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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Sara Mohamed Atef Mustafa

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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-κB	:	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
PLCγ	:	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

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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 andare characterized by elevated production of interlukin-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 interlukin-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 Tregactivity 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.