



<http://researchspace.auckland.ac.nz>

### *ResearchSpace@Auckland*

#### **Copyright Statement**

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

#### **General copyright and disclaimer**

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

**THE STRUCTURE, REGULATION AND  
FUNCTION OF THE LEUKOCYTE  $\beta$ 7  
INTEGRINS AND CG-1**

A thesis submitted to the University of Auckland in partial fulfillment of  
the requirements for the degree of Doctor of Philosophy

by

Euphemia Yee Fun Leung

## Abstract

This thesis examines the structure, regulation and function of the leukocyte  $\beta 7$  integrins and a novel mouse protein termed CG-1.

Members of the integrin family of adhesion molecules are involved in cell-cell and cell-matrix interactions, and play fundamental roles in diverse biological processes. The  $\beta 7$  integrins (LPAM-1, HML-1 and M290) appear to play a special role in mucosal immunity and inflammation. They bind to cell adhesion molecules (CAMs) including MAdCAM-1, VCAM-1 and fibronectin which are expressed on inflamed endothelia and epithelia, and thereby contribute to the processes of leukocyte emigration and retention. An analysis of the  $\beta 7$  gene promoter was undertaken to understand the regulation of expression of the  $\beta 7$  integrins on different leukocyte subtypes. Primer extension and rapid amplification of cDNA ends (RACE) analysis revealed that the start of transcription was confined to nucleotide positions +1 and +4. The 5' site contains the highly conserved CA motif found at the transcriptional initiation sites of eukaryotic genes. Transient transfection assays revealed that a small 292bp 5'-flanking region of the  $\beta 7$  gene is responsible for cell-specific transcription. This promoter appears to be very compact with an array of potential and often overlapping cis-elements clustered around the transcription start site. The types of cis-elements in the  $\beta 7$  gene promoter are reminiscent of those found in the transcriptional enhancers of the TCR, CD3, integrin  $\alpha 4$  subunit, and integrin Leu-CAM genes, in accord with the predominantly leukocyte-restricted distribution of the  $\beta 7$  integrins in normal cells. However, proximal promoter activity was quite weak, and it is likely that distal enhancer elements are required to drive expression of the  $\beta 7$  gene. The presence of an enhancer region located between nucleotide positions -690 and -397 was detected by both

deletion analysis and DNase I hypersensitivity assay using the EL4 cell line. Functional studies of the trans-acting factors which bind the cis-acting elements contained within the  $\beta 7$  promoter will aid in unravelling the signals imparted to leukocytes in blood vessels and in gut-associated lymphoid tissues that regulate M290/HML-1 and LPAM-1 expression.

CG-1 is a coiled-coil leukocyte protein that is characterized by having extensive heptad repeats. It is immunologically related to, associated with, or identical to CD100, a cell surface antigen which shares a costimulatory function with integrins. CD100 influences the proliferation and differentiation of immune cells. Complementary DNAs encoding the mouse homologue of CG-1 were obtained by screening various mouse cDNA libraries with a human CG-1 cDNA probe. The deduced amino acid (aa) sequence of mouse CG-1 displays 83% identity with the human homologue, and the coiled-coil regions have been stringently conserved. CG-1 transcripts are expressed in a diverse range of cell lines and tissues, and are not restricted to haematopoietic cells. A complex pattern of insertions and/or deletions within CG-1 RNAs was revealed by comparison of the sequences of various mouse cDNA clones isolated from different cells and tissues. The deduced amino acid sequences of mouse and human CG-1 are similar to the sequence of the chicken cardiac morphogenic protein ES/130, suggesting that CG-1 and ES/130 are potentially related members of a gene family.

## Acknowledgements

I am extremely grateful to my PhD supervisor Dr. Geoffrey W. Krissansen for his male intuition, enthusiasm, support, and limitless patience in directing my project and in writing this thesis. I would also like to thank Prof. James D. Watson (currently Director of Research, Genesis Research and Development Corporation Ltd), my other PhD supervisor, for initially giving me the opportunity to do a PhD in the Department of Molecular Medicine, and also for his animal magnetism, and encouragement in my research. I am very grateful to Dr. David Parry (Department of Physics, Massey University) for analysing the protein structure of the CG-1 gene.

