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Development of anti-Bw6 reactivity in patients receiving r-GCSF: a preliminary report

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Recombinant granulocyte colony-stimulating factor (r-GCSF) is used in autologous bone marrow/peripheral blood progenitor cell transplantation (ABMT/PBPC) to shorten the period of neutropenia. As these patients require platelet transfusions, their sera may be monitored for the presence of platelet/human leukocyte antigen (HLA) antibodies. Sera of some patients on r-GCSF (16 µg/kg/day) became difficult to evaluate in vitro for the presence of HLA and platelet antibodies because of apparently anomalous reactions in solid phase red cell adherence (SPRCA) assays. SPRCA tests were positive only when platelets were adhered to the polystyrene plates in the presence of glucose; when other simple sugars were used, the patients' sera failed to react. HLA Bw6-positive platelets were more likely than HLA Bw6-negative platelets to be reactive ($p < .001$). The transfusion of HLA Bw6-positive platelets to patients displaying this in vitro reactivity (positive patients) resulted in a 50 percent lower corrected count increment (CCI) than those given to patients without it (negative patients; $p = <.001$). When all transfusions were considered, the CCI for those of the positive patients was decreased 73 percent when compared to the control patients ($p = .0005$). The presence of the antibody also was associated with a twofold increase in the number of platelet transfusions given ($p = .0005$). ABMT/PBPC patients receiving r-GCSF may develop unexpected reactions on SPRCA antibody screens and poor responses to transfusion of Bw-6-positive platelets. *Immunohematology* 2001;17:63-69.

Key Words: r-GCSF, marrow/progenitor cell transplant, anti-Bw6

Potent hematopoietic stimulants offer important clinical benefits in chemotherapy and marrow transplant patients. Analogs of naturally occurring hormones and cytokines have reduced morbidity and these patients have shortened hospital stays, but they may elicit undesirable immunologic side effects. This study describes the discovery, investigation, and presumptive resolution of an unexpected serologic finding associated with use of a common hematopoietic stimulant.

Because of the frequency and clinical significance of refractoriness to platelet transfusion associated with the development of human leukocyte antigen (HLA) and/or platelet-specific alloantibodies, some institutions regularly screen patients receiving platelet transfusions to detect the formation of these antibodies and redirect

hemotherapy. We encountered a group of patients undergoing autologous bone marrow/peripheral blood progenitor cell transplantation (ABMT/PBPC) who appeared to develop blunted responses to platelet transfusion while receiving recombinant granulocyte colony-stimulating factor (r-GCSF); all demonstrated anomalous serologic activity in HLA/platelet antibody screening associated with development of a novel glucose-dependent antibody. The investigation of this phenomenon and its resolution is the subject of this report.

All hematology-oncology patients (including those undergoing ABMT/PBPC) treated at this institution are routinely tested by a solid phase red cell adherence (SPRCA) assay for the development of HLA- and/or platelet-specific antibodies. Platelet crossmatching is performed when positive HLA/platelet antibody screens are found or when the patient has an unsatisfactory corrected count increment (CCI)¹ following two consecutive platelet transfusions. During this routine evaluation, anomalous positive reactions were encountered in the HLA/platelet antibody screen of some patients. These tests were positive only for a short period of time, beginning after r-GCSF therapy was initiated and continuing until approximately 7 days after r-GCSF therapy was discontinued. As these reactions disappeared from the SPRCA assay following chloroquine diphosphate treatment,² the presence of platelet-specific antibodies was ruled out. Concomitantly, an increase in ABO antibody titers was noted in group O patients, unexpectedly poor responses were seen to some platelet transfusions, and there was an apparent requirement for more platelet transfusions. Following these observations,³ a prospective study was initiated.

