Rabbit erythrocyte stroma bind IgM antibodies regardless of blood group specificity

Marks et al. demonstrated that formaldehyde-fixed rabbit erythrocytes were a useful reagent for the adsorption of cold "autoagglutinins", directed at carbohydrate antigens such as I and IH, and could be used to remove these antibodies without reducing the reactivity of alloantibodies.[1] Another study that used both fixed whole erythrocytes and rabbit erythrocyte stroma demonstrated similar adsorption of anti-I and anti-IH but also observed that some examples of anti-D, anti-E and anti-Le^b, were also adsorbed.[2] Antibodies to the Vel blood group antigen are unusual in that they are often a mixture of IgM and IgG and have a wide thermal range. These characteristics hint at the possible carbohydrate nature of the antigen, an observation that was perhaps reinforced by the unusually high incidence of the P_2 phenotype among Vel-negative individuals in Northern Sweden.[3] Following reports both anecdotal and documented,[4,5] that anti-Vel sera were adsorbed with rabbit erythrocyte stroma, we performed a study to investigate this further. Four different anti-Vel sera that had been shown previously to contain both IgG and IgM-specific anti-Vel (data not shown) were adsorbed with rabbit erythrocyte stroma (RESt™, kindly donated for this study by Immucor, Inc. Norcross, GA) according to the manufacturer's instructions. Briefly, aliquots of RESt™ were packed by centrifugation for 5 minutes at 2700 rpm and with no brake. The supernatant buffer was removed and discarded. Antibody (1mL) was mixed with the stroma and incubated on wet ice for one hour, mixing occasionally. The tubes were centrifuged for 10 minutes at 2700rpm with no brake and the adsorbed plasma harvested. Adsorbed and unadsorbed plasma were tested by a saline indirect antiglobulin test (saline-IAT) and a saline direct agglutination test (saline-RT) following 30 minute incubation at 37^{o}C and room temperature, respectively. The results are shown in Table 1.

Approximately a half-grade drop in reactivity was observed in the indirect antiglobulin test (IAT) in all adsorbed anti-Vel sera. While this small difference might be explained by a

dilution effect, more striking was the adsorption of the IgM component of the Vel antibodies identified by the saline agglutination tests. Reactivity was reduced by at least one grade in all anti-Vel sera and reactivity of Vel#2 and #4 were completely removed by adsorption.

To determine if the adsorption was Vel-specific or possibly some non-specific adsorption of IgM antibodies, human polyclonal IgG and monoclonal IgM anti-D and anti-K were analysed in parallel. As seen in Table 2, significant titre reductions were observed with IgM anti-D and anti-K reagents but not with IgG anti-D and anti-K antisera. These results suggest IgM antibodies, regardless of blood group specificity, bind to rabbit erythrocyte stroma. The mechanism for binding was not investigated. There are possible clinical consequences based on this knowledge: inappropriate use of this otherwise useful reagent may remove newly formed IgM antibodies that have the potential to cause a haemolytic transfusion reaction. As always, we stress the importance of identifying an antibody before employing techniques to remove or circumvent it in order to identify other more clinically relevant antibodies.

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Table 1. Reactivity of anti-Vel adsorbed with RESt™

	RBCS*	K-	K+	K-	K+	K-	K+	K-	K+
		Saline-IAT				Saline-RT			
Antibody ID	Specificity	Unadsorbed		Adsorbed		Unadsorbed		Adsorbed	
1	Vel	3+	3+	2+^{s}	2+^{s}	3+	2+^{s}	1+	1+
2	Vel	2+^{s}	2+	2+	2+	2+	2+^{s}	0	0
3	Vel, K	4+	4+	3+	3+^{s}	3+^{s}	3+^{s}	2+	3+
4	Vel, K	1+^{w}	3+	0	3+	1+^{w}	1+^{w}	0	0
5	K	0	3+	0	3+	0	0	0	0

* Both test RBCs were Vel+.

Table 2. RESt™ adsorption of anti-D and anti-K

		ANTIBODY DILUTION (IN 6%BSA/PBS)									
Antibody		1	10	20	40	80	160	320	640	1280	2560
IgM anti-D	unadsorbed	3+^{s}	3+^{s}	3+^{s}	3+^{s}	3+	2+^{s}	2+	2+	1+	1+^{w}
Clone BS226	adsorbed	3+^{s}	2+	1+^{s}	1+	1+^{w}	0	0	0	0	0
IgG anti-D	unadsorbed	3+^{s}	3+^{s}	3+	3+	3+	2+	2+	1+^{w}	0	0
polyclonal	adsorbed	3+	3+	3+	2+	2+	1+	1+	0	0	0
IgM anti-K	unadsorbed	2+^{s}	2+^{s}	2+	1+^{s}	1+	1+^{w}	0	0	0	0
Clone MS56	adsorbed	1+	1+	1+	1+^{w}	0	0	0	0	0	0
IgG anti-K	unadsorbed	3+^{s}	3+^{s}	3+	3+	2+^{s}	2+	1+	1+	1+^{w}	0
polyclonal	adsorbed	3+^{s}	3+^{s}	3+	2+^{s}	2+^{s}	2+	1+	1+	1+^{w}	0

References

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