There are numerous other people that I would like to thank:

- Dr. Cris Print, the most dedicated and helpful fellow student I have met, for his invaluable assistance in the CG-1 project and his delightful fishing (or should I say salacious) stories.
- Dr. Klaus Lehnert, a most enchanting German postdoctoral research fellow, for his kind help in computer analysis, and his witty comments which uplift the standard mundane laboratory conversations.
- Yi Yang, the magnificent Chinese chef, for her assistance in tissue culture especially when I was away on holiday.
- Jane Harrison, the MacDraw Pro painter, for assisting in my diagram drawing.
- Dr. John Fraser, for his advice in completing the 5' RACE.
- Prof. John Marbrook, for improving my lateral thinking, and his spiritual direction in my rough life of being a poor student.
- Dr. Kathy Crosier, for her effort in setting up the PhD courses to broaden my horizon.

- Dr. Paul Mead, Dr. Yuan Qian and Jiang Wei-meng, for their contributions in the initial genomic library screening.
- Dr. Ross Henderson, for his encouragement and mental stimulation (disguised as verbal abuse and cups of cappucino) during my research.
- Dr. Randal Berg, a good natured Canadian postdoctoral research fellow, and Dr. William Abbott, the mad mogul skier with a very kind heart, for critically reading my thesis.

Others in the Department and in the Medical School to whom I wish to convey my appreciation include Dr. Roger Booth for solving most of my computer-associated problems; Mark Shelly for the artistic photography of my data; David Jenkins for managing the Departmental facility flawlessly; Guy Moffatt and Ailsa MacDonald for the endless supply of clean glassware; and Lois Henry (Duncan if she forgets) and Sarah Watson, the departmental secretaries.

Finally, I would like to acknowledge the financial support from the Health Research Council of New Zealand.

" No 'tis not so deep as a well, nor so wide as a church door;  
but 'tis enough, 'twill serve "

Shakespeare  
"Romeo and Juliet, Act III Scene I"

## List of publications

The results described in this thesis and related data have been published or will be published in the following articles.

**Leung, E.,** Mead, P. E., Yuan, Q., Jiang, W. M., Watson, J. D. and Krissansen, G. W. 1993. The mouse  $\beta 7$  integrin gene promoter: transcriptional regulation of the leukocyte integrins LPAM-1 and M290. *Int Immunol* 5: 551-8

Mead, P. E., **Leung, E.,** Yang, Y., Jiang, W. M., Print, C. G., Harrison, J. E., Watson, J. D. and Krissansen, G. W. 1994. Isolation of the 5' region of the human ITGB7 integrin gene. *Immunogenetics* 39: 375-6

Print, C. G., **Leung, E.,** Harrison, J. E., Watson, J. D. and Krissansen, G. W. 1994. Cloning of a gene encoding a human leukocyte protein characterised by extensive heptad repeats. *Gene* 144: 221-8

Yang, Y., Sammar, M., Harrison, J. E. B., Lehnert, K., Print, C. G., **Leung, E.,** Prestidge, R., and Krissansen, G. W. Construction and adhesive properties of a soluble MAdCAM-1-Fc chimera expressed in a baculovirus system: Phylogenetic conservation of receptor-ligand interaction. *Scand J Immunol.* (revised manuscript resubmitted).

Yang, Y., Harrison, J. E. B., **Leung, E.,** and Krissansen, G. W. Regulation of expression of the  $\beta 7$  integrins during myeloid cell differentiation. In preparation.

**Leung, E.,** Print, C. G., Parry, D., Closey, D., Skinner, S. J. N., Batchelor, D. C., Krissansen, G. W. A new gene family of coiled-coil proteins: Cloning of mouse CG-1 and its relatedness to ES/130 and kinectin. In preparation.

**Leung, E.,** Yuan, Q., Print, C., Krissansen, G. W. Location of an upstream enhancer site in the mouse integrin  $\beta 7$  gene promoter: analysis in transformed haematopoietic and nonhaematopoietic cells. In preparation.