Materials and Methods

Patient selection and data collection

A prospective study involving 92 consecutive patients undergoing ABMT/PBPC from 7/3/91 to 7/13/94 was designed. All patients received r-GCSF (Amgen, Thousand Oaks, CA; 16 $\mu\text{g}/\text{kg}/\text{day}$) from 2 to 3 days before marrow reinfusion until their absolute neutrophil count rose above 5000/ μL or until they had received r-GCSF for 21 days. Sixteen patients were excluded due to the presence of factors that might influence their response to platelet transfusions, such as the presence of drug-associated platelet antibodies (2), the receipt of intravenous gammaglobulin (5), multispecific HLA antibodies for which crossmatch-compatible platelets were not available for transfusion (2), platelet-specific antibodies requiring transfusion of platelets collected from family members (4), renal dialysis (1), or transfusion of plasma-depleted platelets due to repeated severe allergic transfusion reactions (2). Data collected included timing of r-GCSF administration for PBPC collections and pregnancy history. A platelet transfusion history was also obtained, which included the following information: number and dates of transfusions, the donor ABO group, donor HLA type (including Bw4 and Bw6 type), and the resultant CCI. In addition, aliquots of all available patient sera were frozen for future testing. The day on which the patient was reinfused with previously harvested bone marrow and/or PBPCs was designated as Day 0.

The presence of anomalous reactivity (that is, reactivity not attributable to reactivity with one or more HLA/platelet antigens) in HLA/platelet antibody screens was used to assign patients to either the study ($n = 25$) or control ($n = 48$) group. All patients evaluated had at least two PBPC collections prior to transplant using standard ablative regimens of radiation and/or chemotherapy. r-GCSF therapy was initiated 4 days prior to the first PBPC collection and continued until the day before the last PBPC collection. Generally, 1 to 3 weeks elapsed between the last PBPC collection and transplantation. The negative patients included 7 male and 41 female patients with the following diagnoses: breast cancer ($n = 36$), Hodgkin's disease ($n = 7$), non-Hodgkin's lymphoma ($n = 4$), and lung cancer ($n = 1$). These control patients received a total of 308 platelet transfusions, with a CCI being calculated for 246 (80%) of the transfusions. The positive patients included 3 male and 22 female patients with the following

diagnoses: breast cancer ($n = 18$), non-Hodgkin's lymphoma ($n = 2$), osteosarcoma ($n = 1$), ovarian carcinoma ($n = 1$), Hodgkin's disease ($n = 1$), rhabdosarcoma ($n = 1$), and germ cell cancer ($n = 1$). The positive patients received a total of 341 platelet transfusions, with a CCI being calculated for 269 (79%) of the transfusions.

Hemotherapy

All patients received one daily prophylactic platelet transfusion using single donor platelet units when their platelet counts were less than 20,000/ μL . Platelet counts were kept above 50,000/ μL in the event of hemorrhage. A 10-minute posttransfusion platelet count was obtained whenever possible, and the CCI was calculated. All platelet and red cell units released for transfusion were gamma irradiated with 25Gy (2500 rad) using a ^{137}Cs Irradiator (CIS-US, Bedford, MA) and leukocyte reduced by bedside adsorption filtration (PL100K and RC50K filters; Pall Biomedical Corporation, Glen Cove, NY). As we had previously demonstrated that there was no effect of storage time on platelet count increment in bone marrow transplant patients,⁴ units were selected from inventory according to outdate, with the oldest units being transfused first. ABO compatibility was considered only when the recipient demonstrated in vitro or in vivo incompatibility with out-of-group platelet units. (ABO incompatibility was defined as the presence on platelets of A or B substance lacking on the patient's red cells; plasma incompatibility was not considered.)

SPRCA HLA/platelet antibody screens

SPRCA HLA/platelet antibody screens were performed at least weekly (Capture-P[®] Ready Screen, Immucor, Inc., Norcross, GA); manufacturer's directions were followed for all testing. In chloroquine diphosphate treatment to remove HLA antigens from reagent platelets (CDP; Gamma Biologicals, Houston TX), two drops of CDP were added to the test strip, and manufacturer's directions were followed to complete the testing. The incubation time, after CDP addition, was increased to 1 hour to allow for sufficient time for the CDP to react with the reagent platelets. The CDP pretreatment was performed twice on each reagent test strip to maximize the effect on the HLA antigens. If a transfusion in a control patient did not result in a CCI < 7500/ μL without demonstrable clinical cause, HLA and platelet antibodies were assumed to be absent.

