



Faculty of Isfahan medicine

A Thesis Submitted in Partial Fulfillment of the Requirement for the
Degree of Ph.D in Isfahan University of Medical Sciences

Title:

**Evaluation of the prophylaxis effects of Derp2 vaccine
with adjuvants (MPLA, CPG, BCG) on acute
atopic asthma in Balb/c mice**

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December 2016

Abstract

Background and Aims: The house dust mite (HDM) is the most inducer of allergies including asthma which consists of several components specially allergens Derp1 and Derp2. The impairment of T helper (Th1), Th2 and T regulatory (Treg) cells play key roles in asthma pathogenesis. Toll like receptors (TLR) agonists such as the monophosphoryl lipid A (MPLA), CpG oligodeoxynucleotides (CPG-ODN) and bacillus Calmette–Guérin (BCG) are the main adjuvants with their effects on the induction of T cells. The aim of the present study was to evaluate the CPG, MPLA and BCG effects on Th1- Th2 and Treg -related parameters on an asthmatic BALB/c mice model.

Materials and Methods: Five groups of BALB/c mice were immunized on days 1, 15, and 22 with Derp2. Three groups pre-treated on days 0, 14, and 21 with CPG, CPG+MPLA or CPG+BCG. Challenge performed with Derp2 on days 28-37. The saline- or Derp2-sensitized control group received saline or Derp2, respectively. Blood samples obtained on days 0, 23 and 40. The serum IL-4, IFN- γ , IgE and IgG2a levels were measured using ELISA. Blood Th1 and Treg cell frequency were detected using flow cytometry. Pathological studies were performed on lungs and bronchoalveolar lavage fluid (BALF) using standard methods.

Results: At the sensitization phase, the, IFN- γ and IgG2a levels, IFN- γ /IL4, IgG2a/IgE and Th1/Treg ratios in CPG plus MPLA group, Treg cell frequency in CPG or CPG plus MPLA groups, IFN- γ /IL4 ratio in groups of CPG, CPG plus MPLA or CPG plus BCG were significantly higher than Derp2-sensitized control mice ($P < 0.05$).

At the challenge phase, the IFN- γ /IL-4, Th1 cells frequency in groups CPG plus MPLA, CPG plus BCG or CPG, Treg cells frequency and Th1/Treg ratios in CPG plus BCG group, IFN- γ levels, IgG2a in CPG plus MPLA group and Th1/Treg ratio in CPG plus MPLA group were significantly higher than Derp2-sensitized control mice. The Th1/Treg ratio in CPG plus MPLA group was also higher than CPG plus BCG group. The Th1/Treg ratio in CPG plus BCG group was lower than CPG group. The lung inflammatory scores in groups of CPG, BCG and CPG plus MPLA were lower than Derp2-control group ($P < 0.05$). The BALF levels of eosinophils and total cells in Derp2-sensitized group pre-treated with CPG, CPG plus MPLA and CPG plus BCG, were significantly lower than in Derp2-sensitized control group ($P \leq 0.001$).

Conclusion: Derp2 vaccine plus BCG, MPLA and CPG modulate Th1- Th2- and Treg-related parameters, improve their imbalances and ameliorate inflammation in a mouse asthma model.

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List of abbreviations

Ab: Antibody
AHR: Airway hyperresponsiveness
ANOVA: Analysis of variance
APCs: Antigen presenting cells
ASM: Airway smooth muscle
BAL: Bronchoalveolar lavage
BCG: Bacillus Calmette-Guerin
CCR: CC chemokine receptor
CD: Cluster of differentiation
CD40L: CD40 ligand
CFU: Colony forming units
CPG-ODN: CPG-oligodeoxynucleotide
Ctrl: Control
Derp2: Dermatophagoides pteronyssinus2
DC: Dendritic cells
DNA: Deoxyribonucleic acid
dsRNA: Double-stranded RNA
ELISA: Enzyme linked Immunosorbent Assay
EDTA: Ethylenediaminetetraacetic acid
EoPs: Eosinophil lineage-committed progenitors
FACS: Fluorescence-activated cell sorting
FCS: Fetal calf serum
FcεRI: Fc epsilon receptor I
FcγRIII: Fc gamma receptor III
FITC: Fluorescein isothiocyanate

