

# Immunohematology

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# In vitro reactions with red blood cells that are not due to blood group antibodies: a review

G. GARRATTY

In vitro reactions not due to blood group antibodies are sometimes encountered when typing red blood cells (RBCs) or performing compatibility testing. Such ABO or Rh typing anomalies can lead to serious errors or incompatible crossmatches, and may cause a delay in supplying blood for transfusion. Many of these problems are because the patient has an antibody that reacts with a chemical present in the commercial RBC suspension media, commercial antisera, or commercial antibody potentiators (e.g., albumin or low-ionic-strength solution [LISS]). Table 1 lists antibodies to such chemicals that have been described in the literature. Some other more unusual causes are also discussed.

## Problems Due to Antibodies to Chemicals Present in RBC-Suspending Media

Table 2 lists the chemicals that are usually present in commercial RBC-suspending media available in the United States. Some of these chemicals are present in the majority of commercial media, whereas others are present in only one or two manufacturers' media. All of the suspending media are modifications of Alsevers solution. Manufacturers often are not willing to reveal the formula of their suspending medium but can usually be persuaded to reveal the chemicals present; sometimes this information is given in the package insert.

### *Antibodies to antibiotics*

The first description of a penicillin antibody came from a hospital blood transfusion service. Before 1965, donor blood was collected into glass bottles. A pilot tube of donor blood was attached to the main unit providing RBCs for crossmatching. As this tube had to be entered several times, it often became contaminated. One practice was to add penicillin to the tube to prevent bacterial contamination. Ley et al.<sup>1</sup> encountered a patient's serum that reacted with RBCs from all donor units, sug-

**Table 1.** Reported antibodies to chemicals added to reagents

<u>Antibiotics</u>	<u>Bacteriostatic/antifungal reagents</u>
neomycin	Paraben®
chloramphenicol	thimerosal
gentamycin	sodium azide
<u>Sugars</u>	<u>Miscellaneous</u>
glucose	EDTA
	inosine
<u>Dyes</u>	citrate
acriflavine	sodium caprylate
yellow #5 tartrazine	

gesting an antibody to a high-frequency antigen. They showed that blood from the main unit was compatible and that only RBCs from the pilot tubes reacted. RBCs in the pilot tube had become coated with penicillin in vitro, and the patient's serum contained a penicillin antibody, the first description of such an antibody. All manufacturers add antibiotics to their RBC suspension media. Since the report by Ley et al.,<sup>1</sup> penicillin is no longer used. Most media contain chloramphenicol and neomycin sulfate, but some manufacturers add gentamicin, tetracycline, or streptomycin. Antibodies to any of these antibiotics can be present in a patient's serum,<sup>2</sup> but only antibodies to neomycin,<sup>3</sup> chloramphenicol,<sup>4,6</sup>

**Table 2.** Chemicals present in commercial RBC-suspending media

<u>Many companies</u>	<u>A few companies*</u>
adenine	adenosine
chloramphenicol	citric acid
glucose	gentamicin
inosine	guanosine
neomycin sulfate	hydrocortisone
sodium chloride	magnesium sulfate
sodium citrate	potassium chloride
	potassium phosphate
	sodium acetate
	sodium bicarbonate
	sodium gluconate
	sodium phosphate
	sodium pyruvate
	sucrose

\* Sometimes a single company

and gentamicin<sup>7</sup> have been described, so far, as causing a problem with blood typing.

The reactions observed are probably because of a similar mechanism used to explain the reactions of antibodies causing drug-induced immune hemolytic anemia and/or positive direct antiglobulin tests (DATs).<sup>2,8,9</sup> Some antibiotics (e.g., penicillin) bind covalently to RBC membrane proteins.<sup>2</sup> Penicillin antibodies react with penicillin-coated RBCs but are inhibited if penicillin is added directly to the serum containing the antibody. Thus, in the phenomenon described by Ley et al.,<sup>1</sup> penicillin antibodies reacted with reagent RBCs after the RBCs were washed free of the penicillin-containing suspension medium, because the washed RBCs were penicillin-coated. In contrast, neomycin, chloramphenicol, and gentamicin do not seem to bond covalently to RBC membranes and are easily washed away. The reports of ABO grouping problems due to these antibiotics<sup>3-7</sup> suggest that the *in vitro* reactions parallel those described for the drugs reacting by the so-called "immune complex" mechanism.<sup>2,8,9</sup> That is to say, the reactions are seen only when the patient's serum, containing the antibody, is added to RBCs in the presence of the antibiotic (i.e., in the commercial RBC suspension medium). If the commercial RBCs are washed before addition to the patient's serum, no reactions are observed.

