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ISOLATION, CHARACTERIZATION AND QUANTITATIVE PROTEOMICS ANALYSIS OF CHICKEN DENDRITIC CELLS FOLLOWING INFECTION WITH INFECTIOUS BURSAL DISEASE VIRUS

NOR YASMIN BINTI ABD RAHMAN

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By

NOR YASMIN BINTI ABD RAHAMAN

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

June 2015
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DEDICATED WITH LOVE AND GRATITUDE TO:

MY LOVELY HUSBAND (MOHD HANIFF BIN ABD KADIR),
SON (MOHAMMAD YAZDAN ANIQUE) AND DAUGHTER
(NUR LUTHFIATUS SOLEHAH)
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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By

**NOR YASMIN BINTI ABD RAHAMAN**

June 2015

Chairperson: Professor Abdul Rahman Omar, PhD  
Faculty: Institute of Bioscience

Infectious bursal disease (IBD) is an extremely contagious and acute disease of young chicken caused by infectious bursal disease virus (IBDV). IBDV can infect B lymphocytes and macrophages. However, study on the involvement of chicken DCs during pathogen infection especially in IBDV infection has not been studied. Hypothesis of this study was chicken DCs are susceptible to IBDV infection and aimed to characterise the interaction between IBDV and chicken DCs as well as the proteomics profiles of chicken DCs during IBDV infections.

DCs were isolated from bone marrow and spleen for in vitro and ex-vivo study, respectively. The isolated DCs were characterized based on morphology, viability and immunophenotyping while IBDV detection were performed based on immunofluorescence antibody test (IFAT), quantitative real-time polymerase chain reaction (qRT-PCR) and flow cytometry. qRT-PCR was also used to detect the expression of selected cytokines from IBDV-infected DCs. Quantitative proteomics using iTRAQ coupled with tandem LC-MS/MS approach and flow cytometry analysis were performed to quantify and validate differentially regulated proteins of BM-DCs.

Morphologically, uninfected BM-DCs were rounded in shape whilst BM-DCs treated with LPS and vvIBDV showed stellate shapes. Both LPS-treated and vvIBDV-infected BM-DCs expressed high levels of CD86 and MHC class II antigens (>20 %) (p<0.05). In addition, vvIBDV-infected BM-DCs showed significantly higher numbers of apoptotic cells compared to LPS. Replication of vvIBDV was detected in the infected BM-DCs as evidenced by the increased in the expression of VP3 and VP4 antigens based on flow cytometry, qRT-PCR and IFAT. LPS was far more potent than vvIBDV in inducing the expression of IL-1β and IL-18, while the expressions of Th1-like cytokines, IFN-γ and IL-12α were significantly increased in vvIBDV treatment group.

iTRAQ analysis coupled with LC-MS/MS analysis, detect the most abundant proteins (~40 %) with a known membranous localization. From the total of 283 proteins that were identified, 55, 47 and 32 proteins were differentially regulated at 3, 6 and 12 hpi, respectively, as a result of vvIBDV infection, with the fold difference ≥ 1.5 or ≤ 0.67 and ProtScore of more than 1.3 at 95 % confidence level. Most of the protein functions that were impaired at 3 hpi were related to signaling, stress response and immune
response, for instance integrin α and β, heat shock proteins (HSPs) especially HSP90α and HSP60. Interestingly, no proteins related to signaling were activated at this time point. These findings give an indication that vvIBDV able to disrupt several important protein functions in order to infect BM-DCs at the early stage.

Control and infected splenic DCs were distinct as infected DCs showed star like shape. In addition, infected splenic DCs in both vaccine strain and vvIBDV strain expressed higher CD86 and MHCII antigens of more than 30 % at day 5 pi. Meanwhile, VP3 and VP4 proteins of IBDV were readily detected in splenic DCs starting from day 3 pi in both vaccine and vvIBDV-infected groups via IFAT, flow cytometry and qRT-PCR, where the expression of these antigens were significantly higher in vvIBDV (p< 0.05). Splenic DCs infected with vaccine and vvIBDV strains also expressed elevated levels of pro-inflammatory cytokines and chemokines such as IL-1β and CXCLi2 as well as Th1-like cytokines such as IL-12α and IFNγ after day 3 onwards.

