Evaluation of recombinant blood group proteins in pre-transfusion and antenatal testing in a RCI Laboratory.

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Background

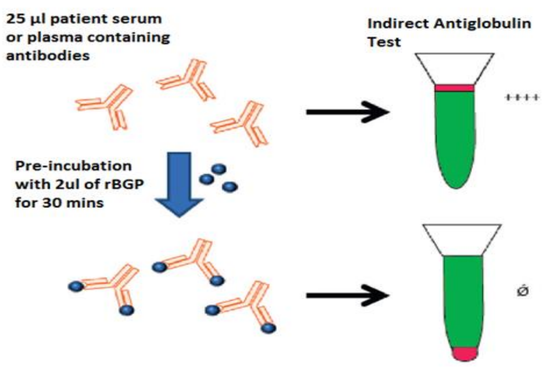
Recombinant blood group proteins (rBGPs) are soluble proteins derived from eukaryotic/prokaryotic expression systems which mimic red cell blood group antigens. They act as inhibiting molecules and are a novel way of elucidating complex antibodies[1]. Current Investigation methods used to resolve complex antibodies such as; multiple antibodies, antibodies to high frequency antigens or high titre low avidity (HTLA) antibodies can be cumbersome and require a great deal of operator expertise. Delays in the provision of suitable red cell units to transfusion dependent patients may result in such cases. This study aimed to evaluate rBGPs for their potential use in a RCI laboratory in the elucidation of complex antibodies in pre-transfusion and antenatal testing.

Materials & Methods

Eleven recombinant blood group proteins sourced from Imusyn were evaluated. The efficacy of the rBGPs was evaluated using 52 samples (n=35 patient samples and n=17 samples received through international exchange programme).

The hemagglutination inhibition assay (HIA) was the method employed with rBGPs as per manufacturer's instructions[2]. Inhibited plasma was then tested with the pertinent red cells by IAT using BioRad gel card method.

Figure 1- Schematic of HIA employed



Source: Ihttps://www.inno-train.de/en/products/rbc-typing/rbc-serology-by-imusyn/recombinant-blood-group-antigens [Accessed 8.10.18]

The study also evaluated how specific rBGPs are to their corresponding antibody. Two case studies received by the RCI laboratory are also described where rBGPs played a central role in antibody investigation and provision of red cell units to patients.

References

[1]Seltsam A *et al* (2014) Recombinant blood group proteins facilitate the detection of alloantibodies to high-prevalence antigens and reveal underlying antibodies: results of an international study. Transfusion 2014 (54), 1823-1830

[2]https://www.inno-train.de/en/products/rbc-typing/rbc-serology-by-imusyn/recombinant-blood-group-antigens [Accessed 8.10.18]

Results: Evaluation of rBGP Efficacy

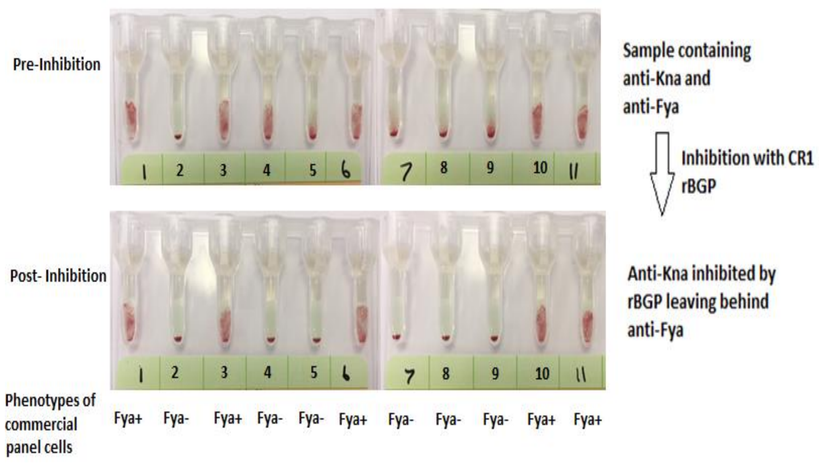
Evaluation of the efficacy of rBGPs is summarised in Table 1

rBPG used	Total no. samples containing corresponding antibody tested n=	No. of samples successfully inhibited n=	% of samples successfully inhibited
HTLA Antibodies			
CR1	15	9	60%
JMH	5	0	0%
Ch(a)	3	3	100%
Rg(a)	-	0	0%
Antibodies to High Prevalence Antigens			
Lu(b)	2	1	50%
Yt(a)	5	4	80%
In(b)	4	3	75%
klkba (cellano)	3	3	100%
Other Specificities			
Fy(a)	9	6	66.6%
Fy(b)	2	0	0%
grKba (Kell)	4	3	75%
Total No. Tested	52	32 (61.5%)	

Table 1- Evaluation of 11 rBGPs listed above against 52 samples containing HTLA type antibodies (n=23), Antibodies to high incidence antigens (n=14) and other specificities (n=15)

Results: rBGP Specificity

Figure 2- Specificity of rBGPs is demonstrated.



