
**IMMUNOREGULATORY EFFECTS
OF VITAMIN D₃ ON MAST CELLS
DURING IMMUNOGLOBULIN E-
DEPENDENT IMMUNE RESPONSES**

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Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to me (Chunping Yu) and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying according to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the digital research repository of the University of Adelaide, the Library catalogue, the Australasian Digital Theses Program and also through web search engines, unless permission has been granted by the University to restrict access for any unforeseen reasons. Finally, I acknowledge that the copyright of the published works listed below resides with the copyright holder(s) of those works.

List of publications

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Dr. Michele Grimaldeston

Abbreviations

$1\alpha,25(\text{OH})_2\text{D}_3$: $1\alpha,25$ -dihydroxyvitamin D_3

25OHD_3 : 25-hydroxyvitamin D_3

6C: 6-*s-cis*

6T: 6-*s-trans*

Ab: antibody

ACD: anticoagulant citrate dextrose

Ag: antigen

APS: ammonium persulphate

ASM: airway smooth muscle

A1AT: α_1 -antitrypsin

BM: bone marrow

BSA: bovine serum albumin

BTK: Bruton's tyrosine kinase

cAMP: cyclic AMP

CCL: CC-chemokine ligand

CHS: contact hypersensitivity

COPD: chronic obstructive pulmonary disease

CTMC: connective tissue mast cell

CYP24A1: 25-hydroxyvitamin D_3 -24-hydroxylase

CYP27B1: 25-hydroxyvitamin D - 1α -hydroxylase enzyme

DAG: Diacylglycerol

DBP: Vit D_3 -binding protein

DC: dendritic cell

DMEM: Dulbecco's modified eagle medium

DMSO: dimethyl sulphoxide

DNP: dinitrophenyl
DT: diphtheria toxin
ECL: enhanced chemiluminescence
EIA: enzyme immunoassay
ELISA: enzyme-linked immunosorbent assay
EMTU: epithelial-mesenchymal trophic unit
ERK: extracellular signal regulated kinase
FCS: fetal bovine serum
FcεRI: high affinity IgE receptor
FDNB: 1-fluoro-2,4,-dinitrobenzene
GAB: growth-factor-receptor-bound protein
GMP: granulocyte/macrophage progenitor
hCBMC: human cord-blood-derived MCs
HDC: histidine decarboxylase
HIV: human immunodeficiency virus
HMEM: Hank's MEM
HRP: horse radish peroxidase
HS: horse serum
HSA: human serum albumin
HSC: haematopoietic stem cell
IFN: interferon
Ig: immunoglobulin
IL: interleukin
IMDM: Iscove's modified Dulbecco's medium
InsP₃: inositol-1,4,5,-triphosphate
ITAM: immunoreceptor tyrosine-based activation motif
LN: lymph node

LT: leukotriene
MAPK: mitogen-activated protein kinase
mBMCMC: mouse bone marrow-derived cultured mast cell
MC: mast cell
MCP: mast cell progenitor
MC_T: tryptase positive mast cell
MC_{TC}: tryptase and chymase positive mast cell
MEK: MAPK kinase
miR: microRNA
MKP: mitogen-activated protein kinase phosphatase
MMC: mucosal mast cell
MMCP: mouse mast cell protease
MMP: matrix metalloproteinase
NEAA: non-essential amino acid
NF- κ B: nuclear factor- κ B
PBS: phosphate buffered saline
PCA: passive cutaneous anaphylaxis
PCR: polymerase chain reaction
Pen/Strep: Penicillin/Streptomycin
PG: prostaglandin
PI3K: phosphatidylinositol-3-OH kinase
PKB (AKT): protein kinase B
PKC: protein kinase C
PL: phospholipase
PLA₂: phospholipase A₂
PLC _{γ} : phospholipase C _{γ}
PMA: phorbol-12 myristate-13 acetate

PtdIns(3,4,5)P₃: phosphatidylinositol-3,4,5,-trisphosphate

PTH: parathyroid hormone

qRT-PCR: quantitative real-time PCR

RIA: radioimmunoassay

RT: room temperature

RT-PCR: real-time PCR

RXR: retinoid X receptor

sAg: specific antigen

SCF: stem cell factor

SDS-PAGE: sodium dodecyl sulfate - polyacrylamide gel electrophoresis

TCR: T cell receptor

TGF: transforming growth factor

Th: T helper

TLR: toll-like receptor

TNF: tumor necrosis factor

T_{reg}: T regulatory cell

TSLP: thymic stromal lymphopoietin

UV: ultraviolet

VDR: vitamin D receptor

VDRE: vitamin D response element

VDR_{mem}: plasma membrane-associated vitamin D receptor

VitD: vitamin D

WT: wild-type

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Thesis summary

Mast cells (MCs) can exert anti-inflammatory effects via production of interleukin (IL)-10 in a number of Immunoglobulin (Ig)E-independent immune responses. Recently, we reported that $1\alpha,25$ -dihydroxyvitamin D₃ ($1\alpha,25(\text{OH})_2\text{D}_3$), the biologically active form of vitamin D₃ (VitD₃), can induce IL-10 production from mouse bone marrow-derived cultured MCs (mBMCMCs). For the current project, we further investigated if the well-recognised pro-inflammatory properties of MCs in IgE-dependent immune settings can be reduced upon $1\alpha,25(\text{OH})_2\text{D}_3$ administration and, if so, which mechanisms are likely to be responsible. In the presence of $1\alpha,25(\text{OH})_2\text{D}_3$, IgE + specific antigen (sAg)-stimulated mBMCMCs exhibited reduced degranulation, as well as decreased production of the pro-inflammatory cytokines, TNF α and IL-6, in a vitamin D receptor (VDR)-dependent manner. Concomitantly, $1\alpha,25(\text{OH})_2\text{D}_3$ significantly up-regulated the production of IL-10. In addition, we demonstrated for the first time the expression of CYP27B1, the enzyme that generates $1\alpha,25(\text{OH})_2\text{D}_3$ from its inactive precursor 25-hydroxyvitamin D₃ (25OHD₃) in both mBMCMCs and human cord-blood-derived MCs (hCBMCs). This enables mBMCMCs to produce endogenous $1\alpha,25(\text{OH})_2\text{D}_3$ and thus granting 25OHD₃ similar VDR-dependent immunosuppressive effects to $1\alpha,25(\text{OH})_2\text{D}_3$ on activated MCs either directly or indirectly.

By employing a mouse IgE-mediated MC-dependent passive cutaneous anaphylaxis (PCA) model as well as four mouse groups with different cutaneous MC profiles in the ears, including wild-type (WT) C57BL/6 mice, MC-deficient C57BL/6-*Kit*^{W-sh/W-sh} mice and C57BL/6-*Kit*^{W-sh/W-sh} mice engrafted with either WT or VDR-deficient (*VDR*^{-/-})

mBMCMCs, we found that topical application of either $1\alpha,25(\text{OH})_2\text{D}_3$ or 25OHD_3 significantly curtailed the magnitude of PCA-associated ear swelling, potentially by reducing the extent of MC degranulation and/or the secretion of various MC-derived cytokines. Notably, these PCA-suppressive effects required the presence of dermal MCs and their expression of VDR.

Taken together, data presented in this thesis provide evidence that $1\alpha,25(\text{OH})_2\text{D}_3$ and 25OHD_3 , the latter likely via its conversion to the active metabolite by MCs, can suppress IgE + sAg-mediated MC activation in a VDR-dependent manner both *in vitro* and *in vivo*. This suggests the therapeutic potential for various VitD₃ analogues to treat MC-dependent IgE-associated allergic disorders.