#### *Antibodies to hydrocortisone*

In 1973, Mann described 25 examples of patients' sera containing IgM antibodies to hydrocortisone.<sup>10</sup> These sera agglutinated RBCs suspended in a commercial medium containing hydrocortisone. Other similar examples have been described.<sup>11-15</sup> Pinto and Rimón<sup>16</sup> showed that 26 percent of healthy adults had IgM antibodies to hydrocortisone in their sera; antibodies were rarely found in children less than 3 years of age. An interesting finding was that 80 percent and 100 percent of patients with syphilis and rheumatoid arthritis, respectively, had antibodies to steroids in their sera.<sup>16</sup> Hydrocortisone antibodies agglutinate RBCs suspended in hydrocortisone-containing media. If the RBCs are washed free of the hydrocortisone-containing medium, they do not react with sera containing hydrocortisone antibodies.

#### *Antibodies to sugars*

In 1964, Gray showed that sera from three blood donors and two patients would agglutinate RBCs that had been exposed to lactose.<sup>17</sup> The RBC suspending medium contained dextrose and lactose. The sugar

appeared to be adsorbed onto the RBC membrane as the antibody reacted with washed RBCs, and, similar to penicillin, when lactose was added to the patients' serum, the antibody was inhibited. Mougey described an antibody that reacted with all RBCs that had been frozen.<sup>18</sup> The antibody appeared to be directed against the sodium lactate that was present in the freezing solution. Lewis et al.<sup>19</sup> detected an antibody that agglutinated all RBCs tested; the antibody appeared to be directed against glucose, which had become adsorbed onto the target RBC membranes during storage. Bird and Roy<sup>20</sup> and Lalezari et al.<sup>21</sup> showed that most human sera contain antibodies to a wide range of sugars (Table 3). Such antibodies will react with RBCs incubated in these sugars and then washed free of the sugar. Using routine procedures, Bird and Roy found only 0.1–0.8 percent of sera reacted with glucose-, galactose-, mannose-, fructose-, lactose-, and dextran-coated RBCs, but 48 percent reacted with melibiose-coated RBCs. Using a more sensitive AutoAnalyzer technique, Lalezari et al.<sup>21</sup> found 2–22 percent of sera reacted with glucose-, galactose-, mannose-, fructose-, lactose-, and dextran-coated RBCs, and 100 percent of the sera reacted with melibiose-coated RBCs.

Morel et al.<sup>22</sup> and Reid et al.<sup>23</sup> described antibodies that showed M and N specificity but only when using RBCs that had been stored in dextrose (glucose)-containing media. The antibodies described by Morel et al.<sup>22</sup> would only react with M+ or N+ RBCs stored in dextrose solutions at a pH above 7.0, thus the RBCs from only one commercial panel showed reactivity. This company added sodium bicarbonate to its suspension medium to stabilize the pH. The antibodies appeared to be detecting an antigen in which interactions between dextrose and the amino group of the N-terminal amino acid(s) of the MN antigens produced a new specificity similar to that seen with formaldehyde-induced antibodies (see below). It was proposed that the antibodies be called anti-M<sup>D</sup> or -N<sup>D</sup> to denote the sialic acid independent expression of M and N conferred on RBCs by exposure to dextrose.<sup>22</sup>

**Table 3.** Antibodies in normal human sera reacting with washed RBCs after incubation in sugar solutions

	Percent sera reacting	
	Bird and Roy <sup>20</sup>	Lalezari <sup>21</sup>
D-glucose	0.3	1.9
D-galactose	0.5	11.8
D-mannose	0.6	27.5
lactose	0.6	22.5
dextrose	0.8	22.0
melibiose	48.1	100.0
sucrose	NT*	0

\* Not tested

#### *Antibodies to inosine*

Vengelen-Tyler et al.<sup>24</sup> described two sera that reacted with RBCs that had been stored in a commercial RBC-suspending medium containing inosine. The reactions did not occur when washed RBCs were used. The antibodies were IgM and caused agglutination of the RBCs at room temperature; one antibody reacted weakly at 37°C. Nason et al.<sup>25</sup> described an anti-P<sub>1</sub> that was inhibited by inosine and did *not* react with RBCs that were suspended in a commercial medium containing inosine.