Above figure illustrates how recombinant CR1 protein specifically inhibits HTLA antibody, anti-Kn^a in a sample leaving behind an anti-Fy^a also present in the sample for detection.

Discussion

This study has reported an overall inhibition rate of 61.5% of 52 samples tested. 52.2% of HTLA antibodies were inhibited by the pertinent rBGP, these antibody types are difficult to inhibit or neutralise due to their "low avidity" nature. Successful inhibition of 78.5% of antibodies to high incidence antigens was demonstrated. Results from this study have shown that success rates associated with rBGPs are variable across the three categories tested ranging from 0 to 100%. The hypothesis exists that rBGP conformation has an effect on how well it inhibits its corresponding antibody. If the rBGP conformation does not allow for the antibody present in the sample to bind to its corresponding epitope present on the rBGP then inhibition will be incomplete or ineffective. This study also demonstrated that rBGP are specific to their corresponding antibody, leaving behind other antibodies present in the sample for detection. The HIA is a faster, more efficient method of antibody identification compared to current methods.

Case Study 1

Patient: Female 70yrs Phenotype: R1r K-, s-, Fya-, Jkb-
History: Previously identified HTLA-type antibody which was successfully inhibited by AB plasma. Referral on this occasion- serology changed and neutralisation with AB plasma no longer successful. Two units requested.
Investigation: The ratio of AB Plasma: Patient Plasma was increased from 2:1 to 3:1. Along with this the ratio of CR1 rBGP: Patient plasma was increased from 2µl:25µl to 6µl:25µl. The increase in both the ratio of AB plasma and rBGP resulted in effective inhibition of the HTLA-type antibody present. Following inhibition by CR1 recombinant protein, an apparent anti-Fya was detected. Inhibited plasma was also used in pre-compatibility testing to supplement results.
Result/Conclusion: The use of rBGP at a ratio of 6µl:25µl (dilution factor 0.24:1) is preferrable as the investigation did not solely rely on plasma diluted 3:1 thus reducing the risk of diluting a clinically significant antibody. The patient was issued **E- K- Fya- Serologically least incompatible red cells.**

Case Study 2

Patient: Female, 89yrs Hb: 8.2gr/dl,Phenotype: R1R2 K- Investigation and 2 units requested urgently.
Investigation: Positivity seen in most reagent panel cells by IAT. Negative reaction detected in one cell by IAT (Dob-) thus anti-Dob suspected. Recombinant Dob protein used in HIA allowed for quick confirmation of anti-Dob present along with the identification of anti-K (cells 2&6) and a white cell antibody (cells 4 &8).

Figure 3- Panel results : Plasma neat and following inhibition with recombinant Dob protein

Test/panel	1	2	3	4	5	6	7	8	9	10	11
Enzyme Panel	4+	4+	4+	4+	-	4+	-	4+	-	4+	4+
IAT Panel	3+	4+	3+	3+	-	3+	-	3+	-	3+	3+
rDob inhibited plasma + IAT	-	4+	-	2+	-	4+	-	0.5 +	-	-	-
PBS Ctl + IAT	3+	4+	3+	3+	-	3+	-	3+	-	3+	3+

Result: The use of rBGPs in this case allowed for fast identification of anti-Dob in the patient sample as opposed to relying on rare antigen negative cells. Dombrock typing of units is not routinely performed and the protocol for anti-Dob is "crossmatch compatible red cells @ 37C". Therefore, fast identification of anti-Dob allowed for the screening/ compatibility testing of 30+ units in order to promptly provide compatible units for the patient.

Conclusion

rBGPs:

- Are specific towards their pertinent antibody
- Allow for fast antibody identification using the HIA
- Reduce the reliance on rare antigen negative cells
- Are however, not effective 100% of the time and therefore cannot replace current methods
- May be implemented as a supplementary method to validated techniques