Nature, 451*(7178), 591-595.

Senapin, S., Jaengsanong, C., Phiwsaiya, K., Prasertsri, S., Laisutisan, K., Chuchird, N., et al. (2012). Infections of MrNV (*Macrobrachium rosenbergii* nodavirus) in cultivated whiteleg shrimp *Penaeus vannamei* in asia. *Aquaculture, 338–341*(0), 41-46.

Seo, S. H., & Webster, R. G. (2002). Tumor necrosis factor alpha exerts powerful anti-influenza virus effects in lung epithelial cells. *Journal of Virology, 76*(3), 1071-1076.

Shtykova, E. V., Baratova, L. A., Fedorova, N. V., Radyukhin, V. A., Ksenofontov, A. L., Volkov, V. V., et al. (2013). Structural analysis of influenza A virus matrix protein M1 and its self-assemblies at low pH. *PloS One, 8*(12), e82431.

Shu, L., Touzjian, N., Nan, D., Kushner, N., Strong, A. J., Zeping, W., et al. (2006). Recombinant hepatitis B large surface antigen, successfully produced in *Escherichia coli*, stimulates T-cell response in mice. *Vaccine, 24*(20), 4409-4416.

Simonsen, L., Clarke, M. J., Schonberger, L. B., Arden, N. H., Cox, N. J., & Fukuda, K. (1998). Pandemic versus epidemic influenza mortality: A pattern of changing age distribution. *The Journal of Infectious Diseases, 178*(1), 53-60.

Slifka, M. K., Rodriguez, F., & Whitton, J. L. (1999). Rapid on/off cycling of cytokine production by virus-specific CD8+ T cells. *Nature, 401*(6748), 76-79.

Smyth, M. J., Cretney, E., Kelly, J. M., Westwood, J. A., Street, S. E., Yagita, H., et al. (2005). Activation of NK cell cytotoxicity. *Molecular Immunology, 42*(4), 501-510.

Solcan, C., Pavel, G., Floristean, V. C., Chiriac, I. S., Slencu, B. G., & Solcan, G. (2015). Effect of ochratoxin A on the intestinal mucosa and mucosa-associated lymphoid tissues in broiler chickens. *Acta Veterinaria Hungarica, 63*(1), 30-48.

Sominskaya, I., Skrastina, D., Dislers, A., Vasiljev, D., Mihailova, M., Ose, V., et al. (2010). Construction and immunological evaluation of multivalent hepatitis B virus (HBV) core virus-like particles carrying HBV and HCV epitopes. *Clinical and Vaccine Immunology, 17*(6), 1027-1033.

Sri Widada, J., Durand, S., Cambournac, I., Qian, D., Shi, Z., Dejonghe, E., et al. (2003). Genome-based detection methods of *Macrobrachium rosenbergii* nodavirus, a pathogen of the giant freshwater prawn, *Macrobrachium rosenbergii* dot-blot, in situ hybridization and RT-PCR. *Journal of Fish Diseases, 26*(10), 583-590.

Stachyra, A., Góra-Sochacka, A., Sawicka, R., Florys, K., Sączyńska, V., Olszewska, M., et al. (2014). Highly immunogenic prime–boost DNA vaccination protects chickens against challenge with homologous and heterologous H5N1 virus. *Trials in Vaccinology, 3*(0), 40-46.

Stahl, S. J., & Murray, K. (1989). Immunogenicity of peptide fusions to hepatitis B virus core antigen. *Proceedings of the National Academy of Sciences of the United States of America, 86*(16), 6283-6287.

Stahl-Hennig, C., Eisenblatter, M., Jasny, E., Rzehak, T., Tenner-Racz, K., Trumpfheller, C., et al. (2009). Synthetic double-stranded RNAs are adjuvants for the induction of T helper 1 and humoral immune responses to human papillomavirus in rhesus macaques. *PLoS Pathogens, 5*(4), e1000373.

Stouffer, A. L., Acharya, R., Salom, D., Levine, A. S., Di Costanzo, L., Soto, C. S., et al. (2008). Structural basis for the function and inhibition of an influenza virus proton channel. *Nature, 451*(7178), 596-599.

