Use of monoclonal Jk^a and Jk^b reagents in phenotyping red cells with a positive direct antiglobulin test

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Twenty-five red cell samples with a positive anti-IgG direct antiglobulin test (DAT) were tested with human monoclonal IgM Jk^a and Jk^b antibodies. Red cell samples were first tested by a 5-minute incubation tube test with the monoclonal antibodies (MAbs). The same red cells were then chloroquine diphosphate (CDP)-treated, and retested. Eleven of the CDP-treated samples were also tested with conventional polyclonal antibodies (PAbs) that required a 37°C incubation for 30 minutes, followed by an indirect antiglobulin test. The Jk^a and Jk^b MAbs consistently gave the same phenotype results both on untreated DAT-positive red cells and on the same cells after CDP treatment. Two of the CDP-treated samples had diminished antigen expression with the MAbs, a finding that may have been caused by the CDP treatment. One untreated sample, which spontaneously agglutinated in a low-protein medium, was incorrectly phenotyped with the anti-Jk^a MAb, but both MAbs and PAbs gave the same correct results with the CDP-treated cells. These findings illustrate that the use of Jk^a and Jk^b MAbs in phenotyping DAT-positive specimens is practical and beneficial. ***Immunohematology*** 1994;10:16.

Red blood cells (RBCs) with a positive direct antiglobulin test (DAT) may be difficult to phenotype correctly. Although some monoclonal, saline, and chemically modified reagents are available, the standard practice for phenotyping DAT-positive samples has been to dissociate the IgG antibody molecules from the RBCs using chloroquine diphosphate (CDP).[1] When successful, phenotyping reagents that require an indirect antiglobulin test (IAT) can be used. However, the CDP procedure does not always effectively remove bound IgG antibody.[2] Manufacturers of the monoclonal reagents state that the reagents do not require the use of diluent controls to correctly phenotype DAT-positive specimens.[3,4] This study was designed to determine if IgM Jk^a and Jk^b monoclonal antibodies (MAbs) could accurately phenotype in vivo-coated DAT-positive samples both before and after CDP treatment. Some of the CDP-treated red cell samples were also tested with conventional Jk^a and Jk^b polyclonal antibodies (PAbs).

Materials and Methods

Twenty-five red cell samples with a positive DAT due to IgG were tested. Samples were selected from donors and patients who had not been transfused in the previous 90 days. Anticoagulated or clotted samples were obtained from local hospitals, local reference laboratories, a community blood center, and an out-of-state reference laboratory. Samples were stored at 2°-8°C until tested, and testing was completed as soon as possible after samples arrived. Because of the time required for shipping, a few samples were 39 days old before testing.

The DAT-positive samples were tested for the Jk^a and Jk^b antigens by three different methods. The first method involved testing untreated DAT-positive RBCs with recently licensed Bioclone™ Jk^a and Jk^b MAbs (Ortho Diagnostic Systems, Inc., Raritan, NJ) using a 5-minute incubation tube test at room temperature. The second method tested CDP-treated RBCs in the same manner. RBCs were treated with CDP using the method described by Edwards et al.[2] Specimens that did not have complete IgG removal after a 2-hour room temperature incubation with CDP were CDP-treated again and incubated at 37°C for 30 minutes. After each CDP treatment, a DAT was performed to assess antibody removal. The RBCs were then phenotyped again with Jk^a and Jk^b MAbs. The third method involved testing 12 of the CDP-treated RBCs with conventional Jk^a and Jk^b PAbs (Ortho), requiring a 30-minute incubation at 37°C followed by an IAT. PAb testing was not performed on all of the CDP-treated RBCs because the reagents were not available at the time of testing.

Agglutination results were read macroscopically and graded ($1+^w$ to 4+).[5] Control Jk(a+b+), Jk(a-b+), and Jk(a+b-) RBCs were included each day testing was done. Jk(a+b+) samples were tested for spontaneous (IgM)

agglutination by incubation in a low-protein medium (6% albumin) for 5 minutes, since a 5-minute room temperature tube test was employed with the MAb reagents. In addition, two cells of known phenotype were coated in vitro with IgG and tested with the MAb reagents.

Results

Twenty-two of the red cell samples had DATs of 1+ agglutination or better.

Twenty-four of the 25 samples gave the same reaction with the MAbs before and after CDP treatment (Table 1). One untreated sample (no. 2) reacted as Jk(a+b+) with the MAb reagents. The same sample, after CDP treatment, reacted as Jk(a–b+) with the MAb reagents. It also reacted as Jk(a–b+) with the PAb reagents after 37°C CDP treatment. The discrepancy was explained when it was found that this sample spontaneously agglutinated at room temperature in a low-protein medium. Of the 25 samples, 13 reacted as Jk(a+b+) using both MAb methods. One Jk(a+b+) sample (no. 1) had weakened antigen expression with the Jk^a MAb after 37°C CDP treatment (Table 1). The IgG was not completely removed by CDP, and it was therefore not possible to test this sample with conventional PAbs. Five samples reacted as Jk(a+b–) and six samples reacted as Jk(a–b+) using both MAb methods. One of the Jk(a–b+) samples (no. 18) also had weakened antigenic expression after CDP treatment. CDP effectively removed bound IgG from this sample, but PAb testing was not performed because reagents were not available at the time of testing. No deterioration of Jk^a and Jk^b antigens was evident on the few samples that had been stored for 39 days.