Anti-A and Anti-B titers

Anti-A and anti-B titers were performed on all available serum/plasma samples (stored frozen) collected from group O patients ($n = 35$).⁵ The results were reported as the reciprocal of the highest dilution that produced at least a 1+ reaction.

Platelets immobilized with various sugars

Platelets in the Capture-P[®] Ready Screen kits are immobilized by the manufacturer to a polystyrene microtiter plate in the presence of glucose. To evaluate the effect of various sugars on the positive patients sera, plates on which platelets had been immobilized with various simple sugars were supplied by the manufacturer. These sugars included arabinose, fructose, galactose, glucose, lactose, maltose, mannose, melibiose, trehalose, and sucrose (Sigma Chemical Company, St. Louis, MO). Platelets from the same donor were immobilized with different sugars so comparisons of the results could be made. Antibody screening using the platelets that had been immobilized with the various sugars was performed as for routine SPRCA-HLA screening.

Antibody neutralization

When positive HLA/platelet antibody screens were identified in routine SPRCA techniques, the testing was repeated using serum or plasma treated with various substances (Immucor, Inc., Norcross, GA), to determine if the observed reactivity could be neutralized. Solutions (0.1M) of glucose, sucrose, fucose, mannose, fructose, galactose, mannose, or galactosamine (all obtained from Sigma Chemical Company) were prepared in deionized water. Undiluted r-GCSF (300 $\mu\text{g}/\text{mL}$) was also used as a neutralizing substance. One hundred μL of each solution were added to 500 μL of serum/plasma. The mixture was allowed to incubate at room temperature (22 to 24°C) for 5 minutes. These treated samples were used to repeat SPRCA HLA/platelet antibody screens; results were compared to those obtained with untreated samples.

Ion exchange chromatography procedure

Ion exchange chromatography, using diethylaminoethyl Affi-Gel Blue agarose gels (Biorad Labs, Hercules, CA) was performed on 18 individual sera collected from positive patients; samples from three control patients were also included. The manufacturer's instructions were followed for use of small volumes of sera to allow separation in a conical centrifuge tube rather than a large column.

The effluent was collected in 1 to 5 mL fractions. The fraction size was determined by the amount of buffer added to the gel during the various steps in the chromatography procedure. The estimated total protein concentration of each fraction was calculated by multiplying the optical density (obtained by spectrophotometric analysis) by the volume and the dilution required to obtain an optical density reading that was < 2. Following total protein determinations, the protein composition of each fraction was evaluated by SDS-PAGE gel electrophoresis following manufacturer's directions (Biorad Labs). Fraction #1 was found to contain the majority of IgG proteins (data not shown). This fraction had a total volume of 1 to 5 mL and was expected to contain the unbound (IgG) protein from the sample. All fractions were placed in a -70°C freezer for use in future testing.

Additional testing

An enzyme-linked immunosorbent assay (ELISA) HLA antibody screening kit (GTI, Brookfield, WI) was used to perform HLA antibody screens on selected fractions obtained from ion exchange chromatography procedures according to the manufacturer's directions (Table 1).

Table 1. Results of ELISA-HLA antibody screening

Patient*	Days from Transplant	Fraction contains†	SPRCA screen with Bw6+ Platelets
1	-51	IgG	neg
	-51	Other	neg
	-21	Other	neg
	-15	Other	neg
	-12	IgG	neg
	-12	Other	neg
	-2	IgG	neg
	-2	Other	neg
	0	IgG	neg
	0	Other	neg
	+8	IgG	pos
	+8	Other	neg
	+9	Other	neg
	+13	IgG	pos
	+15	IgG	pos
+15	Other	neg	
+16	Other	neg	
+850	Other	neg	
2	+18	Other	neg
	+18	IgG	pos
	+18	IgG(concentrated)	pos
3	Pooled serum	IgG	neg
	Pooled serum	Other	neg
4	Pooled serum	IgG	neg
	Pooled serum	Other	neg

* = all samples except those from Patients 3 and 4 are from chromatography fractions from positive patients. Patients 3 and 4 are from negative patients (serum pooled to aid in processing).

