Acute hemolysis after intravenous immunoglobulin amid host factors of ABO-mismatched bone marrow transplantation, inflammation, and activated mononuclear phagocytes

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BACKGROUND: Hemolysis may follow intravenous immunoglobulin (IVIG), with product, dosing, and host factors contributing. The importance of recipient features remains unclear.

CASE REPORT: A 52-year-old obese woman, 10 years after ABO-mismatched (recipient O, donor A) marrow transplantation, presented with immune thrombocytopenia (ITP). IVIG at 100 g/day × 2 days was followed by hemoglobinuria and angina and dyspnea, with frank hemoglobinemia and anemia (hemoglobin 12.9 to 8.4 over 24 hr, to a nadir of 6.9 g/dL).

STUDY DESIGN AND METHODS: Serologic methods established ABO, A1, Lewis, and Secretor type, while monocyte monolayer assay (MMA) examined erythrophagocytosis with control or patient monocytes, and the implicated IVIG lot to opsonize control (group A1, A2, B, O) or patient red blood cells (RBCs). Baseline, hemolytic, and convalescent markers (including cytokines) were assessed.

RESULTS: Passive anti-A was identified on reverse type and eluted from sensitized RBCs (immunoglobulin G 1+, C3d–). Le(a–b+) typing and saliva confirmed H Secretor status. MMA revealed significant activity between patient RBCs, monocytes, and IVIG. However, normal A1 cells opsonized with IVIG were not significantly phagocytosed by either normal or patient monocytes. Proinflammatory markers were significantly elevated before and after IVIG.

CONCLUSIONS: Synergizing host factors (including obesity-unadjusted dosing and existing inflammation) marked this severe post-IVIG hemolytic crisis. Group A antigen restriction to myeloid tissues, with H Secretor phenotype, may have contributed, rendering this bone marrow transplant chimera vulnerable to anti-A in a manner analogous to the idiosyncratic effect of therapeutic anti-D in certain D+ ITP recipients. However, MMA suggested a macrophage activation state as contributory, perhaps precipitated by existing inflammation.

Intravenous immunoglobulin (IVIG) is administered in a variety of clinical settings, with wide use in the treatment of primary and secondary immunoglobulin deficiencies,[1] as well as in autoimmune or inflammatory conditions believed to be mediated by autoantibodies or T cells.[2,3] When used as an immunomodulatory agent, IVIG is administered in higher doses, typically at 2 g/kg.[4] High doses of IVIG were first administered for the treatment of pediatric immune thrombocytopenia (ITP).[5] Mechanisms of action remain incompletely defined, although interference of phagocyte Fc receptor–mediated immune clearance is thought to play a prominent role.[6]

Adverse reactions have been associated with IVIG administration, ranging from mild to severe. Common side effects of IVIG infusion include pyrexia, rigors, and

ABBREVIATIONS: BMI = body mass index; ER = emergency room; IL-1ra = interleukin-1 receptor antagonist; ITP = immune thrombocytopenia; MMA = monocyte monolayer assay.

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headache.[7] Less commonly, hemolysis is observed in association with the passive acquisition of immunoglobulin (Ig)G directed against a number of red blood cell (RBC) antigens.[3] The transfer of such alloantibodies, including anti-A, -B, -C, -D, and -K, may manifest with a positive direct antiglobulin test (DAT), which in turn may or may not predict hemolysis.[8,9] Although up to 64% of neuroinflammatory patients receiving IVIG exhibited modest decreases in hematocrit (mean, –1.04%) in one series,[10] it is not always possible to distinguish the effects of hemodilution from accelerated RBC destruction, and cases of severe hemolysis are reported much less frequently.[3,8,11-14] The rate of clinically significant hemolysis from the available literature has been estimated to range from 1.6%[8] to nearly one in three evaluated patients.[15] While IVIG administration in allogeneic stem cell transplant patients is usually reserved for those with hypogammaglobulinemia (trough IgG < 5-7 g/L), or for the treatment of viral infections,[4,16,17] immune globulin treatment of ITP after allogeneic stem cell transplant has also been documented.[18]

Factors involved in severe, idiosyncratic hemolysis after IVIG administration remain unclarified. We present a case where a female allogeneic bone marrow transplant (BMT) recipient demonstrated severe hemolysis of roughly half of her original RBC mass after administration of IVIG. This acute phenomenon, beginning within hours and lasting for days, manifested with visible plasma hemoglobinemia and hemoglobinuria, blood film spherocytosis, and the detection of passively acquired anti-A IgG on direct and indirect testing. We sought to characterize patient and product features associated with this dramatic response.