In conclusion, chicken BM-DCs and splenic DCs are susceptible and permissive to IBDV infection. The virus infects DCs probably via common host proteins that are also found on other cells such as B cells and macrophages.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**ISOLASI, PENCIRIAN DAN ANALISIS PROTEOMIK KUANTITATIF SEL DENDRITIK AYAM SEMASA JANGKITAN VIRUS PENYAKIT BURSA BERJANGKIT**

Oleh

NOR YASMIN BINTI ABD RAHAMAN

Februari 2015

Pengerusi: Profesor Abdul Rahman Omar, PhD  
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Penyakit bursa adalah penyakit yang sangat berjangkit dan akut kepada ayam yang disebabkan oleh virus penyakit berjangkit bursa (IBDV). IBDV boleh menjangkiti sel B dan makrofaj. Walaupun kajian ke atas penglibatan DCs ayam semasa jangkitan patogen termasuk jangkitan IBDV belum dikaji. Hipotesis kajian ini adalah DCs ayam juga terlibat di dalam jangkitan IBDV dan tujuan kajian ini adalah untuk mencirikan interaksi antara IBDV dan DCs serta melibatkan kajian profil proteomik DCs ayam semasa jangkitan IBDV.

DCs diambil daripada sumsum tulang dan limpa untuk kajian in vitro dan ex vivo, masing-masing. DCs yang dijangkiti dicirikan berdasarkan morfologi, peratusan sel hidup, imunofenotip manakala pengesan IBDV dilaksanakan dengan menggunakan ujian antibodi imunopendarfluor (IFAT), reaksi rantai polymerase waktu nyata kuantitatif (qRT-PCR) dan aliran sitometri. qRT-PCR juga digunakan untuk mengesan ekspresi sitokin terpilih daripada DCs yang dijangkiti IBDV. Kajian kuantitatif proteomik yang menggunakan iTRAQ berserta pendekatan LC-MS/MS dan analisis aliran sitometri dilakukan untuk mengukur dan mengesahkan protein BM-DCs.

Secara morfologi, BM-DCs yang tidak dijangkiti menunjukkan bentuk bulat, manakala BM-DCs yang dirawat dengan LPS and vvIBDV menunjukkan bentuk bintang. Kedua-dua BM-DCs yang dirawat dengan LPS dan vvIBDV menunjukkan secara signifikan tahap antigen CD86 dan MHC kelas II yang tinggi (>20 %) (p<0.05). Tambahan pula, BM-DCs yang dijangkiti dengan vvIBDV menunjukkan peningkatan ekspresi antigen VP3 dan VP4 menerusi analisis aliran sitometri, qRT-PCR dan IFAT. vvIBDV dilihat lebih berkesan daripada LPS dalam mendorong ekspresi IL-1β dan IL-18, manakala ekspresi sitokin Th1 iaitu IFN-γ dan IL-12α meningkat secara signifikan di dalam kumpulan rawatan vvIBDV.

Analisis iTRAQ bersama LC-MS/MS mengesahkan kebanyakan protein (~40 %) yang diketahui terletak di membran. Daripada keseluruhan jumlah protein iaitu sebanyak 283 protein yang dikenal pasti, 55, 47 dan 32 ekspresi protein yang diatur secara berbeza selepas 3, 6 dan 12 jam jangkitan, masing-masing, disebabkan jangkitan vvIBDV, dengan perbezaan kali ganda ≥ 1.5 atau ≤ 0.67 dan ProtScore lebih daripada...
1.3 pada 95% tahap keyakinan. Kebanyakan fungsi protein yang terjejas pada 3 jam selepas jangkitan adalah melibatkan fungsi pengisyaratan, respon kepada stress dan tindak balas imun, sebagai contohnya protein integrin α dan β, *heat shock proteins* (HSPs) terutama sekali HSP90α dan HSP60. Menariknya tiada ekspresi protein yang terlibat di dalam fungsi pengisyaratan yang diaktifkan pada masa ini. Penemuan ini menunjukkan yang vvIBDV mampu untuk menjejaskan beberapa fungsi protein yang penting untuk menjangkiti BM-DCs pada peringkat awal.