† = content of fractions determined by SDS-PAGE electrophoresis

Statistics

The results of the study were analyzed by logistic regression to identify predictors for the presence of the antibody. Gender, blood type (A vs. B vs. O), diagnosis (breast cancer vs. other), HLA Bw type (Bw4 vs. Bw6 vs. other), radiation, days on r-GCSF, days on total parental nutrition (TPN), and increase in anti-A and anti-B titer (group O patients only) were included in the model. An analysis of variance (ANOVA), using natural logarithmic transformation, was performed on the total number of transfusions. Factors in this model included presence of the antibody, patients' HLA Bw6 types, antibody by Bw6 interaction (Does the patient's Bw6 type influence the development of the antibody?), diagnosis, and blood type. A *t*-test, using log scale to meet normality assumptions, was performed to compare average CCI per transfusion between the control and the positive patients. Chi-square analysis was used to determine if the observed frequency of HLA Bw6-positive platelets reacting with the sera under study were significantly different than what would be expected based on chance. The observed frequency of patient ABO group and Rh types was analyzed by Yates Chi-square analysis to determine if the incidence observed was significantly different than what would be expected by chance.

Results

Anomalous results in SPRCA HLA/platelet antibody screening

All HLA/platelet antibody screens were evaluated for the presence of unexplained (anomalous) reactivity. A total of 204 antibody screen procedures were performed on the 48 patients in the negative (control) group ($M = 4.25$); 130 procedures were completed on the 25 patients in the positive group ($M = 5.20$). Specific HLA antibodies were identified in eight patients in the control group and in six patients in the positive group ($p > .05$). Eighty-five percent of all the screens done on control patients were negative compared with 58 percent in the positive patients ($p \leq .001$). Thirty-two percent (42/130) of the screens completed on patients in the positive group were found to have anomalous reactivity. All samples in which specific HLA antibodies and/or unexplained reactivity were identified were further tested to determine the cause of the anomalous reactivity. This reactivity was first observed 5 to 16 days ($M = 9.14$) after receiving the first r-GCSF after admission for transplant; this

reactivity was no longer detected 1 to 5 days ($M = 2.17$) after r-GCSF had been discontinued.

Relationship of anomalous reactivity to glucose immobilization of platelets

All samples displaying the presence of unexpected reactivity, as well as randomly selected samples from five patients with a negative HLA antibody screen, were tested with platelets immobilized with arabinose, fructose, galactose, glucose, lactose, maltose, mannose, melibiose, trehalose, or sucrose. Samples known to contain only HLA-specific antibodies were also tested. In all cases (i.e., samples from the "positive" study group) in which the original HLA/platelet antibody screen had exhibited the anomalous reactivity, negative results were also obtained when the sample was tested with platelets immobilized with any sugar other than glucose. Samples that had not exhibited this reactivity continued to show a negative antibody screen when the other sugars were utilized for platelet immobilization. However, the samples from patients whose samples had not exhibited the anomalous reactivity but had defined HLA specificities continued to react in the presence of the other sugars as they had when the reagent platelets had been immobilized with glucose. Similarly, samples from patients demonstrating the anomalous reactivity as well as defined anti-HLA reactivity continued to show the specific anti-HLA reactivity when the reagent platelets were immobilized with other sugars. No reactivity was observed when sera from the positive study group were reacted with glucose in plates that contained no platelets.

