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**INVESTIGATION OF THE LEUCOCYTE ACTIVATION
ANTIGENS CG-1, CD100 AND HML-1**

CRISTIN GREGOR PRINT

**A Thesis submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy**

**Department of Molecular Medicine
School of Medicine
University of Auckland
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This thesis is dedicated to the memory of my father

James Murray Print

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Abstract

This thesis describes the investigation of three novel leucocyte activation antigens. The investigation began with the characterisation of the α HML-1 integrin subunit. Immunoprecipitation analysis and Western blotting indicated that α HML-1 associates with the β 7 integrin subunit to form a typical integrin heterodimer. α HML-1 appears to be post-translationally cleaved into heavy and light chains which remain linked by disulphide bonds. With the aim of isolating the α HML-1 protein, a 45kDa disulphide-linked polypeptide was isolated from leucocytes by two-dimensional non-reducing / reducing gel electrophoresis, and used to raise a polyclonal antiserum.

This polyclonal antiserum detected a protein encoded by a cDNA in a λ gt11 leucocyte expression library. This cDNA was designated *CG-1*. It became apparent that *CG-1* did not encode the α HML-1 protein, but rather encoded a novel protein which is abundantly expressed in activated leucocytes and reproductive tissues, and weakly expressed in all other tissues examined. The *CG-1* gene may be one member of a larger gene family, as two different chicken relatives of *CG-1* have been isolated elsewhere. The deduced *CG-1* amino acid sequence contains potential transmembrane domains and a potential nuclear localisation signal motif. On the basis of its primary structure the *CG-1* protein is expected to form an α helical coiled-coil dimer. A polyclonal antisera raised against a *CG-1* - β -galactosidase fusion protein detected a 300kDa protein on the surface of activated human leucocytes. The apparent molecular weight of this protein is halved following the reduction of disulphide bonds, supporting the contention that *CG-1* is a disulphide-linked cell-surface dimer. This protein appears to be associated with tyrosine kinase activity in an *in vitro* kinase assay.

While participating in the Fifth International Conference on Leucocyte Differentiation Antigens we noted that the protein detected by the anti-*CG-1* antisera was remarkably similar to a novel leucocyte protein designated CD100. CD100 is a T

lymphocyte co-stimulatory antigen capable of signalling in concert with the T lymphocyte antigen receptor to produce T lymphocyte proliferation. Like the protein detected by the anti-CG-1 antisera, CD100 was associated with tyrosine kinase activity in an *in vitro* kinase assay, and appeared to be a 300kDa disulphide-linked cell-surface dimer. Anti-CD100 antibodies were able to remove the protein detected by the anti-CG-1 antisera from leucocyte lysates in immunodepletion experiments. This suggests that CG-1 and CD100 are immunologically related, or that CD100 and the protein detected by the anti-CG-1 antisera are physically associated.

Current research is directed toward determining the relationship between CG-1 and CD100, and investigating the signalling and costimulatory potential of these molecules. The three leucocyte activation antigens investigated in this thesis all appear to mediate leucocyte transmembrane signalling. All three molecules potentially form the basis of therapeutic approaches for immunoregulation of inflammatory and neoplastic disease.

Publications arising from the work described in this thesis

(*) Print, C. G. and Krissansen, G. W. Structural analysis of the costimulatory leucocyte antigen CD100. FEBS. Letters. (Submitted).

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(2) Yang, Y., Sammar, M., Harrison, J. E. B., Lehnert, K., Print, C. G., Leung, E., Prestidge, R. and Krissansen, G. W. (1995). 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.* (In press).

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List of Abbreviations

aa	amino acid
APC	antigen presenting cell(s)
ATP	adenosine triphosphate
ARAM	antigen receptor activation motif
bp	base pairs
B cell	B lymphocyte
BCIG	5-bromo-4-chloro-3-indolyl- β -galactosidase
C	complement factor
CAM	cell adhesion molecule(s)
cAMP	cyclic adenosine 3',5'-monophosphate
CAPS	[cyclohexylamino]-1-propanesulfonic acid
CAR	cell adhesion regulator
cfu	colony forming units
cGMP	cyclic guanosine 3',5'-monophosphate
CIP	calf intestinal phosphatase
Coll	collagen(s)
Con A	concanavalin A
CTP	cytosine triphosphate
d	day(s)
DABCO	1,4-diazabicyclo [2.2.2]octane
DAG	diacylglycerol,
DEC	dendritic epidermal cell(s)
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease
DTT	dithiothreitol
EAE	experimental autoimmune encephalitis
ECM	extracellular matrix
EDTA	ethylenediaminetetra-acetic acid.Na ₂
Fa	coagulation factor
FAK	focal adhesion kinase
Fb	fibrinogen
FISH	fluorescent <i>in situ</i> hybridisation
FITC	fluorescein isothiocyanate

Fn	fibronectin
Fn*	V25 splice variant of Fn
g	grams
GAP	GTP-ase activating protein
h	hour(s)
HEV	high endothelial venule(s)
HSA	heat stable antigen,
I	Iodine
ICAM	intercellular adhesion molecule(s)
IEL	intraepithelial lymphocyte(s)
Ig	immunoglobulin
IL	interleukin,
IP ₃	inositol 1,4,5-triphosphate
IPTG	isopropyl- β -D-thio-galactopyranoside
kb	kilobase, 1000bp
kDa	kilodaltons
l	litre
LAD	leucocyte adhesion deficiency,
LB	Luria-Bertani
Ln	laminin
M	molar
mAb	monoclonal antibody(ies)
MAP	mitogen activated protein
MHC	major histocompatibility molecule complex
min	minute(s)
MIP1 β	macrophage inflammatory protein 1 β
ml	millilitre
MOPS	3(N-morpholino)-propane sulphonic acid
MBM	myocardial basement membrane
NEPHGE	non-equilibrium pH gradient gel electrophoresis
NF-AT	nuclear factor of activated T cells
NF-AT _p	cytoplasmic component of NF-AT
NK	natural killer
NLS	nuclear localisation signal
NR/R	nonreducing / reducing
nt	nucleotide(s)

PA	polyacrylamide
PBM	peripheral blood mononuclear cell(s)
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet derived growth factor
PE	phycoerythrin
pfu	plaque forming units
PHA	phytohaemagglutinin
PI	phosphatidylinositol
PKA	protein kinase A (cAMP-dependent kinase)
PKC	protein kinase C
PLC	phospholipase C
PMA	phorbol 12-myristate 13-acetate
PVDF	polyvinylidene difluoride
RE	response element
RGD	arginine - glycine - aspartic acid tripeptide
RNA	ribonucleic acid
RNAse	ribonuclease
RT	room temperature
s	second(s)
SA	superantigen(s)
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-PA-gel electrophoresis
SH1	Src-homology-1
SH2	Src-homology-2
SH3	Src-homology-3
SREBP-1	sterol regulatory element binding protein-1
T cell	T lymphocyte
TCR	T cell antigen receptor
TEMED	N.N.N'N'-tetramethylethylenediamine
TGF β	transforming growth factor β
Th0	T-helper-0
Th1	T-helper-1
Th2	T-helper-2,
Tm	melting temperature
TPA	tetradecanoyl phorbol 13-acetate

tris	tris(hydroxymethyl) aminomethane
Ts	thrombospondin
U	unit(s)
VAP-1	vascular adhesion protein-1
VCAM	vascular cell adhesion molecule(s)
Vn	vitronectin
vol	volume(s)
vWf	Von Willebrand factor