CASE REPORT AND LABORATORY STUDIES

A 52-year-old obese Caucasian woman presented to the BMT clinic for a recent history of epistaxis and easy bruising. She was remotely parous (P3) and had a history of precursor B-cell acute lymphoblastic leukemia, Philadelphia chromosome negative, for which she underwent myeloablative allogeneic BMT from her histocompatible brother almost 10 years earlier. The graft itself was ABO major mismatched (recipient group O, D+; donor group A1, D+), while both donor and recipient were CMV IgG seropositive. The patient's course after transplant had been complicated by chronic graft-versus-host disease of the skin, liver, and lungs, for which she required immunosuppressive therapy for the first 3 years. Since the BMT, there had been no evidence of mixed chimerism or graft failure or leukemic recurrence at any time. Her last blood component exposure occurred a decade ago before BMT engraftment, and she was consistently RBC antibody screen negative, with no known history of transfusion reactions.

Her recent mucocutaneous bleeding correlated with a platelet (PLT) count of 40×10^9/L but no other cytopenias, and she denied any constitutional symptoms. Marrow examination excluded acute lymphoblastic leukemia relapse, myelodysplastic syndrome, and lymphoproliferative disorder. A diagnosis of ITP was made by exclusion and treatment with IVIG was ordered. Her calculated body mass index (BMI) was 40 kg/m^2 (reference range, 18.5-25; obesity > 30). IVIG was administered at an unadjusted dose of 1 g/kg/day (total dose, 2 g/kg), for 100 g/day.

During the course of the infusions, monitored vital signs were without substantial fluctuations. She did not experience any adverse reaction over the first infusion date (Day +1). After her second infusion date (Day +2), she was discharged in stable condition. However, later that evening she began noticing urinary discoloration, and by the next morning she also noted jaundice, right-sided chest pain, palpitations, and dyspnea.

Her hemoglobin (Hb) level had decreased from the baseline of 12.9 mg/dL (Day –3) to 8.4 mg/dL by the evening of the emergency room (ER) visit (Day +3). A sample of centrifuged whole blood was frankly hemoglobinemic (Fig. 1A), while the urine appeared red. The peripheral blood film demonstrated marked spherocytosis with some agglutination and polychromasia, not present at baseline. Laboratory investigations indicated hemolysis (Fig. 1B, Table 1).

The patient was admitted to the hospital with the diagnosis of severe, subacute IVIG-associated hemolytic anemia, bearing features of extravascular (spherocytic) and possibly intravascular (or overwhelmed extravascular) erythrodestruction. The nadir Hb occurred on Day +9 at 6.9 mg/dL, translating to a 47% loss of RBC mass.

Treatment was supportive. Throughout her 10-day hospitalization, she declined transfusions despite suffering various sequelae of the anemia, such as dyspnea requiring supplemental oxygen, and the progression to atrial fibrillation from compensatory sinus tachycardia. Cardiac investigations included nuclear myocardial perfusion imaging, which showed modest reversible circumflex ischemia, not requiring interventional angiography. Troponin levels had not increased despite tachycardic demands, nor were serious electrocardiogram changes noted.

By the last 2 to 3 days of her admission, an increase in Hb was observed (8.1-8.4 g/dL). Despite the observed extent of hemolysis and hemoglobinemia, urine output and renal function remained normal, and coagulation times were never disrupted.

The IVIG achieved a salutary effect toward the ITP, as the PLT count increased to 171 by Day +3 and had increased further to 333 by Day +12, with no relapse as of Day +316.

A report on this reaction to IVIG was submitted to Health Canada's Health Products & Food Branch as well as

