Evaluation of a complement-dependent anti-Jk^a by various sensitization and detection methodologies: a case report

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Abstract: A 79-year-old woman with a diagnosis of lower gastrointestinal bleeding was found to have a complement-dependent anti-Jk^a in her serum. The anti-Jk^a was evaluated by the antiglobulin technique with polyspecific, anti-C3, and anti-IgG antihuman globulin (AHG). A variety of sensitization and detection methods were used, including the prewarmed saline technique, enzyme treatment of test cells, a low-ionic additive solution (LISS), 22 percent albumin, Polybrene, and an increased serum/cell ratio. The anti-Jk^a was detected only when polyspecific AHG and anti-C3 were used compared to anti-IgG, regardless of the selected enhancement technique. Anti-C3 gave weaker reactions than polyspecific AHG. IgG subclassing was inconclusive. The anti-Jk^a was not detected when plasma was substituted for serum. The use of polyspecific versus IgG AHG in pretransfusion testing is discussed.

In 1951, Allen et al.[1] reported the first case of hemolytic disease of the newborn (HDN) associated with anti-Jk^a. Since then, other cases have been reported in which anti-Jk^a caused severe HDN[2] or, in one case, caused no clinical symptoms.[3] However, the evidence that anti-Jk^a [4-6] and -Jk^b [7] cause acute and delayed hemolytic transfusion reactions is well documented.

Kidd system antibodies are often difficult to identify, as they are rarely potent, can show a dosage effect by reacting more strongly with Jk(a+b-) than with Jk(a+b+) cells, and frequently become nondetectable within a few weeks or months after their initial identification.[8] Some of the enhancement techniques used to identify Kidd system antibodies include the use of enzymes, LISS solutions, Polybrene, and an increased serum:cell ratio.[6, 8-11] To be clearly detected, some rare examples of anti-Jk^a require the use of a polyspecific AHG, which contains an anticomplement fraction in addition to anti-IgG.[12-16]

Standard immunohematology protocols vary among hospitals, blood centers, and reference laboratories with regard to alloantibody detection and the routine use of polyspecific or IgG AHG. There is no one method that will detect all alloantibodies. This case report presents comparative data concerning the detection and identification of a complement-dependent anti-Jk^a. The role of anticomplement in AHG for pretransfusion testing is discussed.

Case Report

A 79-year-old woman was admitted to a hospital with a diagnosis of lower gastrointestinal bleeding. The hemoglobin level dropped from 10 g/dL to 8.6 g/dL within four hours and then stabilized at 9 g/dL. The hematocrit was 26 percent. The patient's medications included Feledene (piroxicam), Carafate (sucralfate), and Trazodone (trazodone hydrochloride). A blood sample was sent to the blood bank for pretransfusion testing. Two of three reagent (screening) red cells, tested with the serum, were positive (1+) at the AHG phase. Two of four units of blood were likewise incompatible in the major crossmatch. The direct antiglobulin test (DAT) performed on the patient's red cells was negative. The sample was forwarded to the blood center reference laboratory for antibody identification.

The patient's medical history indicated that several units of blood were transfused in 1956 while she was being treated for an episode of GI bleeding. The attending physician confirmed that she had not been transfused while under his care for the past three years. The patient had been pregnant at least once and had undergone a hysterectomy several years before. She was placed on iron therapy and was not transfused during this admission.

Materials and Methods

Red cell testing

Initial testing at the reference laboratory consisted of an ABO and Rh phenotype and a DAT with two manufacturers' polyspecific antihuman globulins (PS-AHGs) (BCA-Organon Teknika Corp., Malvern, PA, and Gamma Biologicals, Inc., Houston, TX). A complete antigen profile for the common blood group systems was also determined with a variety of blood grouping reagents.

