The Expression and Localization of Kappa Myeloma Antigen on Malignant and Normal B cells

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

University of Technology, Sydney
NSW, Australia

August 2009
Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the written preparation of the thesis, and all experimental work associated with it, has been carried out solely by me, unless otherwise indicated. Finally, I certify that all information sources and literature used are acknowledged in the text.

Andrew Tasman Hutchinson
August 2009
Acknowledgements

I would first like to thank my supervisors and good friends, Professor Bob Raison and Darren Jones. Bob was instrumental in getting my PhD off the ground with Immune System Therapeutics Ltd and I am grateful for the time and patience he dedicated to my project. He never once held me back from chasing even the most outrageous hypotheses and provided both support, and more importantly, a critical examination during the creative process. Bob, I wish you all the best for your retirement and congratulate you on your recent appointment as Emeritus Professor at UTS.

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C3 complement component 3
CCDA co-stimulatory cell dependent activation
CCIA co-stimulatory cell independent activation
CD cluster of differentiation
CDC complement-dependent cytotoxicity
C-domain constant domain
CDR complementarity determining regions
D diversity
Da dalton
ddH₂O double distilled water
D₀ hydrodynamic diameter
DLS dynamic light scattering
DNA deoxyribonucleic acid
DOPC 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine
DTT dithiothreitol
ECL enzymatic chemiluminescence
ELISA enzyme-linked immunoabsorbent assay
ER endoplasmic reticulum
EWB enzyme-linked immunoabsorbent assay wash buffer
EWB-T enzyme-linked immunoabsorbent assay wash buffer with tween 20
Fab fragment, antigen binding
Fc crystallisable fragment
FITC fluorescein
FLC free immunoglobulin light chain
FxLC free immunoglobulin kappa light chain
FαLC free immunoglobulin lambda light chain
FSC forward scatter
FSW flow cytometry staining wash
g gram or centrifugal force
GC germinal centre
GFP green fluorescent protein
gp41  glycoprotein 41
GPI  glycosylphosphatidylinositol
GRP  glucose-regulated protein
h  hours
HC  heavy chain
HCD  heavy chain disease
HRP  horse radish peroxidase
hsp  heat shock protein
Ig  immunoglobulin
IgA  immunoglobulin A
IgD  immunoglobulin D
IgE  immunoglobulin E
IgG  immunoglobulin G
IgM  immunoglobulin M
IL  interleukin
J  joining
k  kilo
kDa  kilodalton
KMA  kappa myeloma antigen
L  litre
LC  light chain
LCA  light chain amyloidosis
LCDD  light chain amorphous deposition disease
LMA  lambda myeloma antigen
LUV  large unilamellar vesicle
M  molar
mAb  monoclonal antibody
mβCD  methyl β cyclodextrin
mg  milligram
MGUS  monoclonal gammopathy of undetermined significance
MHC II  major histocompatibility complex class II
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<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>RMSD</td>
<td>root mean square deviation</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>reverse transcriptase quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RU</td>
<td>arbitrary response unit</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Term</td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>SAC</td>
<td><em>Staphylococcus aureus</em> cowan I strain</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl sulfate-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>siRNA</td>
<td>small inhibitory ribonucleic acid</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
</tr>
<tr>
<td>SP-A</td>
<td>surfactant protein-A</td>
</tr>
<tr>
<td>SPR</td>
<td>surface plasmon resonance</td>
</tr>
<tr>
<td>SSC</td>
<td>side scatter</td>
</tr>
<tr>
<td>TLR</td>
<td>toll-like receptor</td>
</tr>
<tr>
<td>TMNCs</td>
<td>tonsil derived mononuclear cells</td>
</tr>
<tr>
<td>TX100</td>
<td>triton X 100</td>
</tr>
<tr>
<td>TX114</td>
<td>triton X 114</td>
</tr>
<tr>
<td>V</td>
<td>variable</td>
</tr>
<tr>
<td>V-domain</td>
<td>variable domain</td>
</tr>
<tr>
<td>V(D)J</td>
<td>variable, (diversity), joining</td>
</tr>
<tr>
<td>VH</td>
<td>variable heavy chain</td>
</tr>
<tr>
<td>VL</td>
<td>variable light chain</td>
</tr>
<tr>
<td>WM</td>
<td>Waldenström’s macroglobulinaema</td>
</tr>
<tr>
<td>XBP-1</td>
<td>x-box binding protein 1</td>
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**Abstract**

