MORPHOLOGICAL AND IMMUNOPHENOTYPICAL CHARACTERISATION OF MATURATION-ASSOCIATED CHICKEN DENDRITIC CELLS BY WILD TYPE AND RECOMBINANT FOWLPOX VIRUS INFECTIONS

By

SAKINAH BINTI YUSOF

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

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

MORPHOLOGICAL AND IMMUNOPHENOTYPICAL CHARACTERISATION OF MATURATION-ASSOCIATED CHICKEN DENDRITIC CELLS BY WILD TYPE AND RECOMBINANT FOWLPOX VIRUS INFECTIONS

By

SAKINAH BINTI YUSOF

April 2020

Chair : Mariatulqabtiah Abdul Razak, PhD
Institute : Bioscience

Fowlpox is a common viral disease caused by fowlpox virus (FWPV) which infects birds. In commercial poultry farming, this is considered as economically important disease as it can result in lower production of egg besides increasing the rate of mortality. Compared to other viral infectious diseases, study on the interaction of chicken bone marrow-derived dendritic cells (chBM-DCs) with FWPV remains limited. Therefore, this study aimed to characterize the response of chBM-DCs upon stimulation of wild type (WT) FP9 stain of FWPV compared to recombinant FWPV carrying H5 gene of avian influenza virus (AIV) by morphology and expression of surface marker approach. It is hypothesized that FWPV are susceptible to chBM-DCs and have the ability to drive the maturation of the immune cell. In this study, DCs were isolated from chicken femur and cultured for 6 days in the presence of recombinant chicken granulocyte-macrophage colony-stimulating factor (GM-CSF) and recombinant chicken interleukine-4 (IL-4). The cultured population showed typical morphology of chBM-DCs with the formation of dendrites, which are the key morphological characteristics that defined as a sign of maturation. The result showed that infected chBM-DCs expressed enhanced levels of CD86 and MHC class II in comparison to unstimulated chBM-DC. This indicates the phenotypically mature of chBM-DCs upon stimulation of WT FWPV and rFWPV/H5. The maturation process happened when immature DCs capture and process antigen which followed by up regulation of MHC class II surface marker and co-stimulatory molecules such as CD40, CD80 and CD86. The study has demonstrated that both WT FWPV and rFWPV/H5 were susceptible towards DCs and capable in inducing maturation of DCs. In fact, it was shown that rFWPV/H5 induced the functional maturation of DCs better as compared to WT FWPV, phenotypically. Hence, the study of mechanism process of FWPV immune response enhancement could significantly improve the development of vaccines and therapeutics field.

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PENCIRIAN MORFOLOGI DAN IMUNOFENOTIPIK BAGI KEMATANGAN TERKAIT-SEL DENDRITIK AYAM OLEH JANGKITAN CACAR AIR JENIS LIAR DAN REKOMBINAN