Serum testing

The patient's serum was tested for alloantibodies using a set of two reagent (screening) red cells (Gamma Biologicals, Inc.) and a cord and auto control cell at the immediate spin (IS) and room temperature (RT) phases. Two red cell panels were tested: an untreated inhouse saline panel of donor red cells (WARC 1-86), and a commercial ficin-treated red cell panel (Gamma Biologicals, Inc.). The untreated panel was tested by a strict saline prewarmed technique with polyspecific AHG (Gamma Biologicals, Inc.) as described elsewhere.[9] In this laboratory, saline is prewarmed to 40–41 °C. The ficin panel was tested at IS, 37 °C, and AHG, using IgG-AHG (BCA-Organon Teknika Corp.). A 2–3 percent red cell suspension was used for all test procedures.

Selected Jk(a+b-), Jk(a+b+), and Jk(a-b+) test cells were repeatedly tested with the patient's serum by the prewarmed technique using anti-IgG from three different manufacturers (American Red Cross, Rockville, MD; BCA-Organon Teknika Corp.; and Gamma Biologicals, Inc.). Selected red cells were also tested with anti-C3 (Immucor, Inc., Norcross, GA). Those test cells were read at the antiglobulin phase immediately after centrifugation and also after a 10-minute RT incubation. Different enhancement media tested included: LISS additive solution (Immucor, Inc.), 22 percent albumin (BCA-Organon Teknika Corp.), and Polybrene as described by Lalezari and Jiang.[11] All reagents were prepared in our laboratory. All tests using the LISS additive solution and 22 percent albumin were incubated at 37 °C for 30 minutes.

Plasma was tested with duplicate sets of three Jk(a+b-) red cells and one Jk(a+b+) red cell by the prewarmed technique. PS-AHG was added at the antiglobulin phase to one set, and IgG-AHG was added to the remaining set.

The serum:cell ratio was increased from 2:1 to 4:1 and tested with Jk(a+b−) and Jk(a+b+) red cells. Studies to detect hemolysis were performed as described by Mollison.[17] Ten percent washed cell suspensions of Jk(a+b-), Jk(a+b+), Jk(a-b+), and autologous cells were prepared. One part red cells to two parts patient serum and fresh serum from a group O donor were incubated at RT for one hour. The serum:cell mixtures were then centrifuged and examined for hemolysis. An aliquot of the patient's serum was referred to the Special Services Laboratory at the Holland Laboratories of the American Red Cross for IgG subclassing and monocyte monolayer assay.

All testing followed standard blood banking techniques[9] or was performed according to the manufacturer's instructions. The reactions were graded according to our standardized protocol, similar to that published by Marsh.[18]

Results

The patient's red cells typed as group O, Rh-negative, and had a microscopically positive DAT with polyspecific AHG. The DAT with monospecific anti-IgG (heavy chain specific) and with anti-C3 was negative. An eluate was not prepared, as the patient had not been recently transfused. The red cell phenotype was as follows: C-E-c+e+, M+N-S+s+, P_1+, Le(a-b+), K+, Fy(a-b+), Jk(a-b+).

The antibody screening, cord, and auto control cells were negative at IS and after a 30-minute RT incubation. An anti-Jk^a was detected with the untreated prewarmed panel using PS-AHG. The ficin-treated panel was negative at 37 °C and at the antiglobulin phase using IgG-AHG. Two of 10 ficin-treated red cells were weakly positive at IS but had no apparent specificity. The patient's serum was retested with the ficin-treated panel and a PS-AHG serum was substituted at the antiglobulin phase. An anti-Jk^a was clearly demonstrable (1 to 3+). The anti-Jk^a reactivity did not show dosage with either untreated or ficin-treated panel cells.

The results of the initial study and additional studies done with different manufacturer's IgG-AHG and enhancement media are outlined in Table 1. The same three Jk(a+b-) red cells and one Jk(a+b+) red cell were used for the additional testing to maintain consistent antigen expression. The anti-Jk^a was not detected using the prewarmed panel when three different manufacturer's IgG-AHG were used; however, weak anti-Jk^a reactivity was observed with an anti-C3

reagent. The patient's serum reacted with five of seven Jk(a+b-) red cells at 37 °C with a LISS additive solution, but no reactivity was detected at the antiglobulin phase using IgG-AHG. The anti-Jk^a was not detected with a 22 percent albumin–37 °C–IgG technique, nor by using Polybrene (IS followed by IgG-AHG). Although 22 percent albumin is not truly an enhancement medium for sensitization, it was included in this study because many regional hospitals use it in their testing protocol.