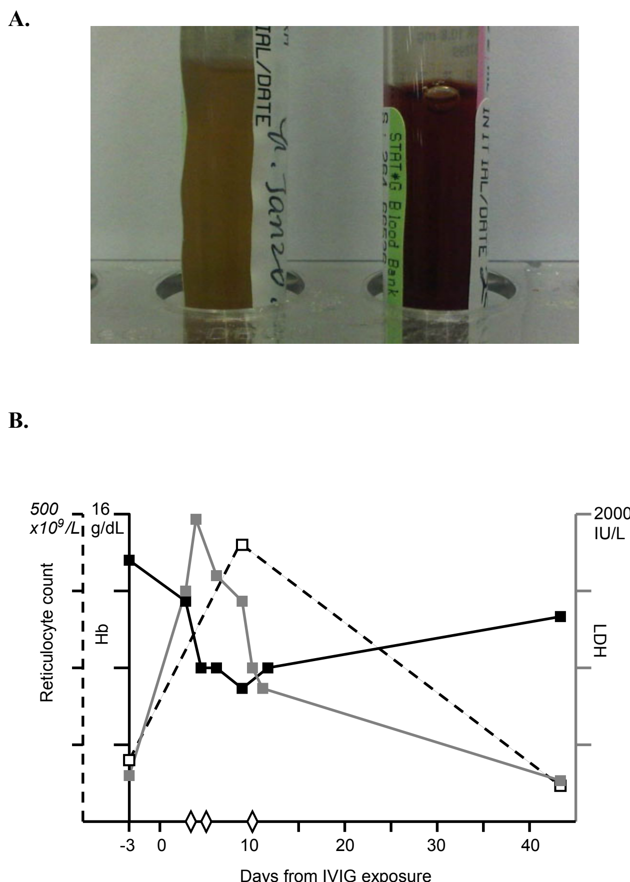

Fig. 1. Clinical course of patient. (A) Visible plasma hemoglobinemia from the ER (morning after Days 1 and 2 of IVIG infusions), compared with pre-IVIG specimen. (B) Course of hemolysis as demonstrated by Hb (■), LDH (■), and reticulocyte count (□) at baseline (Day –3) and at other time points after administration of IVIG. (◇) Instances of verified spherocytosis.

to the manufacturer, as a severe event classifiable as Grade 4 IVIG-associated hemolysis according to criteria published in 2009 (Table 2).[19,20]

MATERIALS AND METHODS

Samples and consent

Specimens from immediately before IVIG (Day –3, peripheral blood EDTA and marrow) and shortly after IVIG (Day +3 to Day +12, peripheral blood) were obtained from the patient. On her outpatient convalescent return (Day +43), both she and her sibling donor agreed to provide peripheral blood and buccal swab material with explicit written consent from each for additional case-relevant analyses and dissemination of anonymized results.

Standard transfusion laboratory testing[21]

Conventional tube technique applied to manual blood typing, the DAT, and acid elution studies, while the indirect antiglobulin test (IAT) for RBC antibodies was performed on a two-cell screen by gel microcolumn, enhanced with low-ionic-strength saline (DiaMed-ID, Ortho Clinical Diagnostics, Raritan, NJ). Immunohematology reagents (Immucor Gamma, Dominion Biologicals Ltd, Dartmouth, Nova Scotia, Canada) included forward typing anti-sera (Novaclone anti-A and anti-B [Series 1] and anti-A,B murine monoclonal IgM; anti-A1 *Dolichos biflorus* lectin; anti-D1 [Series 4] and anti-D2 [Series 5] monoclonal blend [IgM + IgG]; anti-Lea and anti-Leb Gamma-clone); reagent RBCs (for reverse grouping, Referencells A1, A2, B, and O; antibody screening, Panoscreen I and II; and antiglobulin test control, Checkcell); anti-human globulin sera (Novaclone polyspecific anti-IgG,C3d, monospecific anti-IgG, monospecific anti-C3d); and specialty products (Gamma ELU-KIT Plus).

Implicated IVIG lot(s)

The IVIG (Gamunex 10%, Grifols Canada, Mississauga, Ontario, Canada) administered on Day 1 (five bottles × 20 g, Lot 26NK1T1 [Sublots 59, 57, 60, 46, 58]) and Day 2 (five bottles × 20 g, Lot 26NK281 [Sublots 5, 31, 8, 3, 6]) were reviewed by lookback. The former lot elicited no reaction in this or any other recipients. The latter lot (which had been followed within hours by the reported reaction) had been associated with three other reported reactions among a total of 81 recipients (two minor allergic reactions, and another case of Grade 4 passive anti-A hemolysis [Hb 11.2 to 5.4 g/dL in a group A patient who had lupus-associated ITP; not further evaluated]). Additional in vitro studies were thus pursued from Lot 26NK281 due to its inventory availability and circumstantial culpability. The titer of anti-A and anti-B were reported by the manufacturer to be within FDA and Health Canada requirements.

Cytokine analyses

Cytokine analyses were done on both pre-IVIG and post-IVIG plasma samples. The following Th1 and Th2 cytokines were selected for testing: Th1—interleukin (IL)-1β, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α; Th2—IL-4 and IL-13. In addition, IL-1 receptor antagonist (IL-1ra) was tested as an additional, surrogate means of evaluating ongoing inflammation. All cytokine levels were determined by R&D Systems, Inc. (Minneapolis, MN).

