Variable region gene expression and structural motifs of human polyreactive immunoglobulins

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CERTIFICATE

I certify that this thesis has not already been submitted for any degree and is not being submitted as part of candidature for any other degree.

I also certify that the thesis has been written by me and that any help that I have received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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* A copy of this manuscript is bound in the back of this thesis

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Abbreviations

Ab	antibody	**IgM**	immunoglobulin with μ isotype heavy chains
Ag	antigen	**IPTG**	isopropylthiogalactoside
Az	sodium azide	**J**	joining gene
B CLL	chronic B lymphocytic leukaemia	**L**	light chain
BSA	bovine serum albumin	**MFI**	mean fluorescence intensity
C	constant gene/domain	**MHC**	major histocompatibility complex
Cα	*alpha* carbon atom	**PAGE**	polyacrylamide gel electrophoresis
CDR	complementarity determining region	**PBL**	peripheral blood lymphocytes
CIAA	24:1 Choroform:isoamyl alcohol	**PBS**	phosphate buffered saline
CRI	cross reactive idiotype	**PCR**	polymerase chain reaction
D	diversity gene	**RBS**	ribosome binding site (Shine/Dalgarno sequence)
DNA	deoxyribonucleic acid	**RF**	rheumatoid factor
Fab	fragment antigen binding	**rms**	root mean square
Fc	fragment crystalline	**rmsd**	root mean square deviation
Fd	heavy chain fragment of Fab (VH–CH1)	**RNA**	ribonucleic acid
Fr	framework region	**scFv**	single chain variable region fragment (Fv)
Fv	fragment variable	**SDS**	sodium dodecyl sulfate
H	heavy chain	**TBS**	tris-HCl buffered saline
H-bond	hydrogen bond	**TdT**	terminal deoxy-transferase
HEL	hen egg-white lysozyme	**V**	variable gene/domain
Ig	immunoglobulin	**vdW**	van der Waal's
IgG	immunoglobulin with γ isotype heavy chains	**3D**	three-dimensional

* Infrequently used and common abbreviations are not included in this list

Abstract

Polyreactive immunoglobulins (Ig) exhibit a capacity to recognise multiple, structurally dissimilar antigens through a single combining site. This characteristic differentiates these Igs from monoreactive Igs which bind to a single antigen, usually with high specificity and affinity. Chronic B lymphocytic leukaemia (B CLL) is a malignancy identified by the incessant accumulation, in the peripheral circulation, of B lymphocytes of a mature and resting morphology. B CLL malignant cells generally express both surface IgM and the pan T cell antigen CD5. Moreover, the IgM on the surface of these CD5 positive B CLL cells is frequently polyreactive. This thesis examines the structural diversity found in the combining sites of B CLL derived Igs in an attempt to elucidate the structural basis of polyreactive antigen binding displayed by a significant proportion of human Igs. The genes encoding the variable (V) domains of five B CLL derived IgM antibodies (Bel, Tre, Yar, Hod and Jak) were cloned and sequenced (Chapter Two). When the light chain V domain genes were aligned with the closest germline VL and JL coding DNA sequences it was determined that there was either a complete absence of somatic mutation (Tre, Yar and Jak) or a minimal number of mutations (Bel and Hod) present in the rearranged VL domain genes. A remarkable fidelity in the splicing of VL to JL genes was noted suggesting that the diversity, normally introduced through variability of splicing VL to JL, is reduced in Igs expressed by B CLL cells. Furthermore, the markedly reduced primary structural diversity was highlighted when two of the VL domain genes (Yar and Hod) were found to be different in sequence by only four nucleotides and two amino acids. The heavy chain V domain genes of the same five Igs were sequenced in another study (Brock, 1995), however, it was interesting to analyse the sequences of the VH domain genes and compare them with the VL domain genes. The naive or germline nature of the B CLL antibodies was reflected in the VH genes by either an absence or a low frequency of mutations within these sequences compared with germline immunoglobulin gene sequences. No obvious conserved motif, which could be related to polyreactivity, was observed when the primary protein sequence was analysed for distribution of identical or similar amino acids. Thus, homology modelling was used to construct three-dimensional models of the Fv (VL–VH) portions of the five B CLL IgM molecules to examine the structures of the combining sites of these Igs (Chapter Three). Framework regions were constructed using X-ray coordinates taken from highly homologous human variable domain structures. Complementarity determining regions (CDR) were predicted by grafting loops, taken from known Ig structures, onto the Fv framework models. The CDR templates were selected, where possible, to be of the same length and of high residue identity or

similarity. If a single template CDR was not appropriate to model a particular CDR the loop was built from loop stems of known conformation, followed by chain closure with a β-turn. Template models were refined using standard molecular mechanics simulations. The binding sites were either relatively flat or contained a deep cavity at the VL–VH domain interface. Further differences in topology were the result of some CDR loops protruding into the solvent. Examination of the electrostatic molecular surface did not reveal a common structural feature within the binding sites of the five polyreactive Fv. While two of the binding cavities were positively charged the other three structures displayed either negatively charged or predominantly hydrophobic combining sites. These findings suggested that a diversity of structural mechanisms are involved in polyreactive antigen binding. Residues within CDRs which have aromatic side-chains and are partially exposed to solvent were distributed across large regions of the combining sites. It is possible that these aromatic residues are responsible for the conserved binding to mouse Igs observed (Chapter Two) for the B CLL derived polyreactive IgM molecules. Two Fv molecules (Bel and Tre) were cloned as dicistronic constructs, into the bacterial expression vector pFLAG. The expression of the Fvs was fully characterised and unfortunately the VL and VH of Bel and Tre Igs did not associate in an appropriate manner to yield large quantities of purified Fv (Chapter Four). Expression of correctly folded and stabilised fragments of human polyreactive immunoglobulins would enable the structural basis for the polyreactive binding phenomenon to be fully explored using protein crystallography.