No reactivity was observed when the patient's plasma was tested against both Jk(a+b-) and Jk(a+b+) red cells using either PS-AHG or IgG-AHG. The anti-Jk^a was not detected when the serum:cell ratio was increased to 4:1 using IgG-AHG. The hemolysis studies were negative. When the test for IgG subclassing was done, red cells incubated with the patient's serum reacted with heavy chain-specific anti-IgG but did not react with IgG subclass specific reagents. The monocyte monolayer assay was not performed due to insufficient sample volume.

Table 1
Comparative detection of anti-Jk^a according to the antihuman globulin (AHG) used and sensitization technique employed.

Technique	Test cells		
	JK(a+b−)	Jk(a+b+)	Jk(a−b+)
Prewarmed AHG			
PS-AHG	1 to 2+	2+	0
IgG-AHG #A*	0	0	0
#B*	0	0	NT‡
#C*	0	0	NT
Anti-C3	w± to 1+	w± to 1+	0
Ficin pretreatment			
PS-AHG	1 to 3+	1 to 2+	0
IgG-AHG #A	0	0	0
LISS additive			
37 °C	w± to 1+†	0	0
IgG-AHG #A	0	0	NT
22% albumin			
37° C	0	0	NT
IgG-AHG #A	0	0	NT
Polybrene			
IS	0	0	0
IgG-AHG	0	0	0

* Indicative of different manufacturers' reagents
† 5/7 cells reactive
‡ Not tested

Discussion

The importance of the anticomplement fraction in polyspecific antihuman globulin (PS-AHG) in the detection and identification of the Jk^a antibody at the indirect antiglobulin phase (IAT) in this case report is clearly apparent. The antibody was detected only when PS-AHG or anti-C3 were used. The anti-Jk^a was not detected at the IAT phase with a monospecific anti-IgG (heavy chain specific) reagent when enzymes, LISS, 22 percent albumin or Polybrene were used. When plasma was tested instead of serum, anti-Jk^a reactivity was not apparent. Increased reactivity with Jk(a+b-) and Jk(a+b+) cells with anti-C3, although slight, was observed after a 10-minute incubation at RT. These results emphasize the role of anticomplement in the detection and identification of this example of anti-Jk^a at the IAT phase.

Since Löw and Messeter[19] first introduced the LISS procedure, LISS as either an additive or wash solution has become a well-accepted technique for use in detecting alloantibodies. The manual Polybrene procedure has not achieved the same level of routine use as LISS but has been accepted as an alternative technique (DeLong EN. Unpublished data). Lalezari and Jiang[11] reported that an anti-Jk^a titer could be increased from 25 to 100 with Polybrene. A study published by Steane et al.[20] proposed that a manual Polybrene test was a rapid, sensitive, and suitable test for compatibility. The anti-Jk^a presented in this case report was not detectable when either LISS or Polybrene was used. Both of these procedures usually use anti-IgG at the antiglobulin phase to prevent nonspecific agglutination.

Kidd system antibodies are predominantly IgG3, but can also be a mixture of IgG1 and IgG3.[17] This example of anti-Jk^a, when tested at the Holland Laboratory of the Red Cross, was determined to be IgG in composition; however, attempts to further categorize the IgG subclass were unsuccessful.

In the study by Howard et al.,[13] eight antibodies were detected primarily or only by AHG containing anticomplement activity. Anti-IgG gave weak or negative reactions. Seven of the eight antibodies were anti-Jk^a or -Jk^b and three were implicated in delayed hemolytic transfusion reactions or shortened the survival of incompatible ^{51}Cr-labeled red cells. However, Nance et al.[12] studied a complement-dependent anti-

Jk^a in a patient who received five units of Jk(a+) red cells that did not appear to be clinically significant. A flow cytofluorometry method was used to monitor red cell survival. The survival rate of the Jk(a+) transfused cells was found to be comparable to the expected value for normal red cell survival (38% vs. 37%, respectively). In a case described by Mollison and Newlands,[21] a splenectomized patient with a complement-dependent anti-Jk^a slowly but progressively destroyed transfused Jk(a+b+) cells over a three-week period instead of the typical seven days seen with many delayed transfusion reactions.

