UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF MURINE IMMUNE RESPONSES TO H5 DNA VACCINE WITH IRF3 AS GENETIC ADJUVANT AND HIV TAT- CONJUGATED PAMAM DENDRIMER AS THE DELIVERY SYSTEM

AZADEH BAHADORAN

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By

AZADEH BAHADORAN

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

February 2016

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DEDICATION

I dedicate this thesis to my beloved husband

Ali

for his love, endless support and encouragement

to my sweet beautiful love

Aynaz

and

to my **Mother** and **Father**

whose affection, love, encouragement and prayers of day and night make me able to get such success and honour.

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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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Chairman: Abdul Rahman bin Omar, DVM, PhD
Faculty: Institute of Bioscience

Influenza A virus is a major pathogen that represents an ongoing threat to several species as diverse as poultry, swine and mammals including humans health principally through their ability to cause respiratory morbidity and mortality. With the advances in modern vaccine technologies, several different vaccines are currently available against the Influenza A virus subtype H5N1. DNA vaccines have various attributes, which present them distinct advantages over other vaccine technologies in terms of safety, stability, ease of fabrication and immunogenicity. Although DNA vaccines offer a wide range of advantages, there is still a need to improve the delivery of DNA vaccines and to increase the immunogenicity of antigens expressed from the plasmids. Inefficient cellular delivery of DNA plasmids and insufficient stimulation of the innate immune system are considered as the main reasons for the failure of DNA vaccines to induce potent immune responses. In this study the immune responses of a new recombinant DNA vaccine encoding the H5 of avian influenza virus (AIV), green fluorescent protein (GFP) and *Mus musculus* interferon regulatory factor 3 (IRF3) genes (pBud-H5-GFP-IRF3) delivered via a platform for DNA delivery were evaluated. This platform is based on the use of generation 5 polyamidoamine (G5-PAMAM) dendrimers conjugated with HIV transactivator of transcription (TAT) as a cell-penetrating peptide. The expressions of H5 and IRF3 genes based on *in vitro* transfection study and the effect of TAT peptide on expressions of H5 gene *in vitro* transdermal transfection were evaluated as well as the immunogenicity of recombinant DNA plasmid delivered by different delivery systems based on *in vivo* animal study.

The DNA plasmid constructs encoding H5, GFP and IRF3 genes were characterized using restriction enzyme analysis and sequencing prior to *in vitro* study. Effective conjugation of the TAT to the dendrimer was indicated by 1H-NMR and UV-visible spectroscopy. The interaction between PAMAM or TAT-PAMAM and the recombinant DNA plasmid was analyzed through the agarose gel retardation, DNase I protection assays and size, and zeta-potential measurements where all the analysis showed the designed platform able to form a compact and nanometre-sized polyplexes with DNA. *In vitro* study was performed by transient transfection of the constructed plasmids in Vero cell using PAMAM dendrimer followed by qualitative and quantitative analysis of the genes expression. The inserted genes in the DNA plasmid constructs were verified where significant expression of GFP, successfully transcriptional expression of the H5

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and IRF3 genes and the detected expression of H5 and IRF3 proteins were observed by immunofluorescence assay, RT-PCR and Western blotting, respectively. In addition, the ability of PAMAM dendrimer to enhance the delivery of the DNA plasmid constructs was revealed. In order to examine and compare the effects of TAT conjugation structures on the efficacy of PAMAM dendrimers for gene delivery systems, transfection of recombinant plasmid was performed through artificial membrane (Pion PAMPA) followed by quantitative analysis of the genes expression.

