

Role of Vitamin D in the Induction of Regulatory T Cells Producing Interleukin 10 in Children with Cow Milk Allergy

Thesis

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By

Sara Mohamed Atef Mustafa

M.B., B.Ch.

*Master of Clinical and Chemical Pathology
Faculty of Medicine-Ain Shams University*

Supervised by

Professor/ Randa Reda Mabrouk

*Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University*

Professor/ Hanaa Ahmed Amer

*Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University*

Doctor/ Dina Ahmed Soliman

*Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University*

Doctor/ Nesrine Aly Mohamed

*Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University*

Doctor/ Dalia Helmy El-Ghoneimy

*Assistant Professor of Pediatric
Faculty of Medicine – Ain Shams University*

**Faculty of Medicine
Ain Shams University**

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List of Abbreviations

A	: Allergen
APCs	: Antigen Presenting Cells
BA	: Bronchial Asthma
BALF	: Bronchial Alveolar Lavage Fluid
BL	: Base Line
CAST	: Cellular Antigen Stimulation Test
CM	: Cow Milk
CMA	: Cow Milk Allergy
CNS	: Conserved Non-coding Sequence
CRD	: Component Resolved Diagnosis
CTLA-4	: Cytotoxic T Lymphocyte Antigen-4
CYP	: Cytochrome P
DAG	: Di-Acyl Glycrol
DBPCFC	: Double Blinded Placebo Controlled FoodChallenge
DCs	: Dendritic Cells
DN	: Double Negative
DP	: Double Positive
ELISA	: Enzyme Linked Immunosorbent Assay
FCM	: Flow Cytometry
FcεRI	: Fc Epsilon Receptor I
FITC	: Fluorescein Isothiocyanate
Foxp3	: Forkhead Box P3
GALT	: Gut Associated Lymphoid Tissue
ICOS	: Inducible Costimulatory
IELs	: Intra-Epithelial Lymphocytes
IFN-γ	: Interferon Gamma
Ig	: Immunoglobulin
Ig-FLCs	: Immunoglobulin Free Light Chains
IL	: Interleukin
IP3	: Inositol tri-phosphate
ITAMs	: Immune Tyrosine-based Activation Motifs
iTreg	: Induced Regulatory T Cells
IU	: International Unit

List of Abbreviations (Cont.)

Jak	: Janus Kinase
KDOQI	: Kidney Disease Outcomes Quality Initiative
K-EDTA	: K-Ethylene diamine tetra-acetic acid
LAG-3	: Lymphocyte Activation Gene-3
LAT	: Linker of Activated T Cell
LPLs	: Lamina Propria Lymphocytes
MHC	: Major Histocompatibility Complex
MoAb	: Monoclonal Antibody
NFAT	: Nuclear factor of activated T-cells
NF- κ B	: Nuclear Factor Kappa B
nTreg	: Natural Regulatory T Cell
OFCs	: Oral Food Challenges
PAF	: Platelet Activating Factor
PBMCs	: Peripheral Blood Mononuclear Cells
PCR	: Polymerase chain Reaction
PD-1	: Prgrammed Cell Death
PE-Cy5	: Phyco-erythrin Cyanine-5
PIP2	: Phosphatidyl Inositol bi-Phosphate
PLC γ	: Phospholipase C Gamma
PTH	: Parathyroid Hormone
pTreg	: Peripheral Regulatory T Cells
RPMI	: Roswell Park Memorial Institute medium
SHP-1	: Src-homology tyrosine Phosphatase
SIT	: Specific Immune Therapy
sjTRECs	: Single Joint TCR Excision Circle
SOCS	: Suppressor of cytokine signaling
SA-HRP	: Streptavidin-Horse reddish peroxidase
STAT	: Signal transducer and activator of transcription
TCR	: T Cell Receptor
Teff	: T Effector
Tfh	: Follicular Helper T
TGF- β	: Transforming Growth Factor β
Th	: T Helper

List of Abbreviations (Cont.)

TNF	: Tumor Necrosis Factor
Tr-1	: Regulatory T Cell-1
Treg	: Regulatory T cells
TSDR	: Treg-Specific Demethylated Region
tTreg	: Thymus derived regulatory T Cells
TyK	: Tyrosine Kinase
VD	: Vitamin D
VDBP	: Vitamin D Binding Protein
VDR	: Vitamin D Receptor
VDREs	: Vitamin D Response Elements

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Abstract

Role of Vitamin D in the Induction of Regulatory T Cells Producing Interleukin 10 in Children With Cow Milk Allergy

Randa Reda Mabrouk¹, Hanaa Ahmed Amer¹, Dina Ahmed Soliman², Nesrine Aly Mohamed⁴, Dalia Helmy El-Ghoneimy³, sara mohamed Atef

¹Chemical Pathology, ³ Clinical and Chemical Pathology, ³Pediatric, Faculty of Medicine – Ain Shams University

Corresponding author: Sara Mohamed Atef mostafa
e-mail: drsara15181@yahoo.com.