Sudhakaran, R., Ishaq Ahmed, V. P., Haribabu, P., Mukherjee, S. C., Sri Widada, J., Bonami, J. R., et al. (2007). Experimental vertical transmission of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) from brooders to progeny in *Macrobrachium rosenbergii* and artemia. *Journal of Fish Diseases, 30*(1), 27-35.

Sylvan, S. P., Madalinski, K., & Hellstrom, U. B. (2009). Anti-preS responses influence the anti-HBs response in newborns after vaccination with the third generation sci-B-vac vaccine. *Vaccine, 28*(2), 446-451.

Tan, G. H., Yusoff, K., Seow, H. F., & Tan, W. S. (2005). Antigenicity and immunogenicity of the immunodominant region of hepatitis B surface antigen displayed on bacteriophage T7. *Journal of Medical Virology, 77*(4), 475-480.

Tan, W. S., & Ho, K. L. (2014). Phage display creates innovative applications to combat hepatitis B virus. *World Journal of Gastroenterology, 20*(33), 11650-11670.

Tan, W. S., Dyson, M. R., & Murray, K. (1999). Two distinct segments of the hepatitis B virus surface antigen contribute synergistically to its association with the viral core particles. *Journal of Molecular Biology, 286*(3), 797-808.

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. *The Journal of Biological Chemistry, 277*(42), 39880-39886.

Thimme, R., Wieland, S., Steiger, C., Ghrayeb, J., Reimann, K. A., Purcell, R. H., et al. (2003). CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *Journal of Virology, 77*(1), 68-76.

Tolosa Martinez, N., Tenias Burillo, J. M., Perez Bermudez, B., & Bautista Sanchis Alvarez, J. (1998). Factors associated with inadequate response to hepatitis B vaccination in health care personnel. *Revista Espanola De Salud Publica, 72*(6), 509-515.

Towbin, H., Staehelin, T., & Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America, 76*(9), 4350-4354.

Treichel, U., Meyer zum Buschenfelde, K. H., Stockert, R. J., Poralla, T., & Gerken, G. (1994). The asialoglycoprotein receptor mediates hepatic binding and uptake of natural hepatitis B virus particles derived from viraemic carriers. *The Journal of General Virology, 75*(Pt 11), 3021-3029.

Tzeng, H. T., Tsai, H. F., Chyuan, I. T., Liao, H. J., Chen, C. J., Chen, P. J., et al. (2014). Tumor necrosis factor-alpha induced by hepatitis B virus core mediating the immune response for hepatitis B viral clearance in mice model. *PloS One, 9*(7), e103008.

Valenzuela, P., Medina, A., Rutter, W. J., Ammerer, G., & Hall, B. D. (1982). Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature, 298*(5872), 347-350.

Vietheer, P. T., Boo, I., Drummer, H. E., & Netter, H. J. (2007). Immunizations with chimeric hepatitis B virus-like particles to induce potential anti-hepatitis C virus neutralizing antibodies. *Antiviral Therapy, 12*(4), 477-487.

Vila-del Sol, V., Punzon, C., & Fresno, M. (2008). IFN-gamma-induced TNF-alpha expression is regulated by interferon regulatory factors 1 and 8 in mouse macrophages. *Journal of Immunology (Baltimore, Md.: 1950), 181*(7), 4461-4470.

Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., Yokoyama, W. M., & Ugolini, S. (2011). Innate or adaptive immunity? The example of natural killer cells. *Science, 331*(6013), 44-49.

Wan, Y., Wu, Y., Bian, J., Wang, X. Z., Zhou, W., Jia, Z. C., et al. (2001). Induction of hepatitis B virus-specific cytotoxic T lymphocytes response in vivo by filamentous phage display vaccine. *Vaccine, 19*(20-22), 2918-2923.

Wang, R., Jaw, J. J., Stutzman, N. C., Zou, Z., & Sun, P. D. (2012a). Natural killer cell-produced IFN-gamma and TNF-alpha induce target cell cytolysis through up-regulation of ICAM-1. *Journal of Leukocyte Biology,* 91(2), 299-309.

Wang, Y. S., Ouyang, W., Liu, X. J., He, K. W., Yu, S. Q., Zhang, H. B., et al. (2012b). Virus-like particles of hepatitis B virus core protein containing five mimotopes of infectious bursal disease virus (IBDV) protect chickens against IBDV. *Vaccine, 30*(12), 2125-2130.