In vivo study was aimed to evaluate the immunogenicity of recombinant plasmid DNA, pBud-H5-GFP-IRF3 either using different delivery systems, including PAMAM dendrimer and TAT conjugated PAMAM dendrimer or the effect of the IRF3 as the genetic adjuvants in BALB/c mice. Mice were vaccinated intradermal and transdermal with naked DNA, PAMAM/H5-GFP, TAT-PAMAM/H5-GFP and TAT-PAMAM/H5-GFP-IRF3. The expression analysis of H5 gene from the blood by using qRT-PCR confirmed the ability of PAMAM dendrimer as carrier for gene delivery as well as the ability of TAT peptide to enhance the delivery efficiency of PAMAM dendrimer. Serum samples collected from the immunized group with TAT conjugated PAMAM dendrimer/H5-GFP pDNA elicited higher hemagglutination-inhibition (HI) titers compared to PAMAM dendrimer/H5-GFP pDNA exhibited the efficient gene delivery system when the PAMAM was modified with TAT peptide. The highest HI titer was achieved in mice vaccinated with TAT conjugated PAMAM dendrimer/ H5-GFP-IRF3 pDNA revealed the effect of IRF3 as genetic adjuvant on humoral immunity induction. TAT-PAMAM/H5-GFP-IRF3 also elicited CD3+/CD4+ and CD3+/CD8+ T cells in vaccinated mice. The modification of PAMAM dendrimer with TAT peptide resulted in <2-fold increases in the number of CD8+ T lymphocytes. The effects of TAT peptide for CD4$^+$ T cells were not as significant as those for CD8$^+$ T cells. Simultaneously study of the cytokines secreted including pro-inflammatory cytokines (IL-1β, TNF-α, IL-6), Th1 (IFN-γ, IL-2, IL-15, IL-12) and Th2 (IL-4, IL-10) was performed using multiplexed bead-based immunoassays. The highest level of the pro-inflammatory cytokines and Th1 cytokines including TNF-α, IL-6, IFN-γ and IL-12 were observed when PAMAM dendrimer was conjugated with TAT peptide. Additionally, co-administration of IRF3 as a genetic adjuvant showed the significant results in the expression levels of both pro-inflammatory and Th1 cytokines. Hence, administrations of the TAT conjugated PAMAM dendrimer recombinant plasmid H5-GFP-IRF3 (pBud-H5-GFP-IRF3) could induce strong antibody and both CD4+ and CD8+ T cell responses as well as enhanced cytokine productions against AIV in mice. This study provides valuable information for further study to determine the efficacy of the developed DNA vaccine to induce protection against challenge with virulent H5N1 virus.

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

PEMBANGUNAN GERAK BALAS IMUN MURIN TERHADAP VAKSIN H5 DNA DENGAN MENGGUNAKAN IRF3 SEBAGAI ADJUVAN GENETIK DAN PAMAM DENDRIMER BERKONJUGAT HIV TAT SEBAGAI SISTEM PENYAMPAIAN

Oleh

AZADEH BAHADORAN

Februari 2016

Pengerusi: Abdul Rahman bin Omar, DVM, PhD
Fakulti: Institute Biosains

Virus influenza A merupakan patogen utama yang mengancam secara berterusan pelbagai spesis ternakan seperti ayam, babi dan mamalia termasuk kesihatan manusia terutamanya melalui keupayaannya yang boleh menyebabkan kesesakan pada pernafasan dan kematian. Dengan kemajuan dalam teknologi vaksin moden, beberapa jenis vaksin kini boleh didapati untuk menghalang virus H5N1 Influenza A. Vaksin DNA mempunyai kepelbagaian ciri, di mana kelebihannya lebih nyata dibandingkan dengan teknologi vaksin yang lain dari segi keselamatan, kestabilan, kemudahan fabrikasi dan juga keimunogenan. Walaupun vaksin DNA menawarkan pelbagai kelebihan, namun masih ada yang perlu ditambah baik dalam penghantaran vaksin dan juga meningkatkan perungkapan imunogenik antigen daripada plasmid. Penghantaran selular DNA plasmid yang tidak terancang dan rangsangan sistem imun semula jadi yang tidak cukup dianggap sebagai penyebab utama kegagalan vaksin DNA untuk mendorong tindak balas imun yang kuat. Dalam kajian ini, tindak balas imun vaksin rekombinan baru pengabungan pengekodan H5 virus selesema burung (AIV), protein fluoresen hijau (GFP) dan gen *Mus musculus* interferon peraturan faktor 3 (pBud-H5-GFP-IRF3) dihantar melalui platform untuk penghantaran DNA telah dinilai. Platform ini adalah berdasarkan kepada penggunaan generasi 5 polyaminoamine (G5-PAMAM) dendrimer yang dikonjugat dengan pengaktifan trans transkripsi HIV (TAT) sebagai sel penembusan peptida. Perungkapan gen H5 dan IRF3 berdasarkan kajian transfeksi *in vitro* dan kesan peptida TAT ke atas perungkapan H5 dalam transfeksi transdermal *in vitro* telah dinilai dan juga keimunogenan pengabungan DNA plasmid telah dihantar melalui pelbagai sistem penghantaran berdasarkan kajian *in vivo* haiwan.

