Trogocytosis in Multiple Myeloma

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DECLARATION

I declare that no part of the work described in this thesis has been submitted for any other degree nor has it been submitted as part of the requirements for another degree.

As the author of this thesis, I also certify that the thesis has been written by me, any help that I have received in my research work and the preparation of the thesis has been acknowledged. All due acknowledgement has been made where appropriate.

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Karieshma Kabani

DEDICATION

This thesis is dedicated to my family whose love and support encourages me to work hard and do the best I can.

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PUBLICATIONS ARISING FROM THE WORK DESCRIBED IN THIS THESIS

A) Publications in peer reviewed journals

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Brown RD, Spencer A, Ho PJ, Kennedy N, Dolotin M, **Kabani K**, Yang S, Sze D, Aklilu E, Gibson J, Joshua D. (2009) Prognostically significant cytotoxic T cell clones are stimulated after thalidomide therapy in patients with multiple myeloma**.** *Leukaemia and Lymphoma* 50:1860-1864.

B) Conference Abstracts/Presentations

Brown R, **Kabani K**, Yang S, Favaloro J, Ho PJ, Gibson J, Fromm P, Hart D, Woodland N, Nassif N, Joshua D. (2011) HLA-G acquired by bone marrow T cells mediates tumour escape in myeloma, oral presentation to be presented at *Haematology society of Australia and New Zealand (HAA)*, *O16.*

Kabani K, Brown R, Yang S, Aklilu E, Woodland N, Nassif N, Ho PJ, Gibson J, Joshua D. (2011) HLA-G can be acquired by T cells via trogocytosis and is a clinically relevant immunoregulator in patients with myeloma, poster presented at the 13^{th} International myeloma workshop. *Haematologica 96 (s1), S38.*

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Yang S, Brown R, Reid S, **Kabani K**, Aklilu E, Ho PJ, Woodland N, Joshua D. (2009). Characterisation of $CD138^-$ plasma cells in multiple myeloma, poster presented at the *Australasian Flow Cytometry group (AFCG)*, *p66*.

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Kabani K, Brown R, Yang S, Aklilu E, Sze D, Woodland N, Nassif N, Ho PJ, Gibson J, Joshua D. (2008) Trogocytosis in Multiple Myeloma, poster presented at *Haematology society of Australia and New Zealand (HAA),* pA355.

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Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APCs	Antigen presenting cells
APC	Allophycocyanin
ASCT	Autologous stem-cell transplantation
ATCC	American type culture collection
BCR	B cell receptor
BM	Bone marrow
CD	Cluster differentiation
CFSE	Carboxyfluorescein succinimidyl ester
CLL	Chronic lymphocytic leukemia
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytolytic T lymphocyte-associated antigen 4
Cy5.5	Cyanine 5.5
Cy7	Cyanine 7
DC	Dendritic cell
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediamine tetraacetic acid
EFS	Event free survival
FACS	Fluorescent-activated cell sorting
FCS	Foetal calf serum
FGF	Fibroblast growth factor
FISH	Fluorescent *in situ* hybridisation
FITC	Fluorescein isothiocyanate
FLC	Free light chains
FoxP3	Forkhead transcription factor 3
FSC	Forward scatter
GM-CSF	Granulocyte macrophage – colony stimulating factor
Hb	Haemoglobin
HLA	Human leukocyte antigen
IFN	Interferon

Ig	Immunoglobulin
IL	Interleukin
ILT	Immunoglobulin-like transcript receptors
IS	Immunological synapse
ISS	International staging system
KIR	Killer Ig-like receptors
M-protein	Monoclonal protein
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MM	Multiple myeloma
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NF-κB	Nuclear Factor-kappa B
NHL	Non-Hodgkin's lymphoma
NK	Natural killer
OS	Overall survival
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PC	Plasma cell
PCLI	Plasma cell labelling index
PCR	Polymerase chain reaction
PerCP	Peridinin chlorophyll protein
PFS	Progression free survival
PHA	Phytohemagglutinin
pMHC	peptide MHC
R-PE	R-phycoerythrin
rpm	Revolutions per minute
RPMI	Rosewell Park Memorial Institute
RT	Room temperature
Rt-PCR	Reverse transcription polymerase chain reaction
SMM	Smoldering multiple myeloma
SSC	Side scatter
TCR	T cell receptor

TGF	Tumour growth factor
TNF	Tumour necrosis factor
VEGF	Vascular endothelial growth factor
WBC	White blood cells
WM	Walsendtrom macroglobulinemia
β_2M	Beta-2-microglobulin

Abstract

The term trogocytosis is used to describe the fast, cell-to-cell contact-dependent transfer of membrane proteins between cells, with T and B-lymphocytes, natural killer (NK) cells, antigen presenting cells (APC) and tumour cells being the most widely studied. This study aimed to: (1) identify the extent of trogocytosis in patients with multiple myeloma (MM), a malignancy of bone marrow plasma cells, compared with the other B cell malignancies, (2) to identify some of the molecules involved with trogocytosis in these patients and then (3) determine if cells that had acquired molecules had altered function.

