THE STRUCTURE, REGULATION AND FUNCTION OF THE LEUKOCYTE β7 INTEGRINS AND CG-1

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by

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Abstract

This thesis examines the structure, regulation and function of the leukocyte β7 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 β7 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 β7 gene promoter was undertaken to understand the regulation of expression of the β7 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 β7 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 β7 gene promoter are reminiscent of those found in the transcriptional enhancers of the TCR, CD3, integrin α4 subunit, and integrin Leu-CAM genes, in accord with the predominantly leukocyte-restricted distribution of the β7 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 β7 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 β7 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.

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" 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 β7 integrin gene promoter: transcriptional regulation of the leukocyte integrins LPAM-1 and M290. Int Immunol 5: 551-8

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Leung, E., Yuan, Q., Print, C., Krissansen, G. W. Location of an upstream enhancer site in the mouse integrin β7 gene promoter: analysis in transformed haematopoietic and nonhaematopoietic cells. In preparation.

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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
µg	microgram
µl	microlitre
µF	microfaraday
2ME	β mercaptoethanol
mg	milligram
ml	millilitre
mM	millimolar
mm	millimetre
mRNA	messenger RNA
min	minute
M.W.	molecular weight
nt	nucleotide
NTP	nucleotide triphosphate
Ω	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-β	transforming growth factor-β
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