Monocyte monolayer assay

A monocyte monolayer assay (MMA) with control or patient monocytes, and the implicated IVIG lot to

TABLE 1. Serial variables with hemolytic IVIG exposure (administered Days +1 and +2)

Test (reference ranges)	Before IVIG (Day –3)	After IVIG (Day +3 to +12)	After recovery (Day +43)
Blood grouping			
Forward	Anti-A = 4+	Anti-A = 4+	Anti-A = 4+
	Anti-A1 = 4+ MF	Anti-A1 = 4+ MF	Anti-A1 = 2+ MF
Reverse	A1 RBCs = 0	A1 RBCs = 2+	A1 RBCs = 0
	B RBCs = 4+	B RBCs = 4+	B RBCs = 4+
DAT	IgG-, C3d-	IgG 1+, C3d–	IgG–, C3d–
pfHb (<29 mg/L)		431 (Day +5)	
Haptoglobin (0.3-2 g/L)		<0.06	1.77

MF = mixed field; pfHb = plasma-free Hb.

TABLE 2. Criteria for the definition of hemolysis associated with the use of IVIG, as developed by the IVIG Hemolysis Pharmacovigilance Group[19]

Variable	Definition
Onset	Within 10 days of IVIG administration
Laboratory signs	Decrease in Hb of ≥1 g/dL
	Positive result of DAT
	At least 2 of:
	• Increased reticulocyte count
	• Increased LDH level
	• Low haptoglobin level
	• Unconjugated hyperbilirubinemia
	• Hemoglobinemia
	• Hemoglobinuria
	• Presence of significant spherocytosis
Exclusion criteria	History or examination consistent with an alternate cause of anemia, including blood loss, other drug-induced hemolysis, anemia associated with chemotherapy for cancer, or hemolysis associated with an underlying disease
	Negative result of DAT
	Absence of other inclusion criteria, in particular evidence of hemolysis

opsonize control (group A1, A2, B, O) or patient RBCs was performed as previously described,[22] with important modifications to address the use of IVIG in the assay.

Preparation of opsonized RBCs

D antigen–expressing (R_2R_2) RBCs (Canadian Blood Services, Toronto, Ontario, Canada) and reagent reference RBCs (A1, A2, B, O; Immucor Gamma) were washed three times in phosphate-buffered saline (PBS; $425 \times g$, 10 min). Seven parts packed R_2R_2 were added to a microcentrifuge tube containing three parts polyclonal anti-D (Gamma Biologicals, Inc., Houston, TX). The reagent reference RBCs were added to individual microcentrifuge tubes and IVIG (10% wt/vol, Gamunex Lot 26NK281; Gamunex Lot 26NK1T1 was not available for testing) added at a concentration of 20 to 50 mg/mL to reflect a range of IVIG concentrations in vivo of 1 to 3 g/kg for an 80-kg person. The tubes were vortexed gently to mix and incubated (37°C, 5% CO_2, 1 hr). After opsonization, the cells were again washed (3×, $600 \times g$). R_2R_2 cells gave a 2+ to 3+ result by IAT. Alsever's reagent was added 1:1 and the cells stored at 4°C until needed (up to 2 weeks). On the day of use, cells were washed before suspension (1.25%, vol/vol) in RPMI 1640 (supplemented with 10% heat-inactivated fetal bovine serum, 20 mmol/L Hepes; complete RPMI). IVIG-opsonized RBCs were prepared the day before the MMA and were stored (12-18 hr) at 4°C until used. Reference RBCs gave negative to weak results by IAT, with A1 cells the most reactive, likely due to anti-A in the IVIG.

MMA

Patient (freshly drawn on Day +10) and normal whole blood along with the implicated IVIG lot were obtained. MMA was performed as previously described[21] with slight modification. Briefly, isolated peripheral blood mononuclear cells (PBMNCs) were resuspended in complete RPMI medium and allowed to incubate overnight (37°C, 5% CO_2). The PBMNC suspension was centrifuged once ($300 \times g$, 10 min), supernatant removed and the pellet resuspended to produce a suspension of 1.75×10^6 cells/mL. Eight well chamber slides (Thermo Fischer Scientific, Lab-Tek II, Nalge Nunc, Rochester, NY) were seeded with 0.4 mL, 0.7×10^6 PBMNCs and incubated to allow the adherence of monocytes (37°C, 5% CO_2, 1 hr). The chamber slides were then aspirated of their contents and 0.4 mL of opsonized RBC suspension (1.25%, vol/vol) or control nonopsonized RBCs was added to each chamber and further incubated for 2 hours.

After the incubation steps, the upper structure of the chamber was removed according to the manufacturer's protocol. The slides were carefully washed of superfluous RBCs using PBS and allowed to air dry briefly. Once dry, the slides were fixed in methanol for 30 seconds and allowed to fully dry before coverslips were added. Polyvinyl alcohol[23] was used as an adhesive and preservative. Phase contrast microscopy was utilized to analyze the number of RBCs that had undergone phagocytosis by a minimum of 100 monocytes. At least 300 monocytes were counted in each assessment. Anti-D–opsonized R_2R_2-positive controls across chamber slides of matching PBMNCs donor/patient were averaged. As with the remaining samples, the number of RBCs phagocytosed were divided by the number of monocytes observed and then multiplied by 100 to give an index of phagocytosis. The R_2R_2 phagocytic index was determined to be 51 ± 47 (n = 13; range, 3-155). Use of the R_2R_2 anti-D–opsonized RBCs served as a control of the assay conditions, to show that phagocytosis was occurring with the monocytes, so as to allow for interpretation of results using IVIG-opsonized RBCs according to ABO type.

