

**DIFFERENTIAL EXPRESSION OF THE
GMCSF GENE IN THE IMMUNE
SYSTEM IS REGULATED BY
EPIGENETIC FACTORS**

**A thesis submitted in fulfilment of the requirements of the
degree of Doctor of Philosophy**

by

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DECLARATIONS

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information and duly acknowledged in the thesis. To the best of my knowledge and belief this thesis contains no material previously published or written by another person except where due acknowledgment is made in the text.

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University. Animal ethics approval was granted by the University of Tasmania Animal Ethics Committee.

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At the time of writing, the following publication has arisen from data presented in this thesis:

K. H. Brettingham-Moore*, **O. R. Sprod***, X. Chen, P. Oakford, M. F. Shannon and A. F. Holloway (2008). Determinants of a transcriptionally competent environment at the GM-CSF promoter. *Nucleic Acids Res* 36(8): 2639-53. (*joint first author)

Data arising from this thesis has been presented at the following scientific meetings:

O.R. Sprod, K.H. Brettingham-Moore, M.F. Shannon and A.F. Holloway. “The role of epigenetic marks in regulating GM-CSF activation in immune cells”. ComBio 2006, Brisbane, Australia. Poster presentation.

O.R. Sprod, K.H. Brettingham-Moore, M.F. Shannon and A.F. Holloway. “Epigenetic factors lead to differential inducibility of GM-CSF in immune cells”. Epigenetics 2007 Australian Scientific Conference, Perth, Australia. Poster presentation.

O.R. Sprod and A.F. Holloway. “The role of chromatin remodelers and epigenetic marks in regulating GM-CSF expression in immune cells”. 29th

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This thesis is less than 100,000 words in length not including tables, figure legends and bibliographies.

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ABBREVIATIONS

Ab	antibody
Ac	acetylation
AP-1	activator protein 1
APC	antigen presenting cell
Asf1	antisilencing function 1
ATP	adenosine triphosphate
aza or azacytidine	5-aza-2-deoxycytidine
BAF	Brg1/Brm associated factor
BCR	B cell receptor
BL	baseline time point
bp	base pair
Brg1	Brahma-related gene 1
Brm	Brahma
BSA	bovine serum albumin
Ca ²⁺	calcium ion
CBP	CREB binding protein
CD	cluster of differentiation
CD28RE	CD28 response element
CD28RR	CD28 response region
CD28RRm	CD28RR mutant
cDNA	complementary DNA
CHART-PCR	chromatin accessibility by real-time PCR
ChIP	chromatin immunoprecipitation
CHX	cycloheximide
CK-1	cytokine-1 element
CLE0	conserved lymphokine element 0
CO ₂	carbon dioxide
CpG	5' CG 3'
CRC	chromatin remodelling complex
CsA	Cyclosporin A
CTCF	CCCTC-binding factor
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DNMT	DNA methyltransferase
dNTP	deoxynucleotide triphosphate
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid

EZH2	enhancer of Zeste homolog 2
FCS	foetal calf serum
g	gram
g	relative centrifugal field
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GCN5	general control nonderepressible 5
GM0.2	pXP1-mGM0.2 plasmid
GM-CSF	granulocyte-macrophage colony stimulating factor
H2A	histone H2A
H2B	histone H2B
H3	histone H3
H4	histone H4
HAT	histone acetyltransferases
HDAC	histone deacetylase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hGM	human GM-CSF
HMG	high mobility group
HMT	histone methyltransferase
I	calcium ionophore
IFN	interferon
Ig	immunoglobulin
Igf2	insulin-like growth factor 2
IKK	I κ B kinase
IL	interleukin
IP ₃	inositol-1,4,5-trisphosphate
IS	immunological synapse
I κ B	inhibitor of NF- κ B
κ B	NF- κ B binding site in GM-CSF promoter
K	lysine
kb	kilobase
kDA	kilodalton
M	molar
MBD	methyl binding domain
me	methylation
MECP2	methyl CpG binding protein 2
mg	milligram
mGM	mouse GM-CSF
MHC	major histocompatibility complex
mL	millilitre
mM	millimolar
MNase	micrococcal nuclease