Because our patient had not been recently transfused or splenectomized, one cannot determine whether this example of anti-Jk^a would be clinically significant in vivo. It is of interest that this patient's immune system continues to produce a relatively potent Jk^a antibody, considering that the last transfusion was probably over 20 years ago. Issitt reports a case in which an anti-Jk^a, with a titer of 64, continued to be produced by a patient 16 years after exposure to Jk(a+) red cells.[8] The significance of the patient's weakly positive DAT is unknown.

Many hospital blood banks in our region use IgG-AHG, and a few routinely use EDTA plasma for all pretransfusion testing (DeLong EN. Unpublished data). These blood banks would not have detected this example of anti-Jk^a at the IAT phase and, statistically, would have likely transfused Jk(a+) red cells. However, one must keep in mind that a complement-dependent anti-Jk^a detectable solely by PS-AHG is considered to be relatively rare.[14] The advantages and disadvantages of polyspecific and IgG AHG have been debated over the years, most notably by Petz and Garratty,[22] Howard et al.,[13] Wright and Issitt,[15] and Beck and Marsh.[23] The decision to use PS-AHG or IgG-AHG is in part a matter of personal preference and may be based on whether the cost of investigating clinically insignificant complement-binding antibodies would outweigh the risk of missing a rare complement-binding antibody that may be clinically significant. Another aspect of the decision-making process depends on whether the immunohematology testing is being performed in a hospital blood bank, donor center, or reference laboratory. In a hospital, one must consider what is appropriate and practical for a transfusion service and then select methods which will identify the alloantibodies predominantly found in the patient population served. A donor center is primarily interested in detecting clinically significant alloantibodies in the healthy donor population while reference laboratories are prepared to evaluate unusual antibodies that are either not readily identified, react serologically in an unpredictable fashion, or are difficult to isolate from a sample containing multiple antibodies. The resources and methods used in a reference laboratory are more extensive than those used in most hospital blood banks or transfusion services and therefore are more likely to find an unusual antibody of questionable in vivo significance.

It is not the author's intent to recommend one type of AHG or one alloantibody detection technique over another. Rather, it is to remind immunohematologists that, as the trend in pretransfusion testing is toward less testing, as with the abbreviated crossmatch, one must evaluate the current methodologies to determine what is appropriate and practical and what will provide the least risk to the patients.

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Educational and scientific posters

M. S. KENNEDY

Many associations and societies are encouraging poster presentations instead of oral presentations at meetings. However, posters prepared by professionals can be costly ($200 and up). Neat, easy-to-read, and attractive posters that can be prepared at a low cost are desirable. Instructions follow for the preparation of such posters.

Materials and Methods

Decide on a title for the poster presentation and then organize the information to be presented into introduction, materials and methods, results, and conclusions, as for an oral presentation. Write each section concisely, including enough information to clarify the points. Simple tables and diagrams are helpful to the audience. Diagrams may be beyond your artistic skill, but tables can easily be done using methods presented here.

In a commercial artists' supply store (see the yellow pages), buy a package of colored construction paper (all one color). Each sheet of paper is 9″ x 12″, so 30 sheets would be needed to cover a 4′ x 6′ poster display area. For a stiffer backing, poster board (all one color) can be used. One 28″ x 44″ poster board usually costs less than $2. Two and one-half sheets of poster board will cover the 4′ x 6′ display area. If the store has a cardboard cutter, you're in luck; otherwise, a long steel ruler and a knife will be needed. Cut each of the colored poster boards into eight 11″ x 14″ pieces.

Buy transfer letters (Prestype®, Chartpak®) in 84-point type for the title and in 48-point type for the authors and institutions. Helvetica Medium is a good type style to choose. For the title, select white poster board or smooth-finish, white drawing paper. Cut into appropriately sized 6″ x 12″, 6″ x 14″, or 9″ x 11″ pieces, to show a colored border above, below, and at extreme ends when each is glued or pinned to the colored poster board or construction paper (see Fig.