DCs limpa daripada kumpulan yang tidak dijangkiti dan dijangkiti IBDV adalah berbeza kerana DCs yang dijangkiti menunjukkan bentuk bintang. Tambahan pula, DCs limpa yang dijangkiti dengan kedua-dua strain iaitu strain vaksin dan strain vvIBDV mengekspresi antigen CD86 dan MHCII dengan tinggi iaitu lebih daripada 30% pada hari ke-5 selepas jangkitan. Sementara itu, protein IBDV iaitu VP3 dan VP4 dapat dikesan di dalam DCs limpa bermula dari hari ke -3 selepas dijangkiti strain vaksin dan vvIBDV menerusi IFAT, aliran sitometri and qPCR, dimana ekspresi antigen ini adalah lebih tinggi didalam ayam yang dijangkiti vvIBDV (p< 0.05). DCs limpa yang dijangkiti strain vaksin dan vvIBDV juga mengekspresi peningkatan kadar sitokin dan kemokin yang berkait rapat dengan tindak balas keradangan seperti IL-1β dan CXCLi2 serta sitokin Th1 seperti IL-12α dan IFNγ selepas 3 hari dijangkiti.

Sebagai kesimpulan, BM-DCs dan DCs limpa ayam adalah rentan kepada jangkitan IBDV. Virus ini menjangkiti DCs mungkin melalui protein perumah yang umum yang juga ditemui di dalam sel lain seperti sel B dan makrofaj.
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I certify that a Thesis Examination Committee has met on 25 June 2015 to conduct the final examination of Nor Yasmin Binti Abd Rahaman on her thesis entitled “Isolation, Characterisation and Quantitative Proteomics Analysis of Chicken Dendritic Cells following Infection with Infectious Bursal Disease Virus” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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