An *in vitro* model of trogocytosis was established in which plasma cell lines and flow-sorted bone marrow plasma cells ($CD38^{++}$) of patients with MM (n=11) or the malignant B cells from patients with chronic lymphocytic leukaemia (CLL) ($CD5^{+}CD19^{+}$) (n=4) and Waldenstrom macroglobulinaemia (WM) ($CD19^{+}$) were biotinylated and then cultured with either patient or normal mononuclear cells. The acquisition of biotinylated membrane proteins was determined by flow cytometry and confocal microscopy. Screening for potential molecules involved suggested that CD86 and HLA-G were likely candidates for trogocytosis. CD86 is a co-stimulatory molecule at the immune synapse and HLA-G is a non-classical MHC class I molecule which prevents antigen-specific cytolysis by cytotoxic T lymphocytes (CTLs), inhibits the function of circulating NK cells and prevents proliferation of allogeneic $CD4^{+}$ T cells. These observations have lead to the hypothesis that expression of HLA-G may aid in the escape of tumours from immune surveillance.

T cells acquired significantly more biotinylated proteins (mean=13.55%) than B cells (mean=2.43%; $t=2.80$; $p<0.05$) or NK cells (mean=3.15%; $t=2.57$; $p<0.05$). There was no significant difference between levels of biotin transferred to T cells from either plasma cell lines or primary plasma cells and acquisition was the same with autologous and allogeneic T cells. Significantly more trogocytosis was observed in myeloma patients than other B cell malignancies (n=5) as <1% T cells acquired membrane fragments when cultured with malignant B cells from patients with CLL or WM ($t=3.86$; $p<0.05$). Upon culture with biotinylated $CD3^{+}$ flow-sorted normal T

cells, approximately 2% of $CD38^{++}$ plasma cells acquired membrane fragments, suggesting that in patients with myeloma, trogocytosis was predominantly unidirectional.

Although HLA-G expression was found on 0.02 – 0.56% of normal T cells (mean =0.23%), 20% of MM patients (11 of 56) demonstrated a level of HLA-G^{+} $CD3^{+}$ T cells above the normal range. Addition of flow-sorted $CD3^{+}$ HLA-G^{pos} T cells led to a reduction in the proliferation of carboxyfluorescein succinimidyl ester (CFSE)-labelled $CD3^{+}$ HLA-G^{neg} T cells stimulated with anti-CD3/CD28 beads and this inhibition was greater than the inhibition due to $CD38^{++}$ HLA-G^{pos} plasma cells ($t=2.64$; $p=0.046$). The $CD3^{+}$ HLA-G^{pos} T cells acquired inhibitory function but were not natural T regulatory cells as they were $CD25^{neg}$. Overall survival was significantly worse for the 11/46 patients with HLA-G^{pos} plasma cells ($\chi^2=12.4$; $p<0.0004$).

Flow cytometric analysis of $CD38^{++}$ bone marrow plasma cells from MM patients showed varied HLA-G expression ranging from 0.2% to 96% (n=46). The clinical relevance of HLA-G^{pos} plasma cells was demonstrated by a significant reduction in overall survival (n= 46; $\chi^2= 12.4$; $p<0.004$). CD86 expression on T cells of myeloma patients (n=98) ranged from 0 – 30% (normals = 0 – 2.7%; n=10). T cells from myeloma patients (n=7), when co-cultured with CD86 expressing plasma cells, were found to acquire significantly ($p<0.0001$) higher levels of CD86.

This study reports several new findings. It has shown that trogocytosis is more common in multiple myeloma than other B cell malignancies, is primarily unidirectional, HLA independent and T cells are more likely to be involved than other lymphocytes. T cells which acquire tumour antigens may have altered function and it has been demonstrated that HLA-G^{pos} T cells form a new subset of acquired regulatory T cells that inhibit the proliferation of HLA-G^{neg} T cells and therefore protect MM cells against the host's immune defences.