#### *Hemagglutinating properties dependent on polycarboxyl groups*

Sera sometimes contain antibodies that react with RBCs that have been exposed to ethylenediaminetetraacetic acid (EDTA).<sup>26-29</sup> Some companies add EDTA to their A and B RBC-suspension media. An ABO grouping discrepancy may occur when using such RBCs to confirm the ABO types of patients with EDTA antibodies. The antibodies are usually IgM agglutinins. They do not react with washed reagent RBCs. Beck et al.<sup>27</sup> showed that the antibodies react with polycarboxyl groups. Thus, the antibodies will react with a wide range of chemicals with polycarboxyl groups (e.g., citrate, L-tartrate, succinate, acetate, lactate, propionate, valerate, and butyrate).<sup>28</sup> Reports in the earlier literature, of calcium- and citrate-dependent agglutinins, may sometimes be explained by the same mechanism.<sup>30-32</sup> In 1997, Joshi<sup>33</sup> described a citrate-dependent autoantibody that caused an error in ABO grouping. Tedrow and Zeigler<sup>34</sup> described an anti-A<sub>1</sub> that was inhibited by EDTA (i.e., it did not react with the group A RBCs from the manufacturer that uses EDTA in its suspending medium). Green et al.<sup>35</sup> described a patient with cold agglutinin syndrome associated with an IgM anti-Pr<sub>1d</sub>. When commercial unwashed RBCs were used, the cold agglutinin titer was only 16 at 4°C, but when the same RBCs were used after four washes, the titer was > 2,000. The antibody was found to be inhibited by the citrate present in the RBC-suspending medium.

#### **Problems Because of Antibodies to Chemicals Added to Commercial Antisera**

Most of the problems associated with commercial antisera are due to antibodies, present in a patient's serum, to dyes or bacteriostatic agents that are added to the antisera.

#### *Antibodies to dyes*

There have been three patients described with antibodies to acriflavine<sup>36,37</sup> and one patient with antibodies

to yellow #5 tartrazine, a yellow dye added to commercial anti-B.<sup>38</sup> These antibodies caused ABO anomalies if the patients' RBCs were suspended in their own plasma (i.e., containing the antibody to the dye) and added to the anti-B containing the dye. No problems occurred if the patient's washed RBCs were used.

#### *Antibodies to bacteriostatic agents in antisera*

Reviron et al.<sup>39</sup> described an anti-I cold agglutinin that was enhanced (the anti-I reacted at 4°C but not at 22°C, without sodium azide being present) in the presence of sodium azide. This led to difficulty in determining the ABO type of the patient when the patient's unwashed RBCs were tested with ABO-typing sera containing sodium azide. When washed RBCs were used, no problems were encountered. Although no other blood grouping problems because of antibodies to sodium azide have been described, it should be noted that sodium azide or another bacteriostatic agent, thimerosal (merthiolate/sodium ethylmercurithiosalicylate), are sometimes added to LISS.

#### **Problems With Antibodies to Chemicals Added to Commercial Antibody Potentiators**

#### *Bovine albumin*

In 1956, Weiner described three patients having autoagglutinins that only reacted in the presence of bovine albumin.<sup>40</sup> The albumin antiglobulin test was the most popular crossmatching procedure for more than 30 years in the United States, so many other examples were subsequently reported.<sup>41</sup> Problems with Rh typing were also reported.<sup>42,43</sup> Most of the antibodies involved were IgM agglutinins but some reacted only by the indirect antiglobulin test (IAT).<sup>41,45</sup>

In 1969, Golde et al.<sup>46</sup> showed that the "albumin autoagglutinin" phenomenon was due to antibodies in the patients' sera reacting with sodium caprylate, which is added as a stabilizer during the heating phase when bovine albumin is manufactured. Later, Spence et al.<sup>47</sup> and Beck et al.<sup>48</sup> found that the antibodies had a broader specificity and would react with short chain fatty acids. Beck et al.<sup>48</sup> suggested that these antibodies should be called fatty acid-dependent antibodies. They also showed that the patient's serum would react with RBCs in the presence of sodium caprylate without any bovine albumin being present.<sup>48</sup> Two examples of fatty acid-dependent antibodies were reported as having blood group specificity, an anti-c<sup>49</sup> and an anti-e.<sup>50</sup> Hossaini et al.<sup>51</sup> were able to induce caprylate-dependent antibodies in rabbits.

