IMMUNOREGULATORY EFFECTS OF VITAMIN D_3 ON MAST CELLS DURING IMMUNOGLOBULIN E-DEPENDENT IMMUNE RESPONSES

A thesis submitted in partial fulfilment of the PhD degree

in

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Faculty of Sciences
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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 unforseen 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

- Yu, C., Fedoric, B., Anderson, P.H., Lopez, A.F., and Grimbaldeston, M.A., 2011, Vitamin D_3 signalling to mast cells: a new regulatory axis. *The International Journal of Biochemistry & Cell Biology*, Vol. 43, p 41 – 46.
- Biggs, L., Yu, C., Fedoric, B., Lopez, A.F., Galli, S.J., and Grimbaldeston, M.A., 2010, Evidence that vitamin D_3 promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice. *J Exp Med*, Vol. 207, p 455- 463.

- Yip, K., Kolesnikoff, N., Yu, C., Hauschild, N., Taing, H., Biggs, L., Goltzman, D., Gegory, P., Anderson, P., Samuel, M., Galli, S., Lopez, A., and Grimbaldeston, M., 2014, Mechanisms of vitamin D_3 metabolite repression of IgE-dependent mast cell activation. *Journal of Allergy and Clinical Immunology*, published online.

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Chunping Yu Dr. Michele Grimbaldeston

Abbreviations

$1\alpha,25(OH)_2D_3$: 1α,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: $VitD_3$-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

$InsP_3$: 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-κB: nuclear factor-κB

PBS: phosphate buffered saline

PCA: passive cutaneous anaphylaxis

PCR: polymerase change reaction

Pen/Strep: Penicillin/Streptomycin

PG: prostaglandin

PI3K: phosphatidylinositol-3-OH kinase

PKB (AKT): protein kinase B

PKC: protein kinase C

PL: phospholipase

PLA_2: phospholipase A_2

PLC_γ: phospholipase C_γ

PMA: phorbol-12 myristate-13 acetate

PtdIns(3,4,5)P_3: 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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Finally, I want to dedicate this thesis to my husband, Yang Lu, and all my family overseas, as without your deep understanding and unconditional love and support, I would not be able to stay focused and complete my experiments in a three-year time frame.

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 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), the biologically active form of vitamin D_3 ($VitD_3$), 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 1α,25$(OH)_2D_3$ administration and, if so, which mechanisms are likely to be responsible. In the presence of 1α,25$(OH)_2D_3$, IgE + specific antigen (sAg)-stimulated mBMCMCs exhibited reduced degranulation, as well as decreased production of the pro-inflammatory cytokines, TNFα and IL-6, in a vitamin D receptor (VDR)-dependent manner. Concomitantly, 1α,25$(OH)_2D_3$ significantly up-regulated the production of IL-10. In addition, we demonstrated for the first time the expression of CYP27B1, the enzyme that generates 1α,25$(OH)_2D_3$ from its inactive precursor 25-hydroxyvitamin D_3 ($25OHD_3$) in both mBMCMCs and human cord-blood-derived MCs (hCBMCs). This enables mBMCMCs to produce endogenous 1α,25$(OH)_2D_3$ and thus granting $25OHD_3$ similar VDR-dependent immunosuppressive effects to 1α,25$(OH)_2D_3$ 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*$^{W\text{-}sh/W\text{-}sh}$ mice and C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice engrafted with either WT or VDR-deficient (*VDR*$^{-/-}$)

mBMCMCs, we found that topical application of either $1\alpha,25(OH)_2D_3$ 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 $1\alpha,25(OH)_2D_3$ 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 $VitD_3$ analogues to treat MC-dependent IgE-associated allergic disorders.