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LIST OF ABBREVIATIONS

A Absorbance
AC-ELISA Antigen capture-Enzyme-linked immunoabsorbent assay
AI Avian Influenza
ANOVA Analysis of variance
APC Allophycocyanin
APC Antigen presenting cells
ATP Adenosine triphosphate
B Base
BGM-70 Buffalo green monkey kidney
BM Bone marrow
BM-DCs Bone marrow derived dendritic cells
Bp Base pair
BrdU Bromodeoxyuridine
BSA Bovine serum albumin
CAM Chorioallantoic membrane
CAV Chicken anemia virus
CD Cluster of differentiation
cDNA Complementary deoxyribonucleic acid
CEE Chicken embryonated eggs
CEF Chicken embryo fibroblasts
CEK Chicken embryo kidney
cIBDV Classical strain of infectious bursal disease virus
CMI Cell mediated immunity
CMV Cytomegalovirus
CO₂ Carbon dioxide
COS-7 Transformed African green monkey kidney fibroblast cells
Cq Quantification cycle
CXCLi Chemokine (C-X-C motif) ligand
DAMPs Danger associated membrane proteins
DAPI 4',6-diamidino-2-phenylindole
DC Dendritic cells
ddH₂O Double distilled water
DIGE Difference gel electrophoresis
DMEM Dulbecco's Modified Eagle's Medium
DMSO Dimethyl sulfoxide
DNA Deoxyribonucleic acid
dNTP Deoxyribonucleotide triphosphate
Dpi Day post-infection
ds Double stranded
EDTA Ethylene-diamine-tetraacetic-acid
EID₅₀ 50% Egg Infectious Dose
ELISA Enzyme-linked immunoabsorbent assay
ERK Extracellular-signal-regulated kinases
ESI Electrospray ionization
FACS Fluorescence activated cell sorter
FAM 6-carboxyfluorescein
FBS Fetal bovine serum
FDC Follicular dendritic cells
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>G</td>
<td>Gauge</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>g</td>
<td>Gravitational force</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte monocyte-colony stimulating factor</td>
</tr>
<tr>
<td>GO</td>
<td>Gene Ontology</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HPAI</td>
<td>High pathogenic avian influenza</td>
</tr>
<tr>
<td>hpi</td>
<td>hours post infection</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>HPV</td>
<td>Human papillomavirus HSP</td>
</tr>
<tr>
<td>HSP</td>
<td>Herpes simplex virus</td>
</tr>
<tr>
<td>IBD</td>
<td>Infectious bursal disease</td>
</tr>
<tr>
<td>IBD-ICX</td>
<td>IBD-immune complex</td>
</tr>
<tr>
<td>IBDV</td>
<td>Infectious bursal disease virus</td>
</tr>
<tr>
<td>IBV</td>
<td>Infectious bronchitis virus</td>
</tr>
<tr>
<td>ICAT</td>
<td>Isotope-coded affinity tag</td>
</tr>
<tr>
<td>IDC</td>
<td>Interdigitating dendritic cells</td>
</tr>
<tr>
<td>IFAT</td>
<td>Immunoflorescent antibody test</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthases</td>
</tr>
<tr>
<td>iTRAQ</td>
<td>Isobaric tag for relative and absolute quantitation</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>Monopotassium phosphate</td>
</tr>
<tr>
<td>LAMP</td>
<td>Lysosomal-associated membrane protein</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography tandem mass spectrometry</td>
</tr>
<tr>
<td>LL</td>
<td>Lymphoid leucosis</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharides</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>MACS</td>
<td>Magnetic activated cell sorting</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>MD</td>
<td>Marek’s disease</td>
</tr>
<tr>
<td>MDA5</td>
<td>Melanoma differentiation-associated gene 5 -like receptors</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility complex</td>
</tr>
<tr>
<td>MIP</td>
<td>Major intrinsic protein</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mM</td>
<td>Mili Molar</td>
</tr>
<tr>
<td>MOI</td>
<td>Multiplicity of infection</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>MYD88</td>
<td>Myeloid differentiation primary response 88</td>
</tr>
<tr>
<td>n</td>
<td>Sample size</td>
</tr>
<tr>
<td>NaoH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>ND</td>
<td>Not detected</td>
</tr>
<tr>
<td>NDV</td>
<td>Newcastle disease virus</td>
</tr>
<tr>
<td>NF-kB</td>
<td>Kappa-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>NFQ</td>
<td>3' nonfluorescent quencher</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>OK</td>
<td>Ovine kidney</td>
</tr>
<tr>
<td>ORF</td>
<td>Open reading frame</td>
</tr>
<tr>
<td>P</td>
<td>Projection</td>
</tr>
<tr>
<td>p</td>
<td>Sample Proportion</td>
</tr>
<tr>
<td>PAMPS</td>
<td>Pathogen-associated molecular patterns</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>pDCs</td>
<td>Plasmacytoid dendritic cells</td>
</tr>
<tr>
<td>PerCp</td>
<td>Peridinin chlorophyll A protein</td>
</tr>
<tr>
<td>PI</td>
<td>Propidium iodide</td>
</tr>
<tr>
<td>pi</td>
<td>Post infection</td>
</tr>
<tr>
<td>PI</td>
<td>Propium iodide</td>
</tr>
<tr>
<td>PRRs</td>
<td>Pathogen recognition receptors</td>
</tr>
<tr>
<td>PSPEP</td>
<td>Proteomics System Performance Evaluation Pipeline</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Real-time reverse transcription PCR</td>
</tr>
<tr>
<td>R²</td>
<td>Coefficient of correlation</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
</tr>
<tr>
<td>RK-13</td>
<td>Rabbit kidney-13</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>Rpm</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td>RSV</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>SCX</td>
<td>Strong cation exchange liquid chromatography</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>sIgM</td>
<td>Surface immunoglobulin M</td>
</tr>
<tr>
<td>SILAC</td>
<td>Stable isotope labeling by amino acids in cell culture</td>
</tr>
<tr>
<td>sp</td>
<td>Species</td>
</tr>
<tr>
<td>SPF</td>
<td>Specific pathogen free</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical program for social science</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris-acetate-EDTA</td>
</tr>
<tr>
<td>Th-1</td>
<td>T helper-1</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptors</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of flight</td>
</tr>
<tr>
<td>tRNAs</td>
<td>Transfer ribonucleic acid</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>UPM</td>
<td>Universiti Putra Malaysia</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>UPR</td>
<td>Unfolded protein response</td>
</tr>
<tr>
<td>USA</td>
<td>United State of America</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per volume</td>
</tr>
<tr>
<td>Vero</td>
<td>African green monkey kidney</td>
</tr>
<tr>
<td>VNT</td>
<td>Virus neutralization test</td>
</tr>
<tr>
<td>VOPBA</td>
<td>Virus overlay protein blotting assay</td>
</tr>
<tr>
<td>VP</td>
<td>Viral protein</td>
</tr>
<tr>
<td>VRI</td>
<td>Veterinary Research Institute</td>
</tr>
<tr>
<td>VSV</td>
<td>Vesicular stomatitis virus</td>
</tr>
<tr>
<td>vv</td>
<td>Very virulent</td>
</tr>
<tr>
<td>vvIBDV</td>
<td>Very virulent strain of infectious bursal disease virus</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>ºC</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>µl</td>
<td>Microliter</td>
</tr>
<tr>
<td>1D-LCMS</td>
<td>One Dimensional Liquid chromatography–mass spectrometry</td>
</tr>
<tr>
<td>2-DE</td>
<td>Two-dimensional electrophoresis</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
</tr>
<tr>
<td>γ</td>
<td>Gamma</td>
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</table>
Poultry industry in Malaysia is an important livestock sector which represents a major proportion of the industry besides aquaculture and pig productions. Malaysia has been self-sufficient in the poultry meat and eggs production since 2002 with the percentage of self-sufficient for 2011 at 132% (Mohamed et al., 2013). However, the industry is facing constant threat and challenges. Infectious diseases are major threat that cause significant economic losses in terms of mortality, condemnation of carcass, poor performance parameters and increased in the cost of medication (Omar, 2013). In addition, viral infections such as Marek’s disease virus (MDV), retrovirus causing lymphoid leucosis (LL), chicken anemia virus (CAV) and infectious bursal disease virus (IBDV) can impair the host immune responses, hence, causing immunosuppression (Hoerr, 2010). Viral induced immunosuppression is a problematic issue that is challenging to manage in poultry flocks due to its significance impact to the health status of the chickens and can lead to vaccination failure against various diseases.

Infectious bursal disease virus (IBDV) is classified under the family Birnaviridae (Dobos, 1979), a bi-segmented and double-stranded RNA (dsRNA) virus with a single-shelled, non-enveloped virions (MacDonald, 1980; Müller et al., 1979). Serotype I IBDV strains can be grouped into different subtypes/strains namely classical strains, variant strains and very virulent strains (van den Berg, 2000). Among these strains, the vvIBDV strain have been reported in several countries and have caused serious problem in commercial poultry industry due to the inability of maternal antibody from classical IBDV vaccine in inducing complete protection (Williams & Davidson, 2005). The virus cause a disease known as infectious bursal disease (IBD) (Gumboro disease), which is difficult to control in commercial flocks since it able to resist many disinfectants and capable of causing high mortality and inducing immunosuppression in susceptible chickens (Van Den Berg et al., 2004).

Infectious bursal disease virus (IBDV) is a lymphotropic virus which known to target IgM+ B cells (Withers et al., 2006; Rodenberg et al., 1994) and macrophage (Palmquist et al., 2006; Khatri et al., 2005). Moreover, IBDV infection promotes infiltration of T cells in infected organ such as bursa of Fabricus (Rautenschlein et al., 2002; Kim et al., 2000). However, T cells are refractory to IBDV infection (Mahgoub, 2012). Hence, chicken infected with the virus develop immunosuppression due to the depletion of IgM bearing B-lymphocytes and disturbance in the innate and cell-mediated immunity responses due to direct activation of macrophage and indirect activation of T cells which subsequently lead to massive production of proinflammatory cytokines (Ingrao et al., 2013). In contrary, the involvement of other immune cells such as dendritic cells (DCs) during IBDV infection has not been characterized.