Pembinaan konstruk DNA plasmid H5, GFP dan IRF3 telah dicirikan menggunakan analisis sekatan enzim, PCR dan penjujuran sebelum kajian *in vitro.* Keberkesanan kekonjugatan TAT dengan dendrimer telah ditunjukkan oleh 1H-NMR dan spektroskopi jelas UV. Interaksi antara PAMAM atau TAT-PAMAM dan pengabungan DNA plasmid rekombinan dianalisis melalui perencatan gel agarose, asai perlindungan DNase I dan size serta pengukuran potensi zeta dimana semua analisis menunjukkan platform yang direka dapat membentuk polypleks bersaiz padat dan berskala nanometer dengan DNA. Kajian *in vitro* telah dilakukan menggunakan transfeksi sementara konstruk plasmid dalam sel Vero menggunakan dendrimer PAMAM kemudian diikuti dengan analisis kualitatif dan kuantitatif ungkapan gen. Gen dimasukkan ke dalam konstruk plasmid

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DNA telah mengesahkan pengungkapan GFP, seterusnya telah berjaya dalam pengungkapan transkripsi gen IRF3 dan H5 serta pengesanan pada pengungkapan protein IRF3 dan H5 telah dilihat melalui assai imunofluoresen, RT-PCR dan sap Western. Disamping itu, keupayaan dendrimer PAMAM untuk meningkatkan penghantaran konstruk plasmid DNA telah ditunjukkan. Berikutan itu juga, perbandingan dan pemeriksaan terhadap kesan kekonjugatan struktur TAT pada keberkesanan dendrimer PAMAM untuk sistem penghantaran gen, transfeksi plasmid rekombinan telah dilakukan melalui membran tiruan (Pion PAMPA) diikuti oleh analisis kuantitatif pengungkapan gen.

Kajian *in vivo* bertujuan untuk menilai keimunogenan plasmid DNA, pBud-H5-GFP-IRF3 samada dengan menggunakan sistem penghantaran yang berbeza, termasuk dendrimer PAMAM dan TAT dikonjugat dengan dendrimer PAMAM atau kesan IRF3 sebagai perangsang genetik pada tikus baka BALB/c. Tikus-tikus telah divaksin melalui intradermal dan transdermal dengan menggunakan DNA terdedah, PAMAM/H5-GFP, TAT-PAMAM/H5-GFP-IRF3. Hasil analisis ungkapaan gen H5 daripada darah dengan menggunakan qRT-PCR menunjukkan keupayaan dendrimer PAMAM untuk penghantaran gen serta keupayaan peptida TAT untuk meningkatkan kecekapan dalam penghantaran dendrimer PAMAM. Pengumpulan sampel serum daripada kumpulan imunisasi dengan pDNA H5-GFP/dendrimer PAMAM dikonjugasi TAT telah menaruh titrat rencatan hemaglutinasi (HI) lebih tinggi berbanding dengan pDNA H5-GFP/dendrimer PAMAM, menunjukan keberkesanan sistem penghanataran dendrimer PAMAM dikonjugasi peptida TAT. Titrat HI tertinggi yang dicapai di dalam tikus yang divaksin dengan dendrimer PAMAM TAT/-H5-GFP-IRF3 pDNA telah mendedahkan kesan IRF3 sebagai perangsang genetik pada induksi keimunan humoral. TAT-PAMAM/H5-GFP-IRF3 juga telah menaruh kedua-dua sel T CD3+/CD8+ dan CD3+/CD4+ di dalam tikus yang telah divaksin. Pengubahsuaian pada dendrimer PAMAM dengan peptida TAT telah menghasilkan peningkatan >2 kali ganda dalam bilangan limfosit T CD8+. Kesan peptida TAT untuk sel T CD4+ tidak begitu ketara sebagaimana untuk sel T CD8+. Pada kajian yang sama pada rembesan sitokin termasuk sitokin pro-radang seperti (IL-1β, TNF-α, IL-6), Th1 (IFN-γ, IL-2, IL-15, IL-12) dan Th2 (IL-4, IL-10) telah dilakukan berdasarkan imunoasai manik multipleks. Tahap tertinggi aras sitokin pro-radang dan sitokin Th1 termasuk TNF-α, IL-6, IFN-γ dan IL-12 dikesan apabila PAMAM telah dikonjugat dengan peptida TAT. Tambahan itu, bersama-sama IRF3 yang bertindak balas sebagai perangsang genetik telah menujukkan keputusan signifikan pada paras sitokin Th1 dan pro-radang. Oleh itu, hasil penggunaan TAT dikonjugat dengan dendrimer PAMAM plasmid kekombinan H5-GFP-IRF3 (pBud-H5-GFP-IRF3) boleh menaruh tindak balas antibodi dan sel T CD4+ dan CD8+ yang kuat serta peningkatan pada penghasilan sitokin ke atas AIV pada tikus. Kajian ini telah menghasilkan maklumat yang bernilai bagi tujuan kajian lanjutan dalam menentukan keberkesanan vaksin DNA yang telah dibangun dalam mengaruh perlindungan terhadap cabaran virus H5N1 virulen.

