Development of novel alloantibody screening cells – the first example of the addition of peptide antigens to red blood cells using KODE™ technology.

Damien Heathcote, CSL Bioplasma Immunohaematology, 45 Poplar Road, Parkville, Melbourne, Australia, 3052.
Robert Flower, PaLMS, Royal North Shore Hospital, St Leonards, NSW, Australia.
Stephen Henry, KODE Biotech, Auckland, New Zealand.

Aim of Project

To use KODE™ technology to add fully synthetic peptide antigens to alloantibody screening cells. These antigens will be representative of the clinically significant 'Miltenberger' phenotypes and will allow the detection and identification of clinically significant alloantibodies, particularly in Asian populations.

What is KODE™ Technology?

CSL Limited has been successfully marketing products in Australia for over three years that have fully synthetic antigens added to red blood cells using KODE™ technology. The Securacell™ range of quality control products have had carbohydrate A and B antigens added in precisely controlled amounts in order to create sensitivity controls (see Figure 1). These constructs have a Functional group (F), a Spacer (S) and a Lipid tail (L) eg FSL-A or B as used in Securacell™. This project aimed to extend the work done using carbohydrate antigens to peptide antigens such as those found in the 'Miltenberger' series.

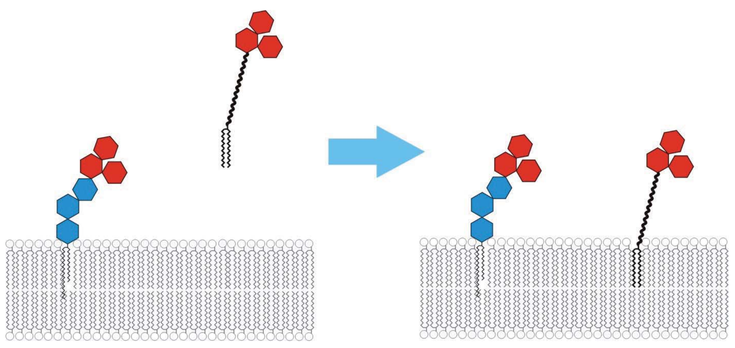

Figure 1 Insertion of Glycolipids : showing an inserted natural glycolipid and the insertion of a synthetic glycolipid.

What is Miltenberger?

Miltenberger (Mi) is not a separate blood group system but was originally a name applied to a group of antibodies that were reactive with variant glycophorins that would normally carry the MNS antigens (vMNS). Miltenberger was originally named from the surname of the first identified case. There is a great deal of confusion about the antigens and antibodies, and a new nomenclature for the phenotypes that result in vMNS's has been proposed. There are currently 11 known genetic variants of glycophorin encompassing what was previously known as the Miltenberger subsystem. Each of these phenotypes has been shown to express from 1 to 6 antigens, and most antigens are common to more than one phenotype. The antigens in this system include Mur, MUT, Hil, Mi^a, Vw and others.

The Miltenberger phenotypes are very rare in Caucasians with an incidence of less than 0.01%. They are very common in all Asian populations studied so far and have been found in people of Chinese, Taiwanese, Thai, Philippino, Indian, Malaysian, Singaporean, Japanese and Sri Lankan descent. The Miltenberger name is now obsolete and the proposed new nomenclature now uses the prefix GP (Glycophorin) followed by a suffix denoting the glycophorin variant such as GP.Mur, GP.Vw, GP.Hut, or GP.Hil. Most of these mutations are crossover events where the resultant glycophorin is a mixture of glycophorins A and B. GP.Mur (Mi III) is the most common phenotype and is found in approximately 10% of the Thai and approximately 5% of the Chinese population.

Antibodies to Variant MNS Antigens

Alloantibodies directed against novel Glycophorin antigens are commonplace in many Asian populations and have been shown to cause both Haemolytic Transfusion Reactions (HTR) and Haemolytic Disease of the Foetus and Newborn (HDFN). The detection of these antibodies is problematic as commercially manufactured screening cells usually lack the antigens necessary to enable these antibodies to be detected. The use of naturally-occurring phenotype positive cells is also problematic, as not all antibodies detected are clinically significant (many antibodies detected are naturally occurring IgM antibodies that don't cause disease), they can't be identified, and the fact that these cells have limited availability.

The main antibodies of interest that bind to these antigen variants are anti-Mi^a, anti-Vw, anti-Mur, anti-Hut, anti-Hil and anti-MUT. Miltenberger-class antibodies commonly have an IgM component and may be a mixture of IgG and IgM. **IgG antibodies against MUT, Mur, Vw, Mi^a and Hil antigens have been shown to be clinically significant and are capable of causing immediate and delayed transfusion reactions and mild to severe HDFN.**

Methods

Preparation and Insertion of KODE™ Molecules

Peptide sequences were selected to represent the Mur, MUT and Hil antigens using published data. Peptides were manufactured by Mimotopes (Clayton, Australia) and attached to lipid and spacer molecules by KODE Biotech. NMR was used to verify the composition and purity of both the peptides and the final constructs. The completed constructs were inserted into vMNS-negative red blood cell membranes at a range of concentrations and checked for transformation efficiency using monoclonal antibodies. Reactivity of test sera to the transformed cell was evaluated using an Indirect Antiglobulin Test in the BioVue™ platform, and confirmed with DiaMed™ and conventional tube techniques.

