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

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

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June 1995

This thesis is dedicated to the memory of my father

James Murray Print

Acknowledgements

Like most scientific research, the research described in this thesis represents the effort of many people. I am grateful to my supervisors Dr Geoff Krissansen (Senior Research Fellow, Department of Molecular Medicine, University of Auckland) and Professor Jim Watson (currently Director of Research, Genesis Research and Development Corp. Ltd, Auckland) for the opportunity to do a PhD in the Department of Molecular Medicine. As my principal supervisor, Geoff Krissansen showed endless patience and great scientific judgement. Geoff's inspired ideas are behind many of the experiments described in this thesis. Geoff also contributed to the immunoprecipitations and non-equilibrium pH gel electrophoresis experiments presented here.

I would like to thank all the members of Geoff Krissansen's research group for their friendship and patient teaching. In particular I would like to thank Qian Yuan for providing the β7 integrin cDNA probe, and Euphemia Leung and Paul Mead for their help with techniques, and for creating an extraordinary lab atmosphere.

I extend my thanks to several others for their advice - in particular John Marbrook and John Fraser (Department of Molecular Medicine) and Ross Prestidge and Greg Murison (Genesis Research and Development Corp. Ltd, Auckland). I would especially like to thank my co-supervisor Jim Watson for the great influence he has had on my thinking toward scientific research. I would also like to acknowledge the generous department members who donated blood for use in these studies.

Professor David Parry from Massey University was tremendously helpful with analysis of the coiled-coil structure of CG-1. Geraldine MacGibbon prepared RNA from the blood of leukaemia patients which was used in the Northern analysis presented in Fig. 33. Dr Nigel Spurr from the Imperial Cancer Research Fund in London supplied the chromosomal hybrid DNA samples used to localise the *CG-1* gene. Christine Morris from the Christchurch School of Medicine performed

fluorescent *in situ* hybridisation to localise the *CG-1* gene in the human genome. Dr Stephen Skinner and Mrs Marilyn Geaney provided help with immunohistochemical staining. Drs. Boumsell, Carriere, Al Saati, Villela and Aversa kindly provided their anti-CD100 monoclonal antibodies for study. The Cancer Society of New Zealand and the Auckland Medical Research Foundation have generously provided financial support.

I am grateful to my friends for their support and understanding, and for encouraging me away from the lab on the sailing and fishing trips which kept me sane through my PhD studies. I would like to thank Randal Berg, Lyndal Print and Jean Taylor for checking the thesis, and Keren Edwards for searching out many of the references. Finally I would like to thank my wife Adele, without who's understanding, friendship and love I could not have done the work presented here.

Abstract

This thesis describes the investigation of three novel leucocyte activation antigens. The investigation began with the characterisation of the αHML-1 integrin subunit. Immunoprecipitation analysis and Western blotting indicated that αHML-1 associates with the β7 integrin subunit to form a typical integrin heterodimer. αHML-1 appears to be post-translationally cleaved into heavy and light chains which remain linked by disulphide bonds. With the aim of isolating the αHML-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 λgt11 leucocyte expression library. This cDNA was designated *CG-1*. It became apparent that *CG-1* did not encode the αHML-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

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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 *in situ* 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)
MIP1β	macrophage inflammatory protein 1β
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
$NF\text{-}AT_p$	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
TGFβ	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