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I certify that a Thesis Examination Committee has met on 17 February to conduct the final examination of Azadeh Bahadoran on his thesis entitled "Development of Murine Immune Responses to H5 DNA Vaccine With IRF3 as Genetic Adjuvant and HIV TAT-Conjugated PAMAM Dendrimer as the Delivery System" 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.

Members of the Thesis Examination Committee were as follows:

Md Zuki Bin Abu Bakar @ Zakaria, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Rasedee @ Mat bin Abdullah, PhD
Professor
Faculty of veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Noorjahan Banu Binti Mohammed Alitheen, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciencees
Universiti Putra Malaysia
(Internal Examiner)

Abdelwahid saeed Ali Babiker, PhD
Professor
King Khalid University
Saudi Arabia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 21 April 2016

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Abdul Rahman Bin Omar, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Mohd Hair bin Bejo, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Mohd Zobir bin Hussein, PhD
Professor
Institute of Advanced Technology
Universiti Putra Malaysia
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BUJANG KIM HUAT, PhD
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LIST OF ABBREVIATIONS

ADCC	antibody-dependent cell cytotoxicity
AI	Avian influenza
AIV	avian influenza virus
ASC	apoptosis associated speck-like protein
APC	antigen presenting cells
BBB	blood–brain barrier
BCIP	bromochloroindolyl phosphate
ca	cold-adapted
CCL2	Chemokine (C-C) ligand
CPP	Cell penetrating peptide
cRNA	complementary RNA
CTL	cytotoxic T cells
DC	Dendritic cells
DEAE-dextran	diethylaminoethyl-dextran
DMSO	dimethylsulphoxide
ECMV	encephalomyocarditis virus
ELISA	enzyme-linked immunosorbent assay
EP	electroporation
ER	endoplasmic reticulum
G5-PAMAM	generation 5 polyamidoamine
Gal	galactose
GFP	green fluorescent protein
GIT	Gastrointestinal

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HA	hemagglutinin
HI	hemagglutination-inhibition
HPAI	highly pathogenic Avian influenza
HGH	human growth hormone
HNMR	proton nuclear magnetic resonance
hr	host range
HSP	Heat shock proteins
IC_{50}	50% inhibitory concentration
ID	intradermal
IFN	interferon
IRES	internal ribosome entry site
IRF	interferon regulatory factor
IM	intramuscular
LB	Luria-Bertani
LN	liposomal nanoparticle
LPAI	low pathogenic Avian influenza
M	Matrix
MFI	Median Fluorescent Intensity
MHC	histocompatibility complexe
NA	neuraminidase
NBT	nitro blue tetrazolium
NCBI	National Center for Biotechnology Information
NDV	Newcastle disease virus
NF-κB	nuclear factor-κB
NGS	next generation sequencing

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NK	Natural killer
NLR	nucleotide binding oligomerization domain (NOD)-like receptors
NO	nitric oxide
NP	Nucleocapsid protein
NS	non-structural proteins
PB	Polymerase basic
PBS	phosphate-buffered sulfate
pDNA	DNA plasmid
PCR	polymerase chain reaction
PCS	photon correlation spectroscopy
PEG	polyethylene glycol
PEI	polyethyleneimine
PRR	pathogen-recognition receptor
PTD	protein transduction domain
qRT-PCR	quantitative real-time reverse transcriptase PCR
RIG-I	retinoic acid inducible gene I
RT-PCR	reverse transcription polymerase chain reaction
SA	sialyloligosaccharide
SPF	specific-pathogen-free
ssRNA	single-stranded RNA
TAT	transactivator of transcription
TCR	T cell receptor
Th	T helper
TLR7	Toll-like receptor 7
ts	temperature sensitive

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UPM	Universiti Putra Malaysia
VLP	virus-like particles
vRNA	Viral RNA