The reagent platelets that showed reactivity on routine antibody screening with positive patients' sera but were negative following neutralization with glucose were evaluated further to determine if there was any specific HLA antigen that appeared more frequently than would be expected. A total of 152 reagent platelets were evaluated. Reactivity was seen with 140 (92%) of HLA-Bw6+ reagent platelets but only 6 (4%) of Bw6- platelets ($p < .001$).⁶ When the patients in the study were phenotyped for HLA-Bw6, 10/48 (21%) of the negative patients were found to be Bw6- compared with 4/25 (16%) of patients in the positive group. This compares with a population distribution of 10 percent HLA-Bw6 negativity (difference = $p > .230$).⁷

Changes in anti-A titer with r-GCSF administration

All patients in the study were evaluated to determine the number of ABO-incompatible platelet transfusions

received. Patients in the negative group received 33 ABO-incompatible transfusions compared to 30 received by patients in the positive group.

Anti-A and anti-B titers were performed on 182 samples from patients in the negative group and on 167 samples from patients in the positive group. The median number of titers performed and the median for the lowest anti-A and anti-B titers were similar in both groups. However, the positive patients showed the median highest anti-A titer of 512 compared to 64 for the negative patients. The median for the highest anti-B titer was slightly higher in the positive patients ($Mdn = 32$) than in the negative patients ($Mdn = 16$). The median change in anti-A titer for the negative patients was 44 (range 0 to 480) compared with 464 (range 14 to 2016) for the positive patients. The median change in the anti-B titer in the negative patients was 12 (range 0 to 120) compared with 22 (range 2 to 480) in the positive patients. The changes in titer were evaluated by the z test for rejection of the null hypothesis of no difference. The z value for changes in anti-A titer in the positive group was > 7 standard deviations from the mean for the negative patients, a significant difference between the two groups. When the changes in anti-B titer were evaluated, the z value was found to be < 1 standard deviation from the control mean; the change in anti-B titer between the two groups was not significantly different.

Antibodies in eluted fractions

After separation using ion exchange chromatography, a total of 144 fractions (each fraction contained 1 to 5 mL) collected from 18 samples from patients with the anomalous reactivity were evaluated. Each fraction was tested with glucose-immobilized donor platelets to determine which fraction contained the anomalous reactivity. Twenty-one of these fractions, including reactive and nonreactive fractions, underwent additional testing; these fractions were the first collected and contained the IgG component of immunoglobulins as determined by SDS-PAGE electrophoresis. These fractions were selected for evaluation as the reactivity undergoing investigation appeared to be IgG (IgG-sensitive indicator red cells in SPRCA). In addition, six study serum samples, showing negative results with glucose-immobilized platelets, were also included in the additional testing for comparative purposes.

IgG-containing chromatography fractions from samples taken from positive patients about the time

when they exhibited the anomalous reactivity clinically showed the same characteristic SPRCA anomalous reactivity. Two of these samples also contained HLA-antigen specific reactivity as previously documented; these specificities were confirmed in ELISA testing. Chromatography fractions containing IgG from negative patient samples shown to contain HLA antibodies displayed antigen-specific reactivity in both SPRCA and ELISA test systems.

When all SPRCA testing was completed, all remaining positive and negative fractions were pooled to allow sufficient volume for additional testing. First, the presence of ABO antibodies was sought by standard techniques. When testing fractions derived from patients who had exhibited the anomalous reactivity, ABO antibodies were not present in the pooled fractions that exhibited the anomalous reactivity (that is, the fractions presumed to contain IgG) but were identified in the pooled fractions that did not show this reactivity (that is, fractions presumed to be of other immunoglobulin classes). Second, glucose neutralization was performed. The anomalous reactivity showed the same neutralization characteristics in these pooled fractions as in the original samples.

Clinical effect of "anomalous" antibody

A logistic regression was fitted to identify predictors of the presence of the antibody from among the following factors: gender, blood type (A vs. B vs. O), diagnosis (breast cancer vs. other), HLA-Bw type (Bw4 vs. Bw6 vs. other) of the patient, radiation (yes or no), days on r-GCSF, days on TPN, increase in anti-A or anti-B titer (group O only).

An ANOVA was performed on the total number of platelet transfusions required by patients. Increased likelihood of the antibody's presence was associated only with the number of platelet transfusions received ($p = .0005$). The geometric mean number of transfusions (with 95% confidence interval) for control patients was 5.01 (4.08, 6.16), whereas that for study patients was 10.49 (7.67, 14.36). No significant differences between the control and positive patients were identified.