Discussion

The reagents used were a blend of human monoclonal antibodies prepared from large-scale tissue culture supernatants.[3] There has been concern that MAbs that recognized a single epitope might fail to demonstrate the same specificity as PAbs.[6] The development of blended MAbs appears to address this concern in

Table 1. Phenotype results with Jk^a and Jk^b MAbs on DAT (IgG)-positive red cells before and after treatment with chloroquine diphosphate (CDP) and with PAbs after CDP treatment

Sample number	MAb (untreated)		MAb (CDP-treated)		PAb (CDP-treated)		Complete IgG removal
	Jk^a	Jk^b	Jk^a	Jk^b	Jk^a	Jk^b	
1	+*	+	+†	+	NT‡	NT	no
2	+§	+	0‖	+	0	+	yes
3	+	+	+	+	QNS¶	QNS	yes
4	+	0	+	0	NT	NT	no
5	+	+	+	+	+	+	yes
6**	0	+	0	+	0	+	yes
7	0	+	0	+	0	+	yes
8	+	+	+	+	+	+	yes
9	+	+	+	+	NT	NT	no
10	0	+	0	+	0	+	yes
11**	+	0	+	0	+	0	yes
12	+	+	+	+	+	+	yes
13	+	+	+	+	+	+	yes
14	+	+	+	+	+	+	yes
15	0	+	0	+	NT	NT	yes
16	0	+	0	+	NT	NT	yes
17	+	+	+	+	NT	NT	yes
18	0	+	0	+††	NT	NT	yes
19**	+	+	+	+	NT	NT	yes
20	+	+	+	+	NT	NT	yes
21	+	+	+	+	NT	NT	yes
22	+	+	+	+	NT	NT	no
23	+	0	+	0	+	0	yes
24	+	0	+	0	+	0	yes
25	+	0	+	0	NT	NT	no

* 1+^{w} or greater agglutination
† Weakened antigen after 37°C CDP treatment
‡ Not tested
§ Sample agglutinated in low-protein medium
‖ Negative
¶ Insufficient sample
** DAT ≤ 1+
†† Weakened antigen after 2-hour room temperature CDP treatment

Note: In vitro IgG-coated and uncoated controls (Jk[a+b+] and Jk[a+b–]) reacted as expected with MAbs.

recent clinical trials of Rh and Kidd MAbs.[3,7] Because these reagents are IgM MAbs suspended in a low-protein medium, they appear promising as "simple direct agglutination system(s)" that Issitt predicted would be possible with advanced MAb technology.[6]

Since these reagents correctly identified the phenotypes of DAT-positive samples, and because similar results were obtained with both untreated and CDP-treated RBCs, these reagents offer the advantages of rapid and accurate results. A 5-minute tube test is simpler and less time consuming than conventional PAb testing following CDP treatment of DAT-positive RBCs. (RBCs from patients who have been recently transfused would require a separation technique before testing in order to demonstrate a true phenotype.)[5] These reagents are not useful with RBCs coated with IgM antibodies, as demonstrated in sample no. 2 (Table 1). Use of a low-protein control is therefore recommended when testing apparent heterozygous samples.

It is believed that the weakened antigen expression of two of the samples (Nos. 1 and 18) was due to CDP treatment (Table 1), even though previous studies did not demonstrate Kidd antigen destruction. However, the MAbs correctly phenotyped five samples that were still IgG-coated after CDP treatment, including those with weakened expression.

As other MAbs are produced and made commercially available, both transfusion services and reference laboratories will benefit from the ability to directly phenotype DAT-positive samples.

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References

1. Laird-Fryer B. Kits and special reagents: what to use and when. In: Ellisor SS, Wallace ME, eds. Blood bank reagents: what to use and when. Arlington, VA: American Association of Blood Banks, 1985:83–4.
2. Edwards JM, Moulds JJ, Judd WJ. Chloroquine dissociation of antigen-antibody complexes: a new technique for typing red blood cells with a positive direct antiglobulin test. Transfusion 1982;22:59–61.
3. Jakway J, Setcavage T, Davies D. Reactivity of monoclonal anti-Jk^a and anti-Jk^b reagents (abstract). Transfusion 1991;31 (Suppl):24S.
4. Two studies comparing the BioClone system of monoclonal antibodies with polyclonal (human-source) reagents and reagents for anti-human globulin testing. Ortho Diagnostic Systems, Inc., 1990 (Publication No. DSI-430).
5. Walker RH, ed. Technical manual. 10th ed. Arlington, VA: American Association of Blood Banks, 1990:528–9, 551–4.
6. Issitt PD. The impact of monoclonal antibodies in blood group serology. Transfusion 1989;29:58–63.
7. Jakway J, Voak D, Setcavage T, Noonan E, Darner J, Davies D. Evaluation of monoclonal C, E, and e reagents (abstract). Transfusion 1991;31(Suppl):24S.

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