Characterisation of Antibodies to vMNS Antigens

Sera that was reactive with GP.Mur-positive cells was obtained from Taiwan and Singapore. These samples were characterised in an assay which could detect IgG anti-peptide antigens. Peptide sequences representing antigens present on variant glycophorins were utilised in an erythrocyte capture ELISA assay to identify the specificity of the antibodies present.

Results

Table 1 Reactivity of ELISA-chacterised sera with red bllod cells transformed with synthetic peptide antigens.

Sample No.	ELISA Specificities	AHG BioVue™ Result		
		Mur	MUT	Hil
1	Hil+Tsen	-	-	-
3	Mur	+	-	-
4	Vw	-	-	-
5	MUT	-	+	-
6	MUT+Mur	+	+	-
7	Mur+Hil+Tsen	+	-	-
8	MUT+Mur	+	+	-

The Mur and MUT peptide antigens displayed the expected reactions whilst the Hil prototype did not detect these two examples of anti-Hil. Transformed cells demonstrated stable antigen levels for at least 10 weeks and there was no change in their ability to detect clinically significant polyclonal alloantibodies (data not shown).

Table 2 Reactivity of sera reactive with natural Mi III cells versus MUT, Mur and Hil transformed red blood cells.

Number Tested	41
IgG Anti-MUT detected	5
IgG Anti-Mur detected	8
IgG Anti-Mur + Anti-Hil detected	3

Table 3 Characterisation of positive serum TAP1.

TAP1 Sera Tested in BioVue™						
BioVue™ card	Natural Mi III cells		Transformed Cells			Control (untransformed cells)
	R_2R_2	R_1R_2	MUT	Mur	Hil	
AHG	10	10	8	0	0	0
Neutral	10*	10*	0	0	0	0

*activity destroyed by DTT treatment.

Table 4 Reactivity of antibody-screen negative sera with MUT transformed red blood cells.

No. of Samples*	500
Antibody screen neg	500
Reactive with MUT + cells	2 (weak)

*samples run on BioVue™ platform at Royal North Shore Hospital, Sydney, Australia.

Conclusions

- KODE™ peptide epitopes have the ability to detect clinically relevant IgG Miltenberger antibodies.
- The non-detection of clinically insignificant naturally-occurring IgM Miltenberger antibodies appears to be a major advantage of this approach.
- Antibody detection is possible using conventional tube haemagglutination methods, as well as in BioVue™ and DiaMed™ platforms.
- Mur and MUT antigens showed acceptable sensitivity and specificity whilst the Hil peptide antigen requires further evaluation to increase its sensitivity.

Acknowledgements

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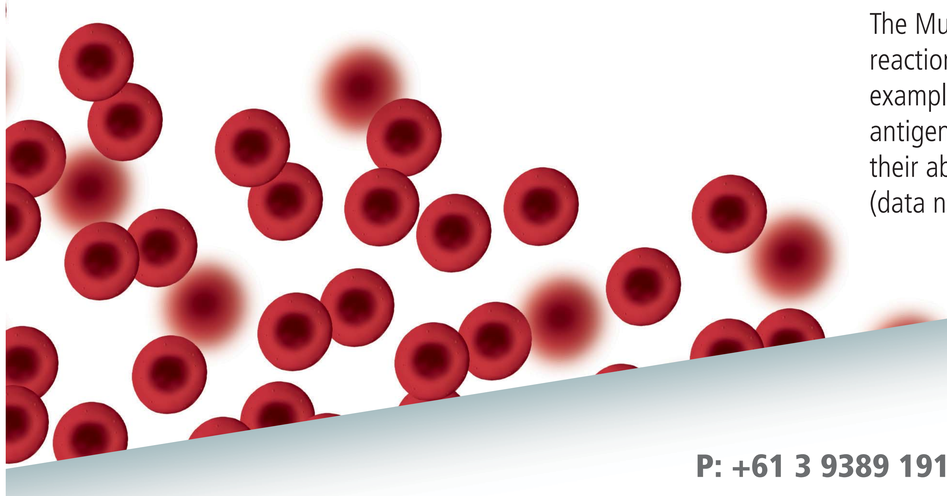

CSL Bioplasma Immunohaematology
P: +61 3 9389 1919 F: +61 3 9389 1646 E: ih@csl.com.au W: www.csl.com.au

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