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CHAPTER 1

INTRODUCTION

Influenza viruses belong to the family Orthomyxoviridae of enveloped viruses and are an important cause of respiratory infections worldwide. Influenza type A viruses can infect humans, birds, pigs, horses and other mammals (Short *et al.*, 2012). Influenza type A viruses are divided into subtypes and named on the basis of two proteins on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). Avian influenza (AI) A virus strains are further classified as low pathogenic (LPAI) or highly pathogenic (HPAI) based on specific molecular genetics and pathogenicity. Most avian influenza A viruses are LPAI viruses that are usually associated with mild disease in poultry. In contrast, HPAI viruses can cause severe illness and high mortality in poultry. Avian influenza A viruses of the subtypes H5 and H7, including H5N1, H7N7, and H7N3 viruses have been associated with HPAI, and human infection with these viruses can cause mild (H7N3, H7N7) to severe and fatal disease (H5N1). Human illness due to infection with LPAI viruses has been documented, including very mild symptoms such as conjunctivitis to influenza-like illness. Examples of LPAI viruses that have infected humans include H7N7, H9N2, and H7N2 (OIE, 2008).

When an outbreak of avian influenza occurs in an area with a high population density, vaccination is one of the most effective and cost-benefit interventions to prevent mortality and reduce morbidity from infectious pathogens (Lee *et al.*, 2014). The expected results of the implementation of a vaccination policy on the dynamics of infection are primarily those of reducing the susceptibility to infection and reducing the amount of virus shed into the environment (OIE, 2008). Many different types of experimental AI vaccines have been described, and some have been licensed for commercial use. Categories of vaccines include the following: inactivated whole virus vaccine, live attenuated vaccine (Yazdanbakhsh & Kremsner, 2009), recombinant subunit vaccines and reverse genetic vaccine (Sedova *et al.*, 2012). While many of these vaccines have been shown to induce protective immunity in the laboratory under optimal conditions, the final proof of protection and efficacy is still derived from field studies. For field use, the overwhelming majority of AI vaccines produced and sold have been oil–emulsion-inactivated whole AI virus vaccines delivered via the parenteral route (subcutaneous or intramuscular) and, less frequently, recombinant vectored vaccines. With the difficulties in controlling the current influenza virus epizootic using existing strategies, including vaccination, there is a clear need to examine alternative vaccine strategies (Luke & Subbarao, 2006).

Over the past few years, numerous studies have shown that DNA vaccines are a simple method to induce humoral and cellular immune responses as well as protection from challenge in animal models. DNA vaccines are considered inexpensive, extremely stable and considerably safer than attenuated viral vaccines (Minigo *et al.*, 2007). Although some experiments showed that DNA vaccines can generate immune responses, but they highlight some needs for enhanced potency if these approaches are to be beneficial. Some reasons have been assumed cause a low potency of DNA-based vaccines include low level of antigen, ineffective DNA plasmid delivery and ineffective innate immunity stimulus (Bolhassani *et al.*, 2011). To address the low immunogenicity of DNA vaccine, different DNA vaccine delivery approaches are currently available that enhance the potency of DNA vaccine by physical delivery, viral and non-viral based delivery methods.

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Several physical methods of delivery have been explored to increase the transfection efficiency of DNA vaccines, including tattooing, gene gun, electroporation (EP), ultrasound and laser (Bolhassani *et al.*, 2011). Viral vectors are recombinant viruses with target genes and a combination of regulatory elements incorporated into their genomes. Viral vectors hold a special position among the existing antigen delivery systems due to the fact that they possess the following properties: a natural mechanism of cellular interaction and penetration delivery of foreign genetic material to the nucleus; providing long-term antigen expression; capsid protecting the antigen-encoding genetic material. Poxviruses, Newcastle disease virus and adenoviruses are those most frequently used today to design viral vector-based influenza vaccines. There are some limitations associated with viral vectors because of their limited capacity for DNA carrying, immunogenicity, toxicity, vector DNA integration into host genome and finally high cost (Bolhassani *et al.*, 2011).

Non-viral vectors provide several advantages as they are safe, simple and easy to manufacture and flexible in the delivery size of transgenes of various sizes. Also, they are able to prevent the DNA degradation and make possible target deliveries to antigen presenting cells (Mills, 2009). Non-viral vectors are classified as follows: lipid-based vectors, polysaccharides, cationic polymers and cell-penetrating peptides (Bolhassani *et al.*, 2011). Cationic polymers like poly-lysine, diethylaminoethyl-dextran, dextranspermine polycations, polyethyleneimine, dendrimers, lipopolyamines and chitosan with special properties to control the delivery of DNA, peptides, proteins and drugs has been recently developed (Masotti & Ortaggi, 2009).