mRNA	messenger RNA
NFAT	nuclear factor of activated T cells
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
nm	nanometre
nM	nanomolar
nmol	nanomole
NS	nonstimulated
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PHO5	acid phosphatase
PHO8	alkaline phosphatase
PI	PMA/ionophore
PKC	protein kinase C
PMA	phorbol myristate acetate
PolIII	RNA polymerase II
PRC2	Polycomb repressive complex 2
qPCR	quantitative PCR
RHD	Rel homology domain
RNA	ribonucleic acid
RNase	ribonuclease
RS	restimulated time point
RT-qPCR	reverse transcription qPCR
RUNX	runt-related
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
Sp1	specificity protein 1
Sp1m	Sp1 binding site mutant
Spt	suppressor of Ty
SR	stimulus removed time point
SWI/SNF	switch/sucrose nonfermentable
T/P	TSA/PI treated time point
TCR	T cell receptor
TE	Tris/EDTA buffer
TF	transcription factor
Th	T helper cell
Thn	naïve helper T cell
TI	total input
TNF	tumour necrosis factor
TNT	Tris/NaCl/Tween buffer
Tris	Tris(hydroxymethyl)aminomethane
TSA	Trichostatin A

TSS	transcription start site
U	unit
V	volt
WT	wild type
μF	microfarad
μg	microgram
μL	microlitre
μm	micrometre
μM	micromolar

ABSTRACT

Granulocyte-macrophage colony stimulating factor (GM-CSF) is a cytokine that stimulates the production of leukocytes as part of an immune response. The role that GM-CSF plays in the immune system is reliant on its tightly controlled expression, both temporally and spatially. The overall aim of this thesis was to investigate the factors that contribute to the correct temporal and spatial expression of *GM-CSF* in immune cells.

It was found that while *GM-CSF* expression can be stimulated in murine T cells but not B cells, key transcription factors involved in *GM-CSF* gene expression were present in both cell types, leading to the hypothesis that epigenetic mechanisms underlie this differential response. In support of this, differences in DNA methylation, histone modifications and the presence of chromatin remodelling proteins were detected at the *GM-CSF* promoter between the two cell types.

DNA methylation levels were higher at a CpG dinucleotide in the *GM-CSF* promoter in T compared to B cell lines, and DNA methylation of the *GM-CSF* promoter blocked expression from a reporter plasmid. Demethylation of the promoter was not sufficient to enable *GM-CSF* gene expression in B cells, although it increased its expression in T cells. The effect of removing the CpG dinucleotide, which is contained in an Sp1 transcription factor binding site, was also examined. In a transiently transfected reporter model, removal of the Sp1 site resulted in loss of

promoter activity. However, in a stably integrated transgene model, the Sp1 mutant promoter exhibited an increased response to stimulation. The differential response of the promoter mutant between the transient and stably transfected models suggests that the chromatin environment of the gene plays an important role in transcriptional regulation.

To further examine the importance of chromatin in *GM-CSF* gene regulation, histone modifications were examined at the *GM-CSF* promoter in T and B cell lines. Several key differences were observed. In T cells, acetylation of histone H3 was increased at the *GM-CSF* promoter relative to B cells. Increasing promoter acetylation levels by treatment with the histone deacetylase inhibitor Trichostatin A (TSA) facilitated expression of *GM-CSF* in the B cell lines in response to stimulation. Furthermore, TSA treatment in combination with DNA demethylation had a synergistic effect on *GM-CSF* expression in both T and B cells. In contrast to histone acetylation, histone H3 lysine 27 trimethylation was lower at the *GM-CSF* promoter in T cells relative to B cells. Finally, the chromatin remodelling protein Brg1, which is known to interact with acetylated histones, was present at the *GM-CSF* promoter in T cells at higher levels than in B cells.

These data suggest that enrichment of histone H3 acetylation and Brg1 and decreased H3K27Me3 contribute to the establishment of a ‘permissive’ chromatin environment at the *GM-CSF* promoter in T cells, which is not present at the promoter in B cells. A ‘permissive’ chromatin environment can be established at the *GM-CSF* promoter

in B cells following treatment with TSA, which increases histone acetylation. This allows remodelling of the promoter chromatin and subsequent gene expression in response to immune signals. However, this induced 'permissive' state is not maintained. Following removal of the inducing stimulus in A20 B cells, the chromatin is reset to its original 'repressive' state and the *GM-CSF* gene becomes unresponsive to subsequent stimulation.