Not all albumin autoagglutinins are due to fatty acid-dependent antibodies. Some of them do not react without bovine albumin being present. Such antibodies appear to be antibodies to bovine albumin or contaminants in the bovine albumin.<sup>52</sup> In 1981, Hirayama et al.<sup>53</sup> showed that 96 percent of sera from pregnant women contained anti-albumin (reacting with bovine and human albumins). In 1993, Atkinson et al.<sup>54</sup> showed that 100 percent of sera from patients with insulin-dependent diabetes mellitus, 10–31 percent of patients with autoimmune disease, and 3 percent of “normal” subjects contained antibodies to bovine albumin. Commercial bovine albumin can be contaminated with bovine gamma globulin.<sup>52</sup> Approximately 1 in 500 healthy individuals have antibodies to bovine proteins.<sup>55</sup> Up to 50 percent of IgA-deficient individuals appear to have antibodies to ruminant proteins in the bovine albumin.<sup>56</sup>

It is interesting to note that sodium caprylate is still commonly used in the manufacture of 5 percent and 25 percent albumin and in plasma protein fraction for intravenous therapy. Albumin (25%) is also used as a stabilizer for freeze-dried plasma protein concentrates (e.g., coagulation factor and intravenous immunoglobulin). So far, no clinical reactions have been attributed to anti-caprylate, but perhaps it should be considered more often in patients who have hemolytic and nonhemolytic reactions following transfusion of albumin or other plasma products.

### LISS

Some antibodies that will only react in LISS have been shown to be reacting with chemicals added to the LISS and are not really ionic-strength-dependent. Antibodies to two preservatives (methyl paraben<sup>57,58</sup> and thimerosal<sup>59-66</sup>) added to commercial LISS were shown to be causing the reactions with LISS-suspended RBCs. Of great interest was that the paraben antibodies all had anti-Jk<sup>a</sup> specificity (i.e., would only react with LISS-suspended Jk[a+] RBCs), and were also complement dependent (i.e., would only react by IAT using antiglobulin sera [AGS] containing anti-C3). The anti-Jk<sup>a</sup> did not react by saline, albumin, or enzyme techniques, nor when the LISS did not contain paraben. Halima et al.<sup>57</sup> showed that the paraben-dependent antibodies had a broader specificity and would react with RBCs in the presence of any methyl esters of hydroxybenzoic acid. It is not known why there is an association with the Jk<sup>a</sup> antigen. There have been seven reports of thimerosal antibodies causing blood bank problems.<sup>59-66</sup> Four of these antibodies were enhanced by thimerosal rather than being thimerosal-dependent.<sup>63,66</sup> One antibody was only

detected by a Polybrene technique when the RBCs had been preincubated with merthiolate.<sup>65</sup> Three examples showed preference for certain blood group antigens (i.e., I,<sup>59</sup> pdl,<sup>60</sup> e/Ce<sup>66</sup>). The thimerosal-enhanced reactions are very interesting, as they suggest that thimerosal may act as a potentiator or modify the RBC membrane so that some antibodies react better. Arndt et al.<sup>66</sup> described a patient with anti-Ce + e that were only detected with ficinized RBCs or with untreated RBCs in the presence of Polybrene<sup>®</sup> (without thimerosal being present) or thimerosal.

Malyska et al.<sup>67</sup> showed that if RBCs were stored without plasma in a low ionic-strength medium containing neomycin, there was a loss of protease-sensitive antigens. Interestingly, there was an increased binding of anti-D and enhanced reactions with agglutinating anti-D. The reactions were only observed in the presence of neomycin, LISS, and white cells (i.e., the buffy coat); all three had to be present. It was pointed out that neomycin, an aminoglycoside, is known to bind progressively to phosphate groups of polyphosphositides, which are present on RBC membranes.

### Miscellaneous Reactions That Are Not Due to Blood Group Antigen–Antibody Reactions

#### *When bovine thrombin is added to plasma in vitro*

Bovine thrombin is used by immunohematologists to convert plasma to serum when it is suspected that coagulation is incomplete and that fibrin clots may interfere with crossmatching. This can sometimes lead to false positive results with all RBCs tested. There are two major causes for this: the patient's plasma may contain antibodies to bovine thrombin, or the bovine thrombin may contain heterophile antibodies against human RBCs. The latter can be controlled by ensuring that only a small amount (< 50 units) of the bovine thrombin is added to the patient's plasma.<sup>68</sup>