The significance of changes in anti-A titer between the control and positive patients was evaluated by ANOVA. Increases in titer in the positive patients was shown to have borderline significance ($p = 0.068$), with an increase in titer of >100 being associated with an increase in the probability of developing the unexpected reactivity. The type of ablative therapy used

had no association with the development of the anomalous reactivity.

A *t* test was performed to compare average CCI per transfusion (on the log scale, to meet normality assumptions) for the positive vs. the negative patients. The difference in CCI between the two groups was statistically significant ($p = .0005$). The geometric mean CCI (with 95% confidence) for the negative patients was 10,822 (9,888;11,844) compared to 7,951 (6,956;9,090) for the positive patients, a 73 percent difference.

Chi-square analyses were used to determine if CCIs following receipt of Bw-6 or Bw-4 positive platelets were significantly different between groups. No statistically significant differences were found when Bw4+6-, ABO-compatible platelets were transfused. However, when Bw4+6+ or Bw4-6+ ABO-compatible units were transfused (168/216 [77%] transfusions in the negative group vs. 205/244 [84%] transfusions in the study group), the CCIs in the study group were significantly lower than those in the control group ($p = <.001$). No significant differences were seen when ABO-incompatible platelet units ($n = 33$ [negative group] compared to $n = 30$ [positive group]) were received.

Yates corrected Chi-square analyses were used to determine if the incidence of ABO groups in both study and negative patients was significantly different between groups and from the expected incidence in the general population. Half (24/48) of the patients in the control group were group O, whereas 64 percent (16/25) of patients with the anomalous reactivity were group O ($p > .1$). The negative patients included 17 group A patients compared to 8 in the positive group ($p > .1$). There were too few patients of other groups to evaluate.

Discussion

This study documented the presence of an IgG antibody giving anomalous reactions in SPRCA. This was seen in all blood groups, was temporarily associated with r-GCSF administration, could be blocked in vitro with addition of glucose to the sera, and reduced platelet transfusion response. This reactivity was also associated with the presence of the HLA Bw6 antigen on the reagent platelets and an increase in ABO titer (in group O patients only).

The unusual serologic findings in the preliminary observations³ prompted a closer, prospective investigation of the apparent phenomenon reported here. The source of the unexpected reactivity was

ultimately traced to an IgG antibody (reactivity observed with IgG-coated indicator cells) that reacted to platelets bound to a polystyrene solid phase in the presence of glucose. The strong association of the reactivity with the presence of the Bw6 antigen on the reagent platelets clearly suggests a role for this epitope in the antibody's specificity. The very specific effect of the use of just one sugar might suggest the former as the more plausible mechanism, but ascribing immunogenicity to such a small and ubiquitous molecule, even through haptenic combination, seems unlikely.

The identification of this reactivity in the SPRCA system but its failure to react in ELISA and lymphocytotoxicity testing systems might initially suggest that this is solely a phenomenon restricted to one particular test system. In itself, such an occurrence would still be important to note because anomalous results in SPRCA antibody screening can be confusing and could delay selection of platelet units for transfusion. However, our finding that the presence of this reactivity was associated with 73 percent lower CCIs demonstrates that the in vitro reactivity may have an in vivo counterpart that is clinically significant. The effect may be associated with the need to administer twice as many platelet transfusions during ABMT/PBPC, thus increasing recipient risk and the procedure's cost. This effect appeared independent of ABO compatibility; however, HLA Bw6+ platelet units were more likely to yield a poor transfusion response in patients with the unexpected reactivity, suggesting a clinical correlation of the increased in vitro reactivity of the positive sera with the presence of HLA Bw6 on platelets.

Red cell antibodies that react only in the presence of certain sugars have been reported.⁸ The presence of this unexpected reactivity with red cells suspended in a variety of sugars, including glucose, galactose, mannose, fructose, lactose, or dextran has been shown to occur in 2 to 22 percent of sera tested.⁹ However, a review of the literature failed to identify prior reports of similar reactivity when testing reactivity with platelets.