Dendrimers as monodisperse three-dimensional and hyperbranched molecules have recently received extensive interest as non-viral vectors for gene delivery because they are capable of forming compact complexes with genetic materials and protecting them from degradation (Wang *et al.*, 2014). Among different types of dendrimers, PAMAM dendrimers promise high ability for efficient transfection of DNA into the different types of cell *in vitro* and the inherent ability to transfect the genes *in vivo* (Eichman et al., 2000).

Cell penetrating peptides (CPP) or protein transduction domain is defined as short sequences of amino acid residues which are able easily to cross the plasma membrane. The potency of CPPs in delivering therapeutic agents into cellular compartments makes them as an effective tool in vaccine design. Various compounds such as drugs, DNA, RNA, peptides, bacteriophages and fluorescent dyes have been conjugated to CPPs for intracellular delivery. The main application of CPP validated for vaccine studies is the delivery of tumor-associated antigen into APCs and as a non-viral delivery system for DNA vaccines (Brooks et al., 2010).

Although PAMAM dendrimers have been evaluated as a vaccine platform and their positive charge allows formation of complex with DNA at physiologic pH, protecting the DNA from nuclease and increasing the transfection efficiency, they showed only modest success *in vivo*. Several modifications have been applied to enhance the efficiency of PAMAM dendrimers such as partial PEGlation, acetylation, alkylation and peptide conjugation (Wang *et al.*, 2014).
In order to enhance immunogenicity, DNA vaccines are designed along with different adjuvants (Gerhard *et al.*, 2006). Adjuvants can be classified as genetic or conventional adjuvants (Sasaki *et al.*, 2003). Genetic adjuvants are expression vectors of cytokines,

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which are able to modulate immune responses when incorporated to a vaccine model. Some cytokines with immune modulatory effects that increases DNA vaccine potency are IL-1, IL-12, IFN-α, IFN-β, IL-6 and IL-8 and IRFs (IRF1, IRF2 and IFR3) (Okuda *et al.*, 2014; Sasaki *et al.*, 2003).

Interferon regulatory factor 3 (IRF3) is a transcription factor, together with IRF7 and nuclear factor-κB (NF-κB), can be activated by antiviral pattern recognition receptors. IRF3 activation is part of the first line of defence against invading viruses, and its activation results in the production of IFN-β. This in turn, induces an amplification loop of type I IFN, which leads to the development of an antiviral state. The importance of IRF3 in the development of antiviral immunity has been shown in IRF3-deficient animals, which are more susceptible to viral infection. In addition, IRF3/IRF7 double-knockouts do not produce IFN-γ in response to viruses and are severely impaired in their antiviral responses (Fitzgerald *et al.*, 2014).

The purpose of this study is to evaluate the immune responses to a new recombinant DNA encoding the H5 of avian influenza virus (AIV) fused to green fluorescent protein (GFP) and co-expressing *Mus musculus* interferon regulatory factor 3 (IRF3) genes on a dendrimer-based platform for DNA delivery. This platform is based on the use of generation 5 polyamidoamine (G5-PAMAM) dendrimers conjugated with HIV transactivator of transcription (TAT) as a cell penetrating peptide (CPP).

The first hypothesis of this study is that the conjugation between PAMAM dendrimer and TAT will improve the efficacy of H5 vaccines, and yield better expression levels of the encoded antigen. The second hypothesis of this study is that expressing H5- IRF3 DNA vaccine with CPP conjugated dendrimer platform can enhance the immune stimulatory potency, therefore enhancing the vaccine-induced immune response.

The objectives of this study were:

General objective

To evaluate the *in vitro* and *in vivo* transdermal transfection of the recombinant DNA plasmid encoding the H5 of avian influenza virus (AIV) and Mus musculus interferon regulatory factor 3 (IRF3) genes based on delivery system of the generation 5 polyamidoamine (G5-PAMAM) dendrimers conjugated with HIV transactivator of transcription (TAT).

Specific objectives

1) To construct a recombinant DNA plasmid encoding the H5 and IRF3 genes and its interaction between dendrimer /TAT conjugated dendrimer
2) To determine the expressions of H5 and IRF3 genes in an *in vitro* transfection study and the effect of TAT on expression of H5 gene in an *in vitro* transdermal transfection.
3) To determine immune responses in mice treated with recombinant H5 DNA vaccine

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