#### *Antibodies to formaldehyde*

In 1972, Howell and Perkins showed that 3 percent of patients undergoing chronic hemodialysis made N-like antibodies; both N+ and N– patients made antibodies.<sup>69</sup> Other authors confirmed these findings.<sup>70-85</sup> It was suggested that (1) the antibodies might be induced by an antigen on foreign surfaces (e.g., membranes) of the extracorporeal circuit; (2) the RBC N antigen might be modified by exposure to foreign surfaces or trauma; and (3) the formaldehyde, used to sterilize the dialysis membranes, might alter the normal N antigen. The latter

hypothesis seems the most likely to explain the findings. Harrison et al.<sup>71</sup> suggested a relationship to the use of reusable dialyzers that had been sterilized with formaldehyde. No antibodies were found in sera of patients using dialyzers sterilized with ethylene oxide. Crosson et al.<sup>72</sup> did not confirm this relationship, finding N-like antibodies in patients who were not using formaldehyde-sterilized equipment. Boettcher et al.<sup>73</sup> showed that two N-like antibodies showed stronger reactivity against formaldehyde-treated RBCs and that sometimes only formaldehyde-treated RBCs reacted. Fassbinder et al.<sup>77</sup> confirmed and extended these findings in 68 sera with N-like antibodies detected in 325 hemodialysis patients. The 68 patients were all using formaldehyde-sterilized dialyzers. Sandler et al.<sup>78</sup> detected N-like antibodies in 27 percent of 22 patients using formaldehyde-sterilized dialysis equipment, but no antibodies were detected in 71 patients using disposable presterilized equipment. Ninety-one percent of the former, and none of the latter, had antibodies that reacted with formaldehyde-treated RBCs (N+ and N- RBCs).<sup>78</sup> The authors suggested that there may be two populations of antibody: anti-N-like and anti-formaldehyde. Sharon<sup>79</sup> showed that formaldehyde antibodies are produced first, about 6 months after hemodialysis, then N-like antibodies appear. These findings would suggest that as the formaldehyde antibodies increase in strength, they become crossreactive, appearing to be anti-N-like. The N-like antibodies appear to be IgM and the formaldehyde antibodies are mainly IgG.<sup>78,83</sup> Dzik et al.<sup>84</sup> showed that 19 percent of patients using formaldehyde-sterilized equipment developed positive DATs because of antiformaldehyde antibodies reacting in vivo with the patient's formaldehyde-affected RBCs. A few cases of decreased RBC survival, probably due to this phenomenon, have been described.<sup>70,77</sup> As early as 1971,<sup>85</sup> an N-like antibody was thought to be the cause of renal transplant failure; in retrospect, this was probably due to the phenomena described above.

#### *Contaminating antibodies in commercial polyclonal antisera*

We have known for years that commercial polyclonal anti-A, anti-B, and anti-A,B could contain anti-T, Tn, etc. This led to problems when typing some patients on whose RBCs these antigens were available for reaction (e.g., patients with infections associated with neuraminidase-producing organisms). Theoretically, the phenomenon could occur with any other typing sera containing anti-T,

etc. The problems are proportional to the degree of antigen activation, the potency of the antibody (e.g., anti-T), and the temperature that is optimal for antigen-antibody reaction (e.g., many anti-T/Tn do not react at 37°C).

Antisera may also contain antibodies to human proteins (e.g., allotypes of IgG). Simmons et al.<sup>86</sup> described difficulty in ABO typing a baby with a positive DAT because the anti-B contained anti-Gm(4), which reacted with the maternal IgG coating the baby's RBCs. In 1974, Wilkinson et al.<sup>87</sup> described a problem due to Bg antibodies contaminating commercial anti-Rh. Many antisera prepared from pregnant women or immunized donors are likely to contain HLA antibodies which may react with strong Bg antigens on RBCs. The Food and Drug Administration requires that anti-D is free of strong Bg antibodies.

As polyclonal blood grouping reagents are being replaced with monoclonal reagents, the problems previously described are becoming rare.

#### *Antibodies to lower oxiranes*

In 1979, Cross et al.<sup>88</sup> described in vitro reactivity of patients' sera with RBCs that had been stored in the plastic packs from one particular manufacturer. It was suggested that the propylene oxide used in the sterilization process may be involved. Callahan et al.<sup>89</sup> showed that such RBCs had a shortened survival in vivo.

Bruce et al.<sup>90,91</sup> described antibodies that reacted with an antigen acquired by RBCs during storage in only some batches of polyvinyl chloride blood donor packs. It was suggested that this was due to sterilization of the outside of the packs with propylene oxide. The antibodies reacted with a wide range of lower oxiranes (propylene, ethylene, and butylene oxides). Sterilization of the outside of the bag with propylene oxide sometimes caused the anticoagulant in the bag to acquire properties that could induce RBCs to acquire a new antigen, termed LOX (lower oxirane). RBCs stored in such packs could be affected, or normal RBCs incubated, in vitro, with anticoagulants from sterilized blood packs would become reactive with patients sera having antibodies to the LOX antigen. The efficiency of the oxiranes in inducing this phenomenon appeared to be related to their molecular weight, lower molecular weight oxiranes being more efficient (ethylene oxide > propylene oxide > butylene oxide).