Not all transplant centers utilize the SPRCA technique and thus may not have serologic findings in patients such as these that correlate with poor platelet transfusion responses in affected patients. If a patient is currently receiving TPN and r-GCSF and has CCIs < 7500 , the patient history should be carefully reviewed and evaluated. As group O patients were 11.4 times more likely to develop the antibody if there was a greater than 100-fold increase in their anti-A titer, this

simple titration test may be important in inferring the possible presence of the antibody. As pretransplant titers are required for this evaluation, it is recommended that a pretransplant sample be frozen and used for isoagglutinin titration when the presence of the anomalous antibody is suspected. If responses to platelet transfusions and patient history indicate that this glucose-dependent reactivity might be present, HLA antibody screen results and determination of the HLA-Bw6 type of transfused platelet units and their CCIs may provide useful information. If the CCIs for HLA-Bw6 negative units are significantly higher than those for HLA-Bw6 positive units without serologic evidence of alloimmunization, the presence of this anomalous antibody should be considered.

It is important to note that all patients included in this study received r-GCSF at a dose of 16 μ g/kg/day. Shortly after the completion of this study, the r-GCSF dose for ABMT/PBPC patients at our hospital was decreased to 5 μ g/kg/day; only two additional examples of this unexpected reactivity have been identified since the r-GCSF dose was decreased. This suggests that the dose of r-GCSF received may contribute to the development of this unexpected reactivity.

Similar anomalous results in SPRCA testing on patients who received r-GCSF have recently been reported.¹⁰ These researchers identified the anomalous reactivity described in this report; however, as the reactivity was observed only in SPRCA, they reported its presence as "false-positivity" and attributed the poor posttransfusion platelet response to the presence of infections and other clinical factors. Our research indicates that r-GCSF recipients should be monitored for the development of a glucose-dependent HLA-Bw6 antibody that may have clinical implications. SPRCA techniques are easy to perform and currently are the only techniques that will detect these significant antibodies. For patients displaying this unexpected reactivity, all platelet units should either be ABO identical or group O, and should be negative for HLA-Bw6 as well, of course, as any HLA antigens to which the patient has been sensitized. This protocol should be followed until at least 7 days after r-GCSF discontinuation.

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Detection of granulocyte antibodies by flow cytometry without the use of pure granulocyte isolates

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Established methods used to detect serum antibodies to granulocytes require the isolation of granulocytes. Flow cytometric analysis of granulocytes with monoclonal antibodies eliminates the need for granulocyte isolation. The purpose of this study was to develop a method to evaluate reactions of antibodies to granulocytes without separating granulocytes from other leukocytes. Three screening cell samples for granulocyte antibody detection were prepared from whole-blood samples in which the red blood cells (RBCs) were lysed and remaining leukocytes tested against sera at 4°C. Binding of human alloantibodies to the screening cells was determined by flow cytometric analysis using phycoerythrin-conjugated antibody to human immunoglobulin. Forward and side scatter were used to analyze granulocytes separately from other leukocytes. The assay was validated by testing granulocytes with reference alloantibodies directed to NA1, NA2, 5b, and Mart antigens. Samples from 32 patients were tested, and the results of the assays were compared with the results of testing the samples in a granulocyte immunofluorescence (GIF) assay performed by a reference laboratory. In the whole-blood flow cytometric (WBFC) assay the mean fluorescence intensities of reference antisera with antigen-positive cells, expressed in arbitrary units, were anti-NA1 = 48 to 221, anti-NA2 = 24 to 69, anti-5b = 13 to 57, and anti-Mart = 42 to 72. In contrast, the mean fluorescence intensity of type AB-negative control sera ranged from 3 to 11. Of the 32 patient sera tested, 23 were positive (range = 12 to 56) and 9 were negative (range = 3 to 10). When compared with the results obtained by the reference laboratory, 27 sera were concordant between the WBFC and the GIF assays. Four of the samples were positive in WBFC (range = 11 to 31) and negative in GIF and one sample was negative in WBFC (range = 5 to 6) and positive in GIF. Leukocytes prepared from whole blood after lysis of RBCs can be used in flow cytometric analysis to detect granulocyte allo-antibodies. The results of testing for granulocyte antibodies with this assay were similar to results of testing sera in GIF. Further comparative studies are indicated to confirm findings and explain the discordant results. *Immunohematology* 2001;17:70-75.