## Table of contents

Abstract	i
Acknowledgements	iii
List of publications	v
Table of contents	vi
List of figures	xi
List of tables	xiii
Abbreviations	xiv
1. Introduction	1
1.1. Integrins	1
1.2. Structure and distribution of integrins	1
1.2.1. General functions of integrins	4
1.2.2. Regulation of integrin genes	5
1.2.2.1. Transcriptional regulation	5
1.2.2.2. Integrin promoters	6
1.2.2.3. $\beta$ 2 gene	6
1.2.2.4. $\alpha$ L gene	8
1.2.2.5. $\alpha$ M gene	8
1.2.2.6. $\alpha$ X gene	9
1.2.2.7. $\beta$ 1 gene	10
1.2.2.8. $\alpha$ 2 gene	10
1.2.2.9. $\alpha$ 4 gene	11
1.2.2.10. $\alpha$ 5 gene	12
1.2.2.11. $\beta$ 3 gene	12
1.2.2.12. $\alpha$ IIb gene	13
1.3. The VLA-4 integrin	15
1.4. The $\beta$ 7 integrin subfamily	16
1.4.1. Distribution and regulation of expression of $\beta$ 7 integrins	17
1.4.1.1. Distribution	17
1.4.1.2. Distribution in the diseased state	21
1.4.1.3. Regulation	21
1.4.2. Ligands of the $\beta$ 7 integrins	22
1.4.2.1. MAdCAM-1	23
1.4.2.2. VCAM-1	24
1.4.2.3. Fibronectin	24
1.4.3. Functions of the $\alpha$ 4 and $\beta$ 7 integrins	25
1.4.3.1. LPAM-1 is a lymphocyte homing	



	receptor	25
	1.4.3.2. LPAM-1 is a synovial homing receptor	26
	1.4.3.3. LPAM-1 and VLA-4 functions are remarkably similar	27
	1.4.3.4. $\alpha E\beta 7$ mediates the retention of lymphocytes at mucosal sites	27
1.5.	Integrin related diseases and potential therapy	28
1.5.1	Congenital defects involving integrins	28
1.5.1.1.	Leukocyte adhesion deficiency	28
1.5.1.2.	Glanzmann's thrombasthenia	28
1.5.2.	Animal models to study integrin function in vivo	29
1.5.2.1.	Gene targeting	29
1.5.2.2.	CD18-mutant mouse for the study of inflammation	30
1.5.2.3.	$\alpha 5$ integrin deficient mice	30
1.5.2.4.	$\beta 1$ knockouts in embryonal cells	31
1.5.3.	Potential applications for integrins in therapy	32
1.5.3.1.	Inflammatory disorders	32
1.5.3.2.	Thrombosis	32
1.5.3.3.	Osteoporosis	33
1.5.3.4.	Wound healing	33
1.5.3.5.	Multiple sclerosis	33
1.5.3.6.	Arthritis	34
1.5.3.7.	Asthma	34
1.5.3.8.	Cancer	34
1.6.	Integrins as costimulatory molecules	36
1.7.	CD100 as a T cell costimulatory molecule	38
1.7.1.	The relationship between CD100 and CG-1	39
1.7.2.	CD100 is physically associated with the CD3/TCR signalling complex	40
1.7.3.	CG-1 is similar to the chicken cardiac morphogenic protein ES/130	40
1.7.4.	CG-1 RNA transcripts are alternatively spliced	41
1.8.	Perspective and Goals	42
2.	Materials and Methods	43
2.1.	Materials	43
2.1.1.	Bacteria	43
2.1.2.	Plasmid vectors for cloning and sequencing	43
2.1.3.	M13 vectors for cloning and sequencing	44
2.1.4.	Bacterial culture medium	44
2.1.5.	Common buffers and solutions	45
2.1.6.	Solutions for $\lambda$ phage libraries	47
2.1.7.	Solutions for RNA	48
2.1.8.	RNA gel solutions	48
2.1.9.	Expression vectors for analysis of promoter /	