These findings caused some concern because ethylene oxide is used to sterilize a wide range of hospital equipment and commercial products.

*Antibodies that only react with RBCs freshly washed or suspended in saline*

Some very unusual antibodies have been described that will react only with RBCs after washing in saline or when freshly suspended in saline. In 1961, Harboe et al.<sup>92</sup> described a panagglutinin that was only detected after washing RBCs with saline following incubation in the patient's serum. The antibody sensitized RBCs equally well at 4°C, 20°C, and 37°C. The antibody did not react with RBCs washed in other solutions (e.g., ACD, trisodium citrate, or 1% human gamma globulin). When purified human gamma globulin was added to the agglutinated RBCs, the agglutination dispersed. Harboe et al.<sup>92</sup> mentioned two other similar reports<sup>93,94</sup> in the earlier literature.

In 1972, Allan et al.<sup>95</sup> described four examples of antibodies that reacted only with RBCs that had been freshly washed in saline. When saline-washed RBCs were left at room temperature for 5–30 minutes before adding sera from the patients, no reactions were observed. However, if the same RBCs were now washed in saline and immediately incubated with the patients' sera, agglutination occurred. RBCs washed in ACD, bovine albumin, or donor serum did not react. The antibodies could be adsorbed from the patients' sera with RBCs freshly washed in saline. Similar results were described by Davey et al.<sup>96</sup> and Scarth and Jones.<sup>97</sup>

Davey et al.<sup>96</sup> described an IgG complement-binding antibody that was detected by IAT only when the RBCs, which were added to the patients' sera, were saline-washed. Thus, RBCs from commercial screening RBCs and antibody identification panels did not react unless washed in saline. <sup>51</sup>Cr-labeled ACD-suspended RBCs had normal 1 hour survival (allogeneic RBCs = 77% survival and autologous RBCs = 93% survival), but survival of saline-suspended RBCs was only 32 percent at 1 hour for allogeneic and 46 percent for autologous RBCs.

Our laboratory has confirmed many of the findings above when investigating several of these very unusual antibodies. No one understands the phenomenon!

*IgG sensitization of RBCs associated with clotted blood but not anticoagulated blood*

In 1980, we reported a strange phenomenon. We investigated several patients who had a positive DAT because of IgG sensitization, but the DAT was positive only when RBCs from clotted blood were used.<sup>98</sup> The patients' sera, but not their plasma, reacted by IAT against all RBCs tested. Eluates prepared from the clot reacted with all RBCs tested by IAT (anti-IgG). All patients had ulcerative colitis but no signs of hemolytic

anemia. When blood was tested immediately (i.e., before clotting), the DAT was negative; when subsampled during the next 30 minutes, the DAT became, after clotting started, progressively more positive. Plasma (which did not react with RBCs), when clotted using thrombin, became reactive by IAT with RBCs. We postulated that the patients had an IgG antibody to an *activated* coagulation factor (i.e., one not present normally in plasma), and that IgG immune complexes formed and attached to RBCs in vitro.

In 1982, Tregellas and Lavine<sup>99</sup> reported studies on an IgG anti-Sc1 that had been described previously as an anti-Sc1 detectable in serum but not plasma.<sup>100</sup> They suggested that the patient might have a population of Ig molecules that were exquisitely sensitive to cleavage by thrombin generated during clotting.<sup>99</sup> Ig molecules, separated from the patient's plasma, reacted with Sc:1 but not Sc:-1 RBCs after the Ig fraction was treated with thrombin or trypsin. The hypothesis was that the serine endoproteases cleaved arginine-lysine bonds, creating Ig molecules with anti-Sc1 activity.<sup>99</sup>

In 1993,<sup>101</sup> results of studying 28 patients with ulcerative colitis showing the IgG-RBC sensitization phenomenon associated with in vitro clotting, as reported in 1980,<sup>98</sup> were summarized. Seventy-one percent had ulcerative colitis or other problems associated with the colon. One interesting finding was that 10 of 11 IgG antibodies tested were of the IgG3 subclass. We found that the phenomenon was not related directly to clotting. We could make a nonreactive plasma reactive (by IAT), without clotting occurring, by adding a variety of serine proteases (e.g., trypsin) to the plasma. We modified our hypothesis to suggest that the antibody in the patient's serum was directed against an epitope present on most serine proteases, including, but not restricted to, some activated coagulation factors.<sup>101</sup>

There is a reported association with ulcerative colitis, positive DATs, and autoimmune hemolytic anemia (AIHA). We believe that this association is distinct from the phenomenon above, as we have encountered many positive DATs and AIHA in ulcerative colitis patients in which the DAT is positive on RBCs from EDTA and clotted samples. It is of interest that three examples of antibodies reacting in serum but not plasma have been described as having blood group specificity (anti-Sc1,<sup>100</sup> anti-Kp<sup>b</sup>,<sup>102</sup> anti-c<sup>103</sup>).