Oleh

SAKINAH BINTI YUSOF

April 2020

Pengerusi : Mariatulqabtiah Abdul Razak, PhD
Institut : Biosains

Cacar air ialah suatu penyakit yang kerap menjangkiti burung disebabkan oleh virus fowlpox (FWPV). Dalam penternakan komersial, ianya dianggap sebagai penyakit yang penting dari segi ekonomi kerana dapat memberi kesan dalam penurunan pengeluaran telur selain meningkatkan kadar kematian burung. Jika dibandingkan dengan penyakit berjangkit virus yang lain, kajian mengenai interaksi sel dendritik dari sumsum tulang ayam (chBM-DCs) dengan FWPV masih begitu terhad. Oleh yang demikian, kajian ini bertujuan untuk mengkaji tindak balas chBM-DCs setelah diberi ransangan oleh FWPV jenis liar (WT) berbanding dengan FWPV rekombinan bersama gen H5 dari virus selesema burung (AIV) dari segi morfologi dan juga pendekatan ekpresi penanda permukaan. Dihipotesiskan bahawa, FWPV mempunyai keupayaan untuk mendorong kematangan sel imun chBM-DCs. Dalam kajian ini, DC diambil daripada tulang paha ayam dan dikultur dengan kehadiran faktor perangsang koloni granulosit-makrofaj rekombinan ayam (GM-CSF) serta interleukin-4 ayam (IL-4) selama 6 hari. Populasi yang telah dikultur menunjukkan morfologi yang biasa bagi chBM-DCs dengan pembentukan dendrit, dimana ianya merupakan ciri morphologi yang utama, yang juga dianggap sebagai tanda utama pematangan sel. Hasil keputusan menunjukkan bahawa chBM-DCs yang dijangkiti mengekspres peningkatan tahap CD86 dan kelas MHC ke II jika dibandingkan dengan chBM-DCs yang tidak diransang. Ini menunjukkan bahawa chBM-DCs adalah matang dari segi fenotipik selepas diransang oleh WT FWPV dan juga rFWPV/H5. Proses pematangan berlaku apabila DCs yang tidak matang menangkap dan memproses antigen diikuti dengan pertambahan penanda permukaan bagi kelas MHC ke II serta molekul rasangan seperti CD40, CD80 dan CD86. Kajian ini telah menunjukkan bahawa kedua-dua WT FWPV dan rFWPV/H5 adalah rentan terhadap DCs dan mampu untuk mendorang kematangan DCs. Tambahan pula, rFWPV/H5 menunjukkan bahawa ianya mampu mendorong pematangan DCs secara finotip dengan lebih baik berbanding WT FWPV. Oleh yang demikian, kajian mengenai proses

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mekanisma bagi peningkatan tindak balas imun FWPV dapat meningkatkan perkembangan bidan vaksin dan terapeutik dengan lebih ketara.

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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 Master of Science. The members of the Supervisory Committee were as follows:

Mariatulqabtiah binti Abdul Razak, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Abdul Rahman bin Omar, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Nor Yasmin binti Abd Rahaman, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature : _____
Name of Member of Supervisory Committee : Prof Dr. Abdul Rahman bin Omar

Signature : _____
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LIST OF ABBREVIATIONS

AIV	Avian influenza virus
APC	Allophycocyanin
APC	Antigen presenting cells
BM	Bone marrow
BM-DCs	Bone marrow derived dendritic cells
bp	Base pair
BSA	Bovine serum albumin
CAM	Chorioallantoic membrane
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CEE	Chicken embryonated egg
CEF	Chicken embryo fibroblast
ch	Chicken
CO_2	Carbon dioxide
COS-7	Transformed african green monkey fibroblast cell
CPE	Cytopathic effect
DAPI	4',6-diamidino-2-phenylindole
DC	Dendritic cell
DMEM	Dulbecco's modified eagle's medium
DNA	Deoxyribonucleic acid
ds	Double stranded
EDTA	Ethylenediaminetetraacetic acid
FACS	Fluorescencce activated cell sorter
FITC	Fluorescein isothiocyanate

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FBS	Fetal bovine serum
FDCs	Follicular dendritic cells
FSC	Forward scatter
FWPV	Fowl pox virus
g	Gram
g	Gravitational force
G	Gauge
GM-CSF	Granulocyte monocyte-colony stimulating factor
h	Hour
HA	Haemagglutinin
HPAI	High pathogenic avian influenza
IACUC	Institutional of animal care and use committee
IBDV	Infectious bursal disease virus
ICTV	International Committee on Taxonomy of Viruses
IDC	Interdigitating dendritic cells
IFAT	Immunofluorescent antibody test
IL	Interleukin
ITR	Inverted Terminal Repeat
IVPI	Intravenous pathogenicity index
K	Potassium
kb	Kilo base pair
LPAI	Low pathogenic avian influenza
LPS	Lipopolysaccaride
M	Matrix
M	Molar
mAb	Monoclonal antibody