GMP: Granulocyte/monocyte progenitor
HDM: House dust mite
H&E: Hematoxylin and eosin
Ig: Immunoglobulin
IFN: Interferon
IKK: I κ B kinase
IL: Interleukin
IL-10R: IL-10 receptor
IN: Intranasal
INF- γ : Gamma interferon
IP: Intraperitoneal
IRFs: Interferon regulatory factors
ISAAC: International study of asthma and allergies in childhood
IV: Intravenous
IRAKs: IL-R1 associated kinases
LPS: lipopolysachardie
LRR: leucine-rich repeat
MAP: mitogen-activated protein
MPLA: Monophosphoryl Lipid A
mAb: Monoclonal antibody
MHC: Major histocompatibility complex
MPLA: Monophosphoryl Lipid A
MyD88: Myeloid differentiation factor-88
NF- κ B: Nuclear factor kappa B
NK: Natural killer
OVA: Ovalbumin
PAMP: Pathogen-associated molecular pattern

PAS: Periodic acid-Shiff reagent
PBS: Phosphate-buffered saline
PPD: Purified protein derivative from *M. tuberculosis*
RNA: Ribonucleic acid
SC: Subcutaneous
SIT: Specific immunotherapy
ss DNA: Single-stranded DNA
STAT-6: Signal transducer and activator of transcription 6
TCR: T-cell receptor
TGF: Tumor growth factor
Th: T helper
TIR: Toll/IL-1
TLR: Toll-like receptor
TNF: Tumor necrosis factor
TRAF6: tumor necrosis factor receptor associated factor 6
Tr cell: T regulatory cell
Treg: T regulatory
UK: United Kingdom
US: United States of America

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ISI Papers resulting from thesis:

2.1. Main aim

Evaluation of the prophylaxis effects of the Der P2 Vaccine (HDM antigen) with adjuvants (MPLA, CPG, BCG) on acute atopic asthma in the Balb/c mice

2.2. Detailed hypothesis

2.2.1. The level of serum IgE is different between studied groups in the sensitization and challenge phases

2.2.2. The level of serum IgG2a is different between studied groups in the sensitization and challenge phases

2.2.3. The level of serum IL4 is different between studied groups in the sensitization and challenge phases

2.2.4. The level of serum IFN γ is different between studied groups in the sensitization and challenge phases

2.2.5. The level of blood TH1 is different between studied groups in the sensitization and challenge phases

2.2.6. The level of blood Treg is different between studied groups in the challenge phase

2.2.7. The level of BAL fluid neutrophil cells is different between studied groups in the challenge phase

2.2.8. The level of BAL fluid macrophage cells is different between studied groups in the challenge phase

2.2.9. The level of BAL fluid eosinophil cells is different between studied groups in the challenge phase

2.2.10. The level of inflammatory scores is different between studied groups in the challenge phase

2.2.11. The level of mucus secretion scores is different between studied groups in the challenge phase

2.3. The research question

2.3.1. How much is the level of serum IgE in the sensitization and challenge phases in studied groups?

2.3.2. How much is the level of serum IgG2a in the sensitization and challenge phases in studied groups?

2.3.3. How much is the level of serum IL-4 in the sensitization and challenge phases in studied groups?

2.3.4 .How much is the level of serum IFN γ in the sensitization and challenge phases in studied groups?

2.3.5. How much is the level of bloodTh1 cells in the sensitization and challenge phases in studied groups?

2.3.6 How much is the level of blood Treg cells in the sensitization and challenge phases in studied groups?

2.3.7. How much is the level of BAL fluid neutrophil cells in the challenge phase in studied groups?

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2.3.10. How much is the level of inflammatory scores in the challenge phase in studied groups?

2.3.11. How much is the level of mucus secretion scores in the challenge phase in studied groups?

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