When the above phenomenon occurs, it can be confusing to the blood banker as the patient's sera may react with all the screening RBCs, all RBCs on the panel, and all crossmatched units; but if the plasma is used, no RBCs

will be reactive. If serum is used, then the auto control also will be positive, and the patient will appear to have an autoantibody. The investigator may become confused when the DAT is found to be negative on an EDTA blood sample. This result may delay a patient receiving a needed blood transfusion. It is always wise to be alert to the patient's diagnosis. If a patient with ulcerative colitis gives the reactions above, one should immediately repeat the tests using plasma. If the plasma is nonreactive and the serum is reactive, compatibility can be performed with the plasma (Standards of the American Association of Blood Banks allow plasma to be used for compatibility testing<sup>104</sup>). We have transfused several patients successfully using this approach.

#### *Antibodies reacting only, or stronger, with stored RBCs*

There have been several reports of antibodies that appear to react better with stored RBCs.<sup>105-112</sup> These antibodies were usually cold agglutinins; a mixed-field appearance was common. Most of the antibodies reacted well with enzyme-treated RBCs, even at 37°C. RBCs that were prematurely aged by incubating at 37°C for at least 24 hours reacted as strongly as RBCs aged at 4°C for weeks. It was also observed that autoantibodies causing lysis of enzyme-treated RBCs would often agglutinate nonenzyme-treated RBCs but not fresh RBCs.

In 1989, this author and colleagues reported a patient with warm AIHA associated with an autoantibody that reacted preferentially with stored RBCs.<sup>113</sup> The antibody showed similar characteristics to some other antibodies described previously in that it reacted well with enzyme- and heat-treated RBCs. It also showed the characteristic mixed-field appearance described by Stratton et al.<sup>106</sup> and Ozer and Chaplin.<sup>108</sup> We separated "young" and "old" RBCs from fresh blood by a capillary centrifugation method and showed that the stored RBC antibody reacted preferentially against the older RBCs in fresh blood. This suggested to us that the target antigen might be similar or identical to the senescent cell antigen (SCA) described by Kay.<sup>114</sup> Kay has suggested that senescent cells are cleared from the circulation because they develop SCAs that react with naturally occurring IgG autoantibodies to SCA. These IgG-sensitized cells are then removed by macrophages.<sup>114</sup> Kay has also characterized the SCA biochemically; it is present on band 3 of the RBC membrane. We were able to demonstrate that the RBC autoantibody that reacted preferentially with stored and old RBCs could be inhibited in vitro with synthetic SCA that was provided by Dr. Kay. Thus, we believe that the stored RBC antibody is reacting against the SCA and that

the ubiquitous anti-SCA, which is usually present in very low levels, like ubiquitous anti-I, can become pathogenic on occasions.

Branch et al.<sup>115</sup> performed DATs on age-fractionated RBCs of 24 DAT+ patients. Seventy-nine percent of the DATs were stronger when the older RBC fraction was tested (Type D); 37 percent of the reticulocyte-enriched fractions were DAT-. Twenty-one percent of the autoantibodies seemed to show no preference for old or young RBCs (Type II). Branch et al.<sup>115</sup> suggested that Type I warm autoantibodies might be recognizing an as yet unidentified RBC antigen, possibly a cryptantigen closely associated with the Rh peptide but not fully expressed on very young RBCs. They hypothesized that the Type I autoantibody might represent augmented production of the physiologic autoantibody responsible for clearing senescent RBCs. We suggest that the antibody against stored RBCs (and older RBCs), that we showed was anti-SCA,<sup>113</sup> is identical to some of the Type I autoantibodies<sup>115</sup> and that these are indeed directed against SCA.

#### **Conclusions**

When a patient's serum reacts with all RBCs, or an ABO/Rh typing anomaly is encountered, we usually assume an antibody to a blood group antigen is involved, but it is obvious from the above that such reactions can be unrelated to blood group antigens. Sometimes these problems are easily solved by using washed RBCs, but at other times this does not help. When marked differences are observed with reagents from different sources, RBCs of different age, or clotted versus anticoagulated blood samples, this should be a stimulus to take note of the phenomena described in this review.