enhancer activity	49
2.1.10. DNA libraries	50
2.1.11. Transfection reagents	51
2.1.12. Cell activating agents	52
2.1.13. Restriction enzymes and DNA modifying enzymes	53
2.1.14. Autoradiographic film	53
2.1.15. Isotopes	53
2.1.16. Synthetic oligonucleotides	54
2.2. Methods	55
2.2.1. Isolation of plasmid DNA	55
2.2.1.1. Small scale preparation of plasmid DNA	55
2.2.1.1.1. Phenol extraction method	55
2.2.1.1.2. Wizard miniprep DNA purification system	56
2.2.1.2. Large scale preparation of plasmid DNA	56
2.2.1.2.1. The lithium chloride method	56
2.2.1.2.2. Cesium chloride gradient centrifugation	57
2.2.2. Isolation of single-stranded M13 DNA	57
2.2.3. Preparation of high titer $\lambda$ phage lysates	58
2.2.4. Isolation of $\lambda$ phage DNA	58
2.2.5. Isolation of RNA	59
2.2.6. Labelling of DNA	59
2.2.6.1. Random oligonucleotide priming	59
2.2.6.2. 5'-end labelling of synthetic oligonucleotides	60
2.2.7. Hybridization	60
2.2.8. Screening $\lambda$ phage libraries	61
2.2.8.1. Bacteria culture	61
2.2.8.2. Phage titers	61
2.2.8.3. Library screening	61
2.2.9. Restriction endonuclease mapping and Southern hybridization	62
2.2.10. Subcloning $\lambda$ phage DNA inserts into pUC18 or M13	63
2.2.11. Transformation of recombinant vectors into E.coli	63
2.2.11.1. Plasmid transformation	64
2.2.11.2. M13 transformation	64
2.2.12. Subcloning of PCR fragments by T and A tailing	64
2.2.12.1. T-tailing of vector DNA	65
2.2.12.2. A-tailed PCR products	65
2.2.13. Sequencing of DNA inserts	65
2.2.14. Cell culture	66
2.2.15. Transfection	67
2.2.15.1. Transfection using DEAE-dextran	67
2.2.15.2. Transfection by electroporation	67

2.2.16.	Luciferase and $\beta$ -galactosidase assay of promoter/enhancer activity	68
2.2.16.1.	Luciferase assay	68
2.2.16.2.	$\beta$ -galactosidase assay	68
3.	Results	69
	Part A: Analysis of the transcriptional regulation of the $\beta$ 7 gene	69
3.1.	Characterization of the mouse $\beta$ 7 gene promoter	69
3.1.1.	Isolation of a mouse $\beta$ 7 genomic clone	69
3.1.2.	Mapping the 5' region of the mouse $\beta$ 7 gene	69
3.1.3.	The mouse $\beta$ 7 gene is lacking exon 2 of its human homologue	70
3.1.4.	Mapping the transcriptional start site of the $\beta$ 7 gene	72
3.1.4.1.	Primer extension	72
3.1.4.2.	Anchor PCR	73
3.1.5.	The 5'-flanking region of the $\beta$ 7 gene: a paradigm for inducible lymphoid-restricted genes	74
3.1.6.	Construction of mouse $\beta$ 7-luciferase fusion genes for assays of promoter activity	75
3.1.7.	The 5'-flanking region of the $\beta$ 7 gene displays promoter activity	76
3.1.8.	DNase I hypersensitivity assay	77
3.1.9.	DNase I-hypersensitive sites in the 5'-flanking region of the $\beta$ 7 gene	77
3.1.10.	Construction of mouse $\beta$ 7-luciferase fusion genes for deletion analysis	78
3.1.11.	Deletion analysis of the $\beta$ 7 gene promoter	79
3.1.12.	Expression of $\beta$ 7 mRNA transcripts	80
3.1.13.	Increased levels of $\beta$ 7 integrin mRNA transcripts in oncogenically-transformed cell lines	80
3.1.14.	DNase I-hypersensitive sites in the 5'-flanking region of the $\beta$ 7 gene in the $\psi$ 2 p2 and $\psi$ 2 $\Delta$ rm cell lines	81
	Part B: Cloning of the mouse CG-1 gene	83
3.2.	Cloning and characterization of the mouse CG-1 antigen	83
3.2.1.	Isolation of mouse CG-1 cDNA clones	83
3.2.2.	Identification of the 5'-end of mouse CG-1 transcripts by rapid amplification of cDNA ends (RACE)	84
3.2.3.	CG-1 sequence analysis	84
3.2.4.	Expression of CG-1 transcripts	86
4.	Discussion	87