Ward, J. W., & Byrd, K. K. (2012). Hepatitis B in the United States: A major health disparity affecting many foreign-born populations. *Hepatology (Baltimore, Md.), 56*(2), 419-421.

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. *Journal of Interferon & Cytokine Research : The Official Journal of the International Society for Interferon and Cytokine Research, 30*(6), 439-449.

Wieland, S., Thimme, R., Purcell, R. H., & Chisari, F. V. (2004). Genomic analysis of the host response to hepatitis B virus infection. *Proceedings of the National Academy of Sciences of the United States of America, 101*(17), 6669-6674.

World Health Organization. (2014a). *Influenza (seasonal) fact sheet.* Retrieved 1/29, 2015, from http://www.who.int/mediacentre/factsheets/fs211/en/

World Health Organization. (2014b). *Hepatitis B.* Retrieved 2/4, 2015, from http://www.who.int/mediacentre/factsheets/fs204/en/

Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., et al. (2012). Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife, 3*, e00049.

Yang, L. P. (2013). Recombinant trivalent influenza vaccine (flublok(®)): A review of its use in the prevention of seasonal influenza in adults. *Drugs, 73*(12), 1357-1366.

Yap, W. B., Tey, B. T., Alitheen, N. B., & Tan, W. S. (2012). Display of the antigenic region of Nipah virus nucleocapsid protein on hepatitis B virus capsid. *Journal of Bioscience and Bioengineering, 113*(1), 26-29.

Yoon, K. Y., Tan, W. S., Tey, B. T., Lee, K. W., & Ho, K. L. (2013). Native agarose gel electrophoresis and electroelution: A fast and cost-effective method to separate the small and large hepatitis B capsids. *Electrophoresis, 34*(2), 244-253.

Yum, J. S., Ahn, B. C., Jo, H. J., Kim, D. Y., Kim, K. H., Kim, H. S., et al. (2012). Use of pre-S protein-containing hepatitis B virus surface antigens and a powerful adjuvant to develop an immune therapy for chronic hepatitis B virus infection. *Clinical and Vaccine Immunology, 19*(2), 120-127.

Zanetti, A. R., Tanzi, E., Manzillo, G., Maio, G., Sbreglia, C., Caporaso, N., et al. (1988). Hepatitis B variant in europe. *Lancet, 2*(8620), 1132-1133.

Zanetti, A. R., Tanzi, E., Romano, L., & Grappasonni, I. (1993). Vaccination against hepatitis B: The italian strategy. *Vaccine, 11*(5), 521-524.

Zhou, C., Zhou, L., & Chen, Y. H. (2012). Immunization with high epitope density of M2e derived from 2009 pandemic H1N1 elicits protective immunity in mice. *Vaccine, 30*(23), 3463-3469.

Zuckerman, J. N., & Zuckerman, A. J. (2003). Mutations of the surface protein of hepatitis B virus. *Antiviral Research, 60*(2), 75-78.

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LIST OF PUBLICATIONS

Tan, W. S., Ho, K. L., **Yong, C. Y.**, Goh, Z. H., Yeap, S. K., & Omar, A. R. (2014). Virus-like particles of *Macrobrachium rosenbergii* nodavirus capsid as a carrier for immunogenic components. (Patent Application Number: PI2014702540).

Yong, C. Y., Ho, K. L., Tey, B.T., & Tan, W. S. (2012). Cloning and expression of small-hepatitis B surface antigen in *Pichia pastoris*. *37th Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology (MSBMB)*, Sime Darby Convention Centre, Kuala Lumpur, Malaysia.

Yong, C. Y., & Tan, W. S. (2015). Microbiology monograph: Beneficial microorganisms in medical & health applications: Production of hepatitis B vaccines by beneficial microorganism. Accepted.

Yong, C. Y., Yeap, S. K., Goh, Z. H., Ho, K. L., Omar, A. R., & Tan, W. S. (2015). Induction of humoral and cell-mediated immune responses by hepatitis B virus epitope displayed on the virus-like particles of prawn nodavirus. *Applied and Environmental Microbiology, 81*(3), 882-889.

Yong, C. Y., Yeap, S. K., Ho, K. L., Omar, A. R., & Tan, W. S. (2015). A recombinant vaccine against influenza A virus based on M2e displayed on nodaviral capsid nanoparticles. *International Journal of Nanomedicine, 10*, 2751-2763.

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