Kappa Myeloma Antigen (KMA) is a plasma membrane associated form of free immunoglobulin kappa light chain (F\(\kappa\)LC) expressed on malignant B cells from patients with multiple myeloma (MM), Waldenström’s macroglobulinaema (WM) and non-Hodgkin’s lymphoma (Walker et al. 1985). KMA is recognized by the murine monoclonal antibody (mAb) mKap, and its human-mouse chimeric equivalent, cKap, which is currently undergoing clinical trials as a therapy for kappa type MM (Boux et al. 1983; Raison et al. 2005).

Earlier expression studies on KMA suggested that the antigen is not expressed by normal B cells *in vivo*. However, *in vitro* activation of tonsillar B cells induced expression of KMA on a subset of cells. Like their KMA expressing malignant counterparts, these were presumed to be F\(\kappa\)LC secreting plasma cells or plasmablasts but, due to the lack of B cell lineage specific markers at the time, these cells were not phenotyped (Walker et al. 1985). Furthermore, given the extremely low frequency of plasmablasts and plasma cells in normal tissues, it was not possible to exclude the presence of a ‘normal’ KMA positive cell population *in vivo*.

The first section of this thesis expands upon this earlier work. By utilizing *in vitro* activation protocols on peripheral blood CD19+ B cells, KMA expression was induced on a subset of cells. Phenotypic analysis revealed that the majority of KMA positive cells were CD27++ CD38+- plasmablasts and CD38++ plasma cells. Analysis from normal human tissues found that a subset of plasma cells in the tonsils expressed the antigen. These cells co-expressed CD45, indicating that they are at an immature stage of plasma cell differentiation. In contrast, peripheral plasma cells, considered to be more fully mature cells in transit from secondary lymphoid organs to plasma cell niches in bone marrow or spleen, did not express KMA. This implies that KMA expression, *in vivo*, is limited to a small subset of immature plasma cells in secondary lymphoid organs such as the tonsils.
Despite cKap's current assessment in clinical trials for the treatment of MM, very little is known about its molecular target KMA. Previous studies have showed that KMA is comprised of FκLC (Goodnow and Raison 1985); however it was never determined as to how FκLC is associated with the plasma membrane. Since FκLC is a secreted molecule, it was initially presumed that it associated with a proteinaceous 'membrane receptor' (Goodnow and Raison 1985). However membrane extraction studies, as described in the second part of this thesis, reveal that FκLC directly associates with the plasma membrane through a combination of hydrophobic and electrostatic forces to form KMA. Further investigations confirmed that FκLCs can bind directly to cellular and artificial membranes. Moreover, this binding is likely dependent on self-association processes, which suggest that KMA consists of aggregated, membrane associated FκLCs.

Lipid binding studies revealed that FκLCs associate specifically with saturated phosphocholine species such as sphingomyelin in membranes, and KMA expression was positively correlated with sphingomyelin expression in FκLC secreting cell lines.

The final section of this thesis examines how FκLCs might interact with saturated phosphocholine lipids. Molecular modeling of dimeric FκLC suggests they are able to weakly associate with phosphocholine in the conventional antigen binding pocket formed by the κLC variable domain (V-domain). Since FκLC aggregation is a feature of KMA, then the avidity effects of multi-valent binding likely increases the strength of the proposed FκLC-phosphocholine interaction. This hypothesis explains the observation of both electrostatic and hydrophobic interactions by FκLC, as KMA, with the plasma membrane - the electrostatic component, governed by single FκLC molecules interacting with the charged phosphocholine headgroups, and the hydrophobic component, due to self-association of adjacent FκLC molecules.

Finally, a model of KMA expression by FκLC secreting cells is proposed. FκLC is synthesized in the endoplasmic reticulum (ER) then transported to the golgi-apparatus and encapsulated into vesicles destined for secretion. There FκLCs interact with saturated
phosphocholine lipids, such as sphingomyelin, and undergo aggregation resulting in stable association on the inner vesicular membrane. Fusion of the vesicle with the plasma membrane during exocytosis allows for membrane associated FxLC to become exposed on the extracellular face as KMA.