Low-incidence MNS antigens associated with single amino acid changes and their susceptibility to enzyme treatment

M. E. REID AND J. R. STORRY

MNS antigens are carried on glycophorin A (GPA), glycophorin B (GPB), or their variants. Antigens at the N-terminus of GPA are sensitive to cleavage by ficin, papain, and trypsin but are resistant to α -chymotrypsin. Antigens at the N-terminus of GPB are sensitive to cleavage by ficin, papain, and α -chymotrypsin but are resistant to trypsin treatment. These characteristics have been used to aid in the identification of blood group alloantibodies. Recent molecular analyses have identified changes in amino acids that are associated with several low-incidence antigens in the MNS blood group system. This review relates the molecular studies with the susceptibility or resistance of these antigens to treatment of intact red blood cells by proteolytic enzymes. *Immunohematology* 2001;17:76-81.

PEG adsorption of autoantibodies causes loss of concomitant alloantibody

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Use of polyethylene glycol (PEG) to promote adsorption of autoantibodies is reported to give good recovery of concomitant alloantibodies. In initial experiments, PEG and ZZAP (Ficin and DTT) adsorption procedures were compared for removal of autoantibody and recovery of alloantibody. Postadsorption studies ($n = 11$) were performed and hemagglutination scores compared. In subsequent studies, equal volumes of alloantibody containing sera, PEG, and antigen-negative red blood cells (RBCs) were used in twofold adsorption experiments. Saline was substituted for PEG for control purposes. Postadsorption titers and immunoglobulin levels were determined. Autoantibodies were completely removed by both methods ($n = 5$); better by PEG ($n = 3$); better by ZZAP ($n = 1$); and not adsorbed ($n = 1$), and partially adsorbed by both ($n = 1$). Alloantibody recovery was comparable in three cases (E, K, Jk^a) but weaker by at least one reaction grade in four (K, E, Jk^a, and antibody to low-frequency antigen). The latter anti-Jk^a reacted 1+ with Jk(a+b+) RBCs after ZZAP adsorption but was nonreactive with the same RBCs following PEG adsorption. Titers of six alloantibodies adsorbed with antigen-negative RBCs in PEG were markedly weaker (range 2 to 8) compared to saline controls (range 4 to 32). IgG levels for PEG adsorbed (range 128 to 243 mg/dL) were 50% lower than controls (range 265 to 505 mg/dL). Although PEG adsorption is effective in removing autoantibody, the precipitation of immunoglobulin by PEG may result in failure to detect underlying alloantibody. *Immunohematology* 2001;17:82–85.

Selecting an acceptable and safe antibody detection test can present a dilemma

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The Transfusion Service at Duke University Hospital has changed antibody detection methods from the use of albumin in indirect antiglobulin tests to low-ionic-strength solution (LISS), and from LISS to polyethylene glycol (PEG) in an effort to enhance the rapid detection of clinically significant antibodies. In 1996, staffing issues required the consideration of automation. Although previous studies indicated that the gel test was not as sensitive as PEG for detection of clinically significant antibodies, we chose to implement the gel test to be used with the Tecan MegaFlex-ID. We performed a retrospective analysis of identified antibodies and transfusion reactions to compare the outcomes of one year's experience with gel and PEG. We found comparable detection of potentially clinically significant antibodies by both methods and significantly fewer unwanted or clinically insignificant antibodies detected with the use of gel. Fewer delayed serologic transfusion reactions and no transfusion-associated hemolytic events occurred in the year that gel was used. Although we initially found the selection of the gel test to be a dilemma, our ultimate decision appears to have successfully protected patient safety and balanced sensitivity with specificity. *Immunohematology* 2001;17:86–89