4.1.	Analysis of the mouse $\beta 7$ gene promoter	87
4.1.1.	Comparison of the mouse and human $\beta 7$ gene promoters	88
4.1.2.	Comparison of the 5'-flanking region of the mouse $\beta 7$ gene with other integrin genes	89
4.1.3.	Expression of the mouse $\beta 7$ gene may be induced by oncogenes	90
4.1.4.	Regulation of expression of the mouse $\beta 7$ gene	91
4.2.	Cloning and characterization of the mouse homologue of CG-1	93
4.2.1.	CG-1 is related to a chicken cardiac morphogenic protein	94
4.2.2.	Expression of CG-1 RNA transcripts	95
4.2.3.	CG-1 RNA transcripts undergo alternative splicing	95
4.2.4.	Potential model for the structure of CG-1	96
4.3.	Future directions	98
4.3.1.	Confirming the transcriptional start site of the mouse $\beta 7$ gene	98
4.3.2.	Identification of cis-regulatory elements in the $\beta 7$ gene	98
4.3.3.	Targeted disruption of the mouse $\beta 7$ gene	99
4.3.4.	Analysis of the expression of alternatively spliced CG-1 transcripts	100
4.3.5.	Mapping of the mouse CG-1 gene	100
4.3.6.	Identification of a ligand for CG-1	101
4.3.7.	Immunotherapy approach to cancer treatment using costimulatory molecules	101
5.	Epilogue	103
6.	References	104

## List of figures

following page

Fig. 1	Integrin subunit associations.....	1
Fig. 2	Schematic structure of a typical integrin.....	1
Fig. 3	The molecular basis of transcription.....	5
Fig. 4	Schematic illustrations of the structures of MAdCAM-1, VCAM-1 and fibronectin.....	23
Fig. 5	Costimulation with the TCR.....	36
Fig. 6	Partial restriction enzyme map of the integrin $\beta 7$ subunit gene.....	69
Fig. 7	(A) Comparison of the 5' untranslated regions of human and mouse integrin $\beta 7$ cDNA sequences.....	70
	(B) Partial restriction enzyme map of the 5' region of the integrin $\beta 7$ subunit gene contained in pMuR3.....	70
Fig. 8	DNA sequence of the 5'-end and flanking region of the $\beta 7$ subunit gene.....	70
Fig. 9	(A) Schematic diagram depicting the partial alignment of the human and mouse $\beta 7$ cDNAs, and positions of intron-exon boundaries.....	70
	(B) Schematic diagram depicting the partial alignment of deduced human and mouse $\beta 7$ protein sequences, and positions of intron-exon boundaries.....	70
Fig.10	Mapping of the transcription initiation sites of the mouse $\beta 7$ gene by primer extension analysis and 5' RACE.....	72
Fig. 11	The 5'-flanking region of the $\beta 7$ subunit gene displays promoter activity.....	76
Fig. 12	(A) DNase I-hypersensitivity analysis of the $\beta 7$ gene promoter.....	78
	(B) Partial restriction map of the 5'-end of the $\beta 7$ gene showing the location of the DNase I-hypersensitivity sites.....	78
Fig. 13	Deletion analysis of the $\beta 7$ gene promoter.....	79
Fig. 14	Analysis of the expression of mouse $\beta 7$ and $\alpha M 290$ mRNA transcripts in 5 different cell lines.....	81