Background: Various populations of regulatory T cells play a central role in the development of peripheral tolerance to allergens. Culturing of CD4⁺ T cells isolated from peripheral blood of allergic patients with vitamin D induces the generation of stable IL-10 producing CD4⁺CD25⁺ Treg cells suppressing the proliferation of T helper cells obtained from the same patients. The immune regulatory role of vitamin D in allergic patients has been controversial and obviously needs a more clarifying research work.

Aim of the work: to determine the percentage of induced T regulatory cells producing interleukin 10 after stimulation of T regulatory cells with cow milk allergen in the presence of vitamin D in culture. This aims to further in-vitro study the immune regulatory role of vitamin D in cow milk allergic patients.

Results: there is association between decreased level of vitamin D and milk-allergy, as serum level of 25(OH) D3 was insufficient in 16 (80 %) patients (10- 29.9 ng/ml) while 4 (20%) patients were sufficient (30-100 ng/ml).

Addition of calcitriol, in culture, induces the production of CD4⁺ CD25^{hi} Foxp3⁺ IL10⁺

Treg cells within PMNCs isolated from allergic children who had insufficient vitamin D, but not in allergic children who had normal level of vitamin D.

Conclusion: this work provides further evidence for an important role of 1,25(OH)2D3 as an immune-modulatory molecule and suggests that supplementation of vitamin-D-deficient individuals, who are reported to have reduced numbers of circulating and Foxp3⁺ IL10⁺ Treg cells, may represent an attractive therapy for enhancing endogenous populations of Treg cells in allergy.

Key words: Regulatory T cells, calcitriol, food allergy.

Introduction

Various populations of regulatory T cells play a central role in the development of peripheral tolerance to allergens and also in successful clinical improvement in allergen-specific immunotherapy which represents the single curative treatment in allergic diseases (*Akkoc et al., 2011*).

Regulatory T cells are classified to natural T regulatory cells and induced T regulatory cells. The natural T regulatory cells (nTreg) are self antigen specific CD4⁺T cells that express CD25⁺ in high levels and forkhead box protein (Fox) that are selected in the thymus, and become regulatory T cells in the periphery. The induced-T reg cells (iTreg) are converted from naïve T cells after encountering specific antigen in the periphery and are characterized by elevated production of interleukin-10 (*Jutel and Akdis, 2011*).

Regulatory T cells inhibit the activation of the T-helper2 cells (Th2), mast cells, basophils and eosinophils thus minimizing the production of interleukin-4 (IL-4) and IL-5 which are essential cytokines during the allergic reactions. In addition, Treg also act on B lymphocytes to suppress the production of allergen-specific immunoglobulin-E (*Maggi, 2010*).

Cow milk allergy is believed to be either IgE-mediated in which activation of Th cells leads to the production of milk-specific IgE, or non IgE-mediated that may include T cell/mast cell interaction with secretion of

inflammatory cytokines including IL-4 and IL-5. Decreased Treg activity has been identified as a factor in both allergy mechanisms (*Fiocchi et al., 2010*).

Shreffler and colleagues (2009) showed that children, who were allergic to cow milk and became tolerated, had high percentage of milk-specific CD4⁺CD25⁺ Treg cells in their peripheral blood with high in-vitro proliferation activity when stimulated with cow milk protein.

Xystrakis et al. (2006) previously reported that culturing of CD4⁺T cells isolated from peripheral blood of atopic patients with vitamin D induced the generation of stable IL-10 producing CD4⁺CD25⁺ Treg cells suppressing the proliferation of T helper cells obtained from the same patients. They confirmed the immune regulatory role of vitamin D in atopic patients.

However the role of vitamin D in allergy has been controversial and obviously needs a more clarifying research work (*Searing and Leung, 2010*).

Aim of the work

The aim of this work is to determine the percentage of induced T regulatory cells producing interleukin 10 after stimulation of T regulatory cells with cow milk allergen in the presence of vitamin D in culture. This aims to further in-vitro study the immune regulatory role of vitamin D in cow milk allergic patients.