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# The immunoglobulin molecule

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Antibodies, or immunoglobulins, have been used for many years in immunohematology and yet the complexity of these molecules is rarely considered. This review concentrates on IgG and IgM molecules, as these are most usually found in transfusion laboratories. The basic structure and function of the immunoglobulin molecule are addressed at both the protein and the gene level, and isotypes, allotypes, and idiotypes are introduced. Although the antibody molecules secreted by each B cell have a unique binding site, a diverse array of immunoglobulin is produced not only by random recombination of the genetic components during assembly of the molecule but also due to somatic mutation. These events, as well as gene restriction, which is often seen in IgM antibodies to blood group antigens, are discussed and some of the differences between monoclonal and polyclonal antibodies are addressed. *Immunohematology* 1998;14:12-18.

# Evaluation of the GTI-ASP-1 platelet antigen genotyping kit for the determination of the *HPA-1* genotype

T.J. LEGLER, C. HAGNER, AND M. KÖHLER

The human platelet antigen system HPA-1 is involved in most cases of neonatal alloimmune thrombocytopenia and posttransfusion purpura, and occasionally causes refractoriness to platelet transfusions. Complete concordance was obtained in genotyping for HPA-1 in all samples tested with the HPA-1 genotyping kit and a ligation-based typing method. However, the genotyping kit is less sensitive than ligation-based typing, which could be of importance when testing cells from amniotic fluid or from chorionic villi. *Immunohematology* 1998;14:19-21.

# K phenotyping using a PK-7200 automated analyzer

M.C.Z. NOVARETTI, S.P. NAVARRO, P.E. DORLHIAC-LLACER, AND D.A.F. CHAMONE

K (Kell) is one of the most immunogenic of the red blood cell (RBC) antigens. In order to select K- RBC units, we developed K phenotyping on the Olympus PK-7200 equipment to save labor, time, and costs. The Olympus PK-7200 is fully automated equipment used primarily for blood typing and syphilis screening. We tested 3,587 blood donor samples in EDTA using a commercial anti-K serum diluted in HP Hemagen Power Solution<sup>®</sup>(1:40). The equipment was set to prepare a 1.7% RBC suspension in bromelain and to dispense 25 $\mu$ L of the mixture (diluted serum and HP Hemagen Power Solution<sup>®</sup>) in terraced microplates. After mixing, the microplates were incubated for 1 hour at 30°C. Reading was performed by a C.C.D. camera and the results were automatically transferred to the mainframe computer. We found 185 K+ blood samples and 3,402 K- samples. Four samples, K+ by the PK-7200, were confirmed as K- by tube test. The use of bromelain with the PK-7200 may have caused the falsely positive tests. The Olympus PK-7200, used for K phenotyping, saves labor time and costs. It also reduces handling and thus promotes less contamination risk for laboratory personnel. *Immunohematology* 1998;14:22-25.

# Warm autoimmune hemolytic anemia associated with an IgM autoanti-Ge

T. SERERAT, D. VEIDT, P.A. ARNDT, AND G. GARRATTY

A 28-year-old male with a prior history of Hodgkin's disease and a recent upper respiratory tract infection presented with autoimmune hemolytic anemia (AIHA). The patient's red blood cells (RBCs) were spontaneously agglutinated after room temperature and 37°C washes. Dithiothreitol-treated RBCs reacted strongly with anti-C3 and were nonreactive with anti-IgG, -IgM, and -IgA; they reacted with anti-IgM ( $\kappa$  light chains only) by flow cytometry. The patient's serum was nonreactive. An acid eluate was only weakly reactive, but a 56°C heat eluate strongly agglutinated untreated RBCs (3+). Ficin-treated RBCs were nonreactive. En(a-) RBCs were strongly reactive, but Ge- RBCs were nonreactive. The anti-Ge in the eluate was IgM. The patient's untreated RBCs were shown, by flow cytometry, to be weakly Ge+. This is the first report of IgM-mediated warm AIHA associated with autoanti-Ge. *Immunohematology* 1998;14:26-29.

# The first case of the p phenotype in a Gurkha Nepalese

C.K. LIN, K.H. MAK, C.K. CHENG, AND C.P. YANG

A serum sample from a Gurkha Nepalese soldier, residing in Hong Kong, was found to cause hemolysis of reagent ABO red cells (RBCs) in the reverse blood grouping test. Subsequent follow-up studies revealed that he was of the p phenotype, with potent anti-PP1P<sup>k</sup> that was strongly hemolytic both at room temperature and 37°C. The anti-PP1P<sup>k</sup> was composed of IgG and IgM, and its various components were separable. *Immunohematology* 1998;14:30-32.