Fig. 15	DNase I-hypersensitivity analysis of the 5'-flanking region of the $\beta 7$ gene in EL4, $\psi 2$ p2, and $\psi 2 \Delta$ rm cell lines.....	82
Fig. 16	Partial restriction enzyme map of the mouse CG-1 cDNA.....	83
Fig. 17	The nt and deduced aa sequences of CG-1.....	84
Fig. 18	Analysis of mouse CG-1 mRNA transcripts.....	86
Fig. 19	DNA sequence encoding the 5' region of the mouse $\beta 7$ subunit gene, and comparison with the human $\beta 7$ gene.....	88
Fig. 20	Schematic comparison of potential transcription factor binding sites in the 5'-flanking regions of the human $\alpha 4$ , and human and mouse $\beta 7$ subunit genes.....	88
Fig. 21	Relatedness of an Inr-like element near the transcription start site of the integrin genes.....	89
Fig. 22	Principal structural features of the CG-1 protein.....	93
Fig. 23	Designation of heptad repeats within the deduced CG-1 aa sequence.....	94
Fig. 24	Comparison of the deduced mouse CG-1 aa sequence with deduced sequences encoding human CG-1 (Z22551) and the chicken cardiac morphogenic protein ES/130.....	94
Fig. 25	Comparison of the deduced aa sequence of mouse CG-1 and its insertion version, with the deduced amino acid sequence of human CG-1, and human Z22551.....	95
Fig. 26	Models for the structure of CG-1.....	96

## List of tables

	page
Table 1	The tissue distribution of integrins and their ligands.....3
Table 2	Potential transcription factor-binding sites in integrin gene promoters.....7
Table 3	Alpha subunits found associated with the $\beta 7$ subunit .....17
Table 4	Distribution of mouse $\beta 7$ RNA transcripts as determined by Northern analysis.....19
Table 5	Distribution of $\alpha E$ and human $\beta 7$ RNA transcripts as determined by Northern analysis.....20
Table 6	Intron-exon junctions identified in the 5'-flanking region of the mouse $\beta 7$ subunit gene.....71
Table 7	Distribution of mouse $\beta 7$ RNA transcripts as determined by Northern analysis.....80

## Abbreviations

aa	amino acid
bp	base pairs
cDNA	complementary DNA
DNA	deoxyribonucleic acid
ECM	extracellular matrix
F.W.	formular weight
g	gram
h	hour
HEV	high endothelial venule
IEL	intraepithelial lymphocyte
Inr	initiator
kb	kilobase
kDa	kilodalton
LAD	leukocyte adhesion deficiency
Leu-CAM	leukocyte cell adhesion molecule
LFA-1	lymphocyte function-associated cell surface molecule-1
LPAM-1	lymphocyte Peyer's patch high endothelial venule adhesion molecule-1
LZ	leucine zipper
M	molar
MAdCAM-1	mucosal vascular addressin cell adhesion molecule-1
Mo-MuLV	moloney murine leukemia virus
$\mu$ g	microgram
$\mu$ l	microlitre
$\mu$ F	microfaraday
2ME	$\beta$ mercaptoethanol
mg	milligram
ml	millilitre
mM	millimolar
mm	millimetre
mRNA	messenger RNA
min	minute
M.W.	molecular weight
nt	nucleotide
NTP	nucleotide triphosphate
$\Omega$	ohm



pfu	plaque forming unit
PBL	peripheral blood lymphocyte
PBS	phosphate-buffered saline
PBM	peripheral blood monocyte
PCR	polymerase chain reaction
PEG	polyethylene glycol
PP	Peyer's patches
RA	retinoic acid
RACE	rapid amplification of cDNA ends
RNA	ribonucleic acid
RT	room temperature
s	second
SDS	sodium dodecyl sulphate
SP	signal peptide
TGF- $\beta$	transforming growth factor- $\beta$
Tdt	terminal deoxynucleotidyl transferase
TM	transmembrane domain
TPA	12-O-tetradecanoylphorbol-13-acetate
u	unit
V	volt
VCAM-1	vascular cell adhesion molecule-1
VLA	very late activation
vol	volume