In mammals, dendritic cells (DCs) are well known as professional antigen presenting cells (APC) linking the innate and acquired immunity during combating infectious diseases (Steinman et al., 2003). DCs progenitors are originated from bone marrow and further differentiated into circulating immature DCs with high ability to capture antigen (Granucci et al., 2003). Once exposed to pathogens, immature DCs migrated to T cell regions of different lymphoid organs and undergo maturation with high antigen presenting capabilities particularly to CD4 helper T cells for the activation of immune responses (Liu, 2001). The activated helper T cells play an important role in activating
other cells such as natural killer cells (NK), eosinophils and macrophages as well as antigen specific cells such as B cells and CD8 cytotoxic T cells. As a result, these cells are recruited to migrate to the damage or infected site in order to prevent the infection from continue to harm the host (Rescigno & Borrow, 2001).

Currently, majority of the studies on the interaction between viruses and DCs are on human virus infection. DCs infected with respiratory syncytial virus (RSV) associated with virus replication and DCs maturation (González et al., 2008). Meanwhile, viruses such as dengue virus, influenza virus and herpesvirus are able to replicate inside DCs but impede the maturation process (Boonnak et al., 2008; Fernandez-Sesma et al., 2006; Novak and Peng, 2005). On the other hand, viruses such as human papilloma virus able to present antigen without replicating inside DCs (García-Piñeres et al., 2006). Hence, characterisation of the interplay between viruses with chicken DCs will provide valuable information in the role of DCs during infection and immunity.

In addition, chicken lack of lymph node as a defense mechanism against infection, yet, chicken also exposed to various kind of pathogen akin to mammals (Wu & Kaiser, 2011). Hence, the involvement of APC are utmost crucial in immune system of chicken. In chicken, DCs progenitor from bone marrow; follicular DCs from secondary lymphoid organs namely spleen, Harderian glands, Payer’s patches and cecal tonsils; as well as Langerhans cells have been studied (Ly et al., 2010; Wu et al., 2010; del Cacho et al., 2008; Igyarto et al., 2006). However, the involvements of chicken DCs during viral infection and immune responses are not well characterized. Thus far, no studies have investigated the role of chicken DCs during IBDV infection. Fundamental study on the interaction of DCs and IBDV will provide valuable information in understanding the role of professional APCs in chickens and their molecular interactions during IBDV infection and vaccination. Since, B cells and macrophages are also APC and are the target of IBDV infection, the hypotheses of this study are:

a) chicken DCs are susceptible to IBDV infection
b) IBDV infected DCs will secrete cytokines resemble other APC such as B cells and macrophages
c) proteomics profiling of IBDV infected DCs will identify differentially regulated DCs proteins that are important during IBDV infection

Hence, in order to address these hypotheses, the specific objectives of this study were:

a) to isolate and compare the bone marrow derived dendritic cells (BM-DCs) response following in vitro stimulation with vvIBDV and lipopolysaccharides (LPS)
b) to determine the expression levels of IBDV, DC activation markers and cytokines production of BM-DCs following in vitro vvIBDV infection
c) to identify the proteome of BM-DCs following in vitro vvIBDV infection based on iTRAQ and LC/MS-MS analysis
d) to characterize in silico and validate the expressions of differentially regulated proteins of BM-DCs following in vitro vvIBDV infection
e) to isolate and compare the splenic DCs responses based on expression levels of IBDV, DC activation markers and cytokines production following vaccine strain and very virulent strain of IBDV inoculation in SPF chickens
BIBLIOGRAPHY


serology with protection of progeny chickens against IBD virus strains of varying virulence. Avian Pathology, 30, 345-354.


Meir, R., Jackwood, D.J. & Weisman, Y. (2001). Molecular typing of infectious bursal disease virus of Israeli field and vaccine strains by the reverse
transcription/polymerase chain reaction/restriction fragment length polymorphism assay. Avian Diseases, 45, 223-228.