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mg	Milligram
MHC	Major histocompatibility complex
mM	Milimolar
MOI	Multiple of infection
MVP	Malaysian Vaccines and Pharmaceuticals
NA	Neuraminidase
NDV	Newcastle's disease virus
NEP	Nuclear export
ng	Nanogram
NP	Nucleoprotein
NS	Non-structure
ORF	Open Reading Frame
PA	Polymerase acidic
PB	Polymerase basic
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PerCp	Peridinin chlorophyll a protein
PI	Post infection
PFU	Plaque forming unit
REV	Reticuloendotheliosis Virus
rFWPV	Recombinant fowlpox virus
rFWPV/H5	Recombinant fowlpox virus strain FP9 expressing H5 gene of AIV
rpm	Revolution per minute
RPMI	Roswell park memorial institute
RNA	Ribonucleic acid

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SD	Standard deviation
SPF	Specific pathogen free
SPSS	Statistical package for social sciences
SSC	Side scatter
TAE	Tris acetate ethylenediaminetetraacetic acid
Th	Helper T cell
$TCID_{50}$	50% tissue culture infectious dose
V	Volt
vv	Very virulent
VV	Vaccinia Virus
v/v	Volume per volume
WT	Wild type
X	Times
%	Percentage
^{o}C	Degree celcius
µg	Micogram
µl	Microliter
µM	Micromolar
µm	Micrometer
*	Asterisk

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CHAPTER 1

INTRODUCTION

Fowlpox is a contagious viral disease which infects domestic and wild birds, caused by fowlpox virus (FWPV). FWPV is a large double stranded DNA virus belonging to genus *Avipoxvirus* of family *Poxviridae* (Gilhare et al., 2015). It has a large size genome up to 300 kb. FWPV replication and maturation occurs exclusively in the cytoplasm of host cell. The replication of this virus is limited to the avian cells only and there is no production of progeny viruses in mammalian cells. This virus is transmitted by biting of insects, mainly mosquitoes (Weli & Tryland, 2011). Fowlpox disease is mainly found in cutaneous form which demonstrates multifocal skin lesions on unfeathered areas of the body. It is usually milder than diptheritic form, which causes fibrous necrotic lesions of the respiratory tracts that results in higher mortality (Zhao et al., 2014). There is a need to focus on this disease as it is an emerging disease that was reported widely and considered to cause serious economic loss.

Avian DCs were first identified in the cecal tonsil which is situated in intestinal mucosa (Olah and Glick, 1979). Dendritic cells are recognized as one of the unique professional antigen-presenting cells (APCs). They are specialized in capture and processing of antigen which plays a central role in initiation of primary immune responses. The maturation and function of this immune cell are triggered by the environmental and microbial stimuli. Previous studies have shown that DCs matured in response with different viruses (Ioannidis et al., 2012; Liang et al., 2015; Vervelde et al., 2013). The maturation of DCs was reported to produce high levels of MHC class II surface marker together with co-stimulatory molecules such as CD40, CD80, CD83 and CD86.

The study on the response of BM-DCs upon virus stimulation is not well known as compared to mammalian DCs (Wu et al., 2010; Yasmin et al., 2015). Therefore, in recent study, the effects of WT FWPV and rFWPV/H5 stimulation on antigen presentation expression of BM-DCs for CD86 and MHC class II cell surface molecules were determined. It is hypothesized that both WT FWPV and rFWPV/H5 were able to induce the maturation of chBM-DCs, similar to previous studies using vvIBDV (Liang et al., 2015; Yasmin et al., 2015) and AIV (Vervelde et al., 2013).

In order to address the general aim, the specific objectives involved in this study are as follows:

1. To propagate WT FWPV and rFWPV carrying H5 gene of AIV
2. To characterize the activities of chBM-DCs during *in vitro* FWPV infection
3. To characterize the expression level of co-stimulatory molecules of stimulated chBM-DCs

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