IVIG- (20 to 50 mg/mL) opsonized A1 RBCs consistently gave the highest phagocytic indices against normal monoctyes (Figs. 2A and 2B; data not shown). Combined results with 21 different normal donor-sourced monocytes against IVIG-opsonized A1 RBCs gave a mean ± standard deviation (SD) of 9 ± 8 for the phagocytic index (data not shown). Based on these results, significant phagocytosis was defined as a phagocytic index of greater than 17.

RESULTS

Acute hemolytic transfusion reaction

Pre-IVIG grouping demonstrated a strong and uniform A, D+ type with only anti-B on reverse typing, while *D. biflorus* lectin testing for A1 expression showed a 4+ mixed field. Lewis antigen typing (Day +3, Le[a–b+]) was consistent with positive Secretor status, while saliva testing (Day +43)[24] confirmed that she was a secretor of H substance alone. Baseline variables for hemolysis were negative (nonspecific changes on peripheral blood film, DAT-negative, with normal bilirubin, lactate dehydrogenase [LDH], and reticulocyte count).

In the ER after her IVIG infusions, the DAT was positive (polyspecific 1+ for IgG with anti-A IgG eluted, negative for C3d), together with the acquisition of a (new) 2+ reaction toward A1 cells on reverse typing (direct agglutination at room temperature). Both of these changes disappeared by the time of retesting (Day +7), and no other anti-RBC IgG plasma antibodies were identified during this period.

Although DAT was negative for C3d deposition, there had been partial serum complement depletion (Day +2, C3 1.57 g/L, C4 0.08 g/L [normal values, C3 0.9-1.8 g/L, C4 0.1-0.4 g/L]), and this finding had in turn recovered to low-normal range by Day +7 (C3 1.29 g/L, C4 0.14 g/L).

Biochemically, the LDH had nearly quintupled to 1460 IU/L in the ER, further peaking at 1970 IU/L (Day +4) before trending down. Bilirubin increased 10-fold from 7 μmol/L at baseline to a peak of 71 μmol/L (indirect fraction 62 μmol/L) in the ER, before falling to 23 μmol/L on discharge. In parallel, the reticulocyte count had increased from 98×10^9 to 457×10^9/L (Day +9). Day +5 plasma-free Hb was 431 mg/L (15× upper limit of normal [≤29 mg/L]), and ferritin had increased from 296 μg/L (baseline) to 646 μg/L (Day +12) (normal range, 22-275 μg/L) with this hemolytic load and/or macrophage activation. Postrecovery testing for paroxysmal nocturnal hemolgoblinuria by flow cytometry[25] was also negative. The course of hemolysis is summarized in Fig. 1 and Table 1.

Chimerism

Donor chimerism on the Day –3 marrow specimen was 97.2%, and at the time of the hemolytic reaction when anti-A was also serologically detected, peripheral blood B lymphocytes continued to reflect 97.6% donor chimerism, with no evidence of a surge in recipient-attributable lymphocyte proliferation.

ABO genotyping

The patient's epithelial (buccal swab) DNA was O/O in genotype, while the peripheral blood of both the remotely transplanted recipient and the donor were A1/O in genotype.

Cytokine profile

There were baseline elevations in both the IL-1ra level (7729 pg/mL; 22-fold increase above normal) and the IL-1β level (5.6 pg/mL; threefold increase) before the IVIG was administered. After IVIG, both IL-1β (20.0 pg/mL; 11-fold increase) and TNF-α (8.9 pg/mL; 1.5-fold increase) were increased. IL-1ra levels decreased slightly after IVIG but remained substantially increased, 19-fold higher compared to normal levels (6927 pg/mL). Other cytokines were at normal levels, both before and after IVIG: IFN-γ (<15.6 pg/mL), IL-13 (<62.5 pg/mL), and IL-4 (<1.6 pg/mL).

MMA results

Highly significant phagocytosis was seen using either patient or normal monocytes when tested with patient RBCs opsonized with the implicated lot of IVIG (Fig. 2C). In addition, using reference RBCs, different patterns of reactivity were noted depending on whether normal

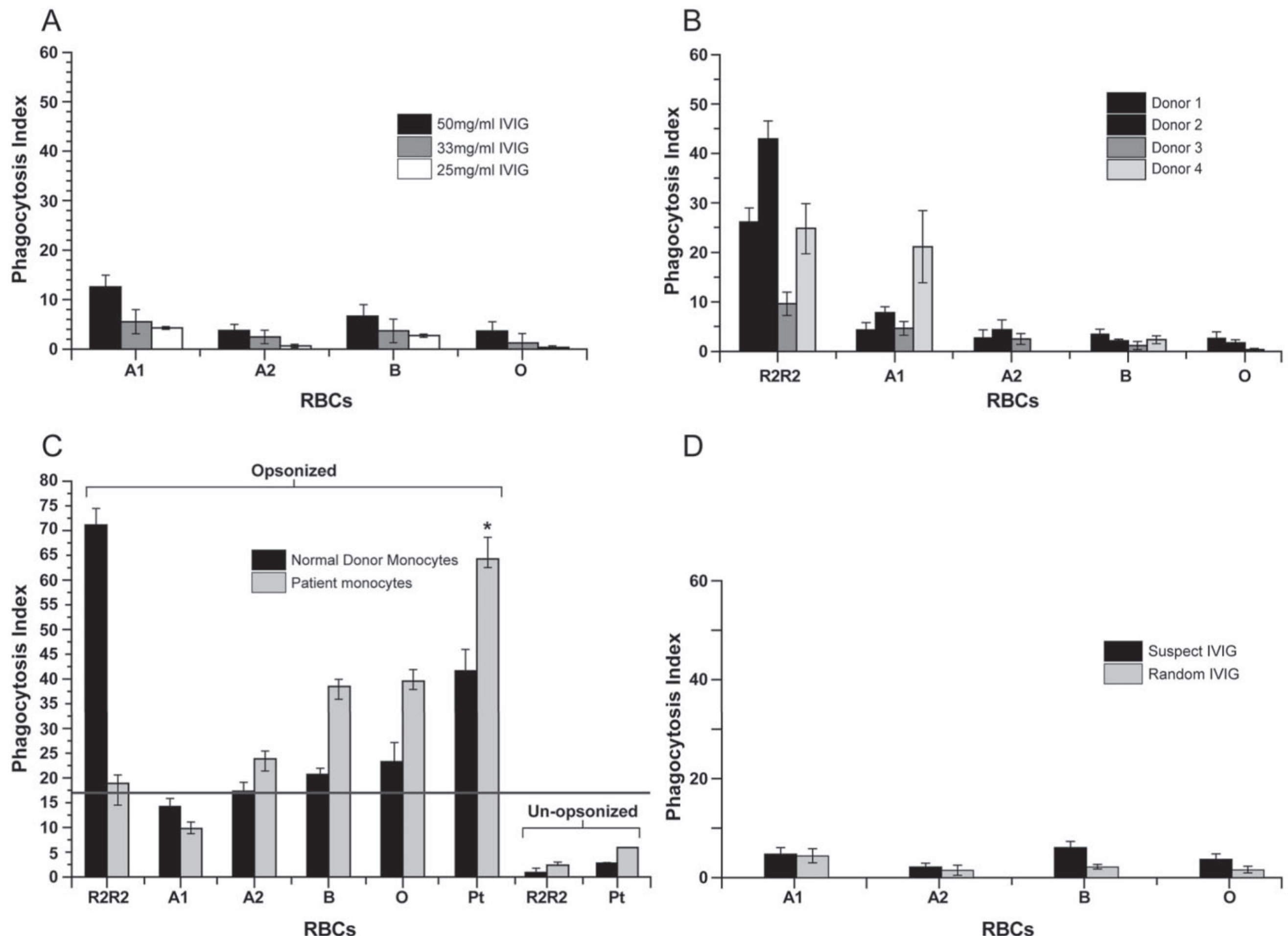

Fig. 2. MMA for phagocytosis of IVIG-opsonized RBCs. (A) Reference RBCs were opsonized with different concentrations of IVIG (50, 33, and 25 mg/mL, respectively, corresponding to approximately 3, 1.5, and 1 g/kg based on an 80-kg person). Opsonized RBCs were incubated with a single normal donor's monocytes and the phagocytic index determined (see Materials and Methods). Results represent the mean ± standard error of the mean (SEM) of triplicate culture wells. (B) Phagocytic indices, using four different normal donors' monocytes against reference RBCs opsonized with 40 mg/mL (equivalent to approx. 2 g/kg based on an 80-kg person). A positive control RBC (R_2R_2) opsonized with anti-D is also shown. Results represent the mean ± SEM of triplicate culture wells. (C) Patient (Pt) and normal (single) donor monocytes are incubated with reference and Pt RBCs, opsonized with the suspect lot of IVIG (40 mg/mL), with phagocytosis compared. A positive control of R_2R_2 RBC opsonized with anti-D is shown as well as negative controls of nonopsonized R_2R_2 and Pt RBCs. Significant phagocytosis is indicated by a phagocytic index above 17 (see Materials and Methods). Results represent the mean ± SEM of triplicate culture wells. (D) The suspect lot or a random lot of IVIG is used to opsonize (40 mg/mL) reference RBCs and then the opsonized RBCs and phagocytic index determined using monocytes from three healthy donors (in triplicate). The results represent the mean ± SEM of the combined phagocytosis indices.

(control) or patient monocytes were used. Normal monocytes did not show any increased phagocytosis of opsonized reference RBCs, regardless of blood group. In contrast, when using patient monocytes, increased phagocytosis of group A2, B, and O RBCs was seen but not with A1 RBCs (representing the patient's own blood group; Fig. 2C). To ensure that the results with the patient and normal monocytes against the patient's RBCs opsonized with the suspected IVIG were not due to an intrinsic property of the IVIG, additional testing with multiple donors against the reference RBCs opsonized with the IVIG were performed showing no significant phagocytosis (Fig. 2D).

DISCUSSION

The IVIG Hemolysis Pharmacovigilance Group of Canada has defined IVIG-related hemolysis as a new hemolytic process within 10 days of IVIG administration, not attributable to other causes (Table 2).[19] While already a well-

recognized phenomenon, the case described is remarkable both for its severity (Grade 4 by the aforementioned case definition, which is rarely reported[7,12]) and for the unusual phenotype of the recipient, a constitutionally group O Secretor with a BMT-derived group A blood type. Extended reference laboratory testing of both the patient and an implicated lot of product provided novel insights into possible mechanisms of observed hemolysis.

Although the hemolysis was apparently intravascular (i.e., visibly and quantifiably hemoglobinemic plasma, with red-tinged urine), true intravascular hemolysis requires completion of the complement cascade to the level of membrane-attack complex formation. Complement levels were transiently decreased in the postinfusion period, but not accompanied by detectable complement binding on patient RBCs. The absence of renal injury or disseminated intravascular coagulation or systemic inflammatory response syndrome, with the increased phagocytosis in the complement-poor environment of the MMA assay together suggested that the plasma-free Hb may have reflected overflow from a predominantly extravascular process.

Another prominent feature in this patient's laboratory investigations was the presence of a transient grouping discrepancy due to a directly agglutinating anti-A. As such discrepancies are not commonly seen in IVIG recipients, an examination of the possibility of rekindled residual host-derived (group O) memory B-cell activity (i.e., a break-of-tolerance insult) was pursued. However, full donor chimerism argued against this hypothesis and it is more likely that the transient anti-A activity observed in the plasma was passively acquired from the IVIG itself. IgG antibodies are generally considered incapable of inducing direct agglutination, raising the possibility that the implicated lot was contaminated with IgM, IgG, and/or IgA dimers. Indeed, the association of one of the implicated lots with a possible hemolytic reaction in another patient would seem to support this possibility, notwithstanding confirmation from the manufacturer that the lot in question had isoagglutinin titers that were within industry standards (maximum, <1:64). Recently, an international collaborative study on the standardization of testing for anti-A and anti-B in IVIG products detected an up to 16-fold variation in anti-A and anti-B titers.[26] To help identify batches of IVIG that may promote hemolysis in patients, cross-matching before infusion has been suggested.[27] Alternatively, the titers may be measured for the purpose of deferring on the use of high-titer lots (e.g., >16)[15] in those with a vulnerable antigen type and/or those at an increased risk of hemolysis.[28]

As IgG directed against RBC antigens that are both densely expressed and relatively elevated from the RBC surface (as is the case with A antigens) can in fact be directly agglutinating, the presence of detectable anti-A in the reverse grouping likely represents a relatively high concentration of IgG in the patient's plasma at the time of testing. This in turn can be ascribed to two possible explanations. One is that the administered dose was based on unadjusted body weight in an obese patient, despite the fact that IgG is relatively lipophobic. Although 2 g/kg is an accepted dose for the treatment of ITP,[29] the initial studies that first revealed the immunomodulatory effects of this dose were conducted in children, who are less likely to have an elevated BMI. In a case series by Daw and colleagues[8] it was reported that patients experiencing hemolytic transfusion reactions from IVIG were more likely to be women and/or receiving doses of 100 g or higher, also suggesting a possible role of elevated BMI. Other groups have noted a decrease in hemolytic transfusion reactions when IVIG doses are adjusted for lean body mass.[29] Had this patient been dosed using a popular online calculator (http://www.transfusionontario.org/dose/), she would have received 140 rather than 200 g of IVIG, possibly mitigating the risk of an adverse reaction.

However, given that even doses 1 g/kg of IVIG have been associated with hemolytic anemia,[28] it is likely that other patient risk factors were at play in this case. The increased risk of patients with non-O blood groups, such as in the case presented, is well recognized, likely reflecting the density of the vulnerable ABO antigen and the affinity of associated isoagglutinins.[30] In addition, the Secretor status of this patient must also be taken into consideration. Born a group O Secretor (of H), she persisted in this phenotype after transplant, as proven by both her Le[a–b+] phenotype as well as the presence of H substance in her saliva.[31,32] Transplantation with a group A graft led to the expression of A antigen which was confined to her myeloid/erythroid mass, without extrapolated soluble A substance by which to competitively quench infused anti-A and thus potentially mitigate the severity of the anti-A effects.[33] A recently reported case of fatal passive hemolytic transfusion reaction (from a group O single donor apheresis PLT) occurred in an adult whose hematopoiesis had similarly converted from native group O to graft group A after transplantation,[34] illustrating the vulnerability created by tissue-restricted expression of A. We speculate that the gap in differential (or soluble) A antigen expression had imitated the particular vulnerability of D+ recipients with respect to the effects of large-dose anti-D infusions in the treatment of ITP, which at times may associate with severe intravascular hemolysis.[35]

It is also notable that our patient had evidence of an existing state of immune activation, as suggested by the pre-IVIG cytokine levels (IL-1ra and IL-1β) and inflammatory biomarker profile. This may have reflected her underlying diagnosis of ITP,[22,36,37] and unlike most adult ITP patients, she had not been primarily treated with glucocorticoids and had instead received IVIG as solitary therapy. Similar cytokine responses, as well as elevations in TNF (noted after IVIG infusion in this patient) have

been shown to occur in response to IgG-coated RBCs, supporting a central role for these cytokines in the pathophysiology of IVIG-related hemolysis.[38] One possible explanation for the severity of hemolysis noted in this case could therefore be that the patient's existing, steroid-unmitigated[39] inflammatory state anticipated the same pathophysiologic processes that underlie a hemolytic transfusion reaction; a similar "two-hit" model of IVIG-mediated hemolysis (i.e., existing inflammation followed by the infusion of incompatible isoagglutinins) has been proposed by others as well.[8]

MMA testing appeared to indicate that the severity of the hemolysis was at least partially attributable to patient activated mononuclear phagocytes, in that elevated phagocytosis was observed when the patient's own phagocytes were tested. However, the results of the MMA assays performed in this case also suggest that there may be a subsequent phase of the hemolysis that was not dependent upon anti-A. Specifically, in these assays patient RBCs appeared to be more prone to IVIG-mediated hemolysis than reference RBCs of the same blood group. Conversely, patient monocytes demonstrated increased phagocytosis of IVIG-opsonized reference RBCs without apparent blood group preference (although the phagocytosis profile of O > B > A2 > A1 cells suggested anti-H specificity). Other groups have hypothesized that IVIG-mediated hemolysis may involve a bystander mechanism whereby immune complexes provoke complement deposition on the RBC surface, with subsequent erythrophagocytosis through the macrophage CR1 receptor. Such a mechanism might explain the apparently increased risk of IVIG hemolysis in multiparous females, who are more likely to have antibodies reacting with the high-molecular-weight moieties present in IVIG, thereby activating complement.[8] Alternatively, certain IVIG formulations may themselves contain high-molecular-weight IgG complexes which are capable of activating complement.[40] In a recently published murine model of incompatible RBC transfusion, it was also observed that while Fcγ receptors were required for the sequestration of incompatible RBCs and the generation of a cytokine storm, the phagocytosis of incompatible RBCs required neither C3 nor Fcγ receptors.[41] The authors of this study hypothesized that RBC antibody binding induced expression of an "eat me signal" (e.g., phosphatidylserine), recognized by other scavenger receptors. A complementary explanation is that macrophages, once activated by IgG-bound RBCs, enter a state of hyperactivation in which all RBCs (regardless of their blood group) have accelerated clearance.

Given the remaining uncertainties regarding the mechanism of IVIG-mediated hemolysis, recommendations for secondary preventions cannot be made with certainty. In this particular patient, however, subsequent courses of therapy are to be administered at lower doses (1 g/kg IVIG at body weight adjusted for BMI). Because the patient is D+, there is also the option of IV anti-D, although this therapy would remain contraindicated in the setting of active immune hemolysis and/or a positive DAT.[42] Regardless of whether IVIG or anti-D are administered, however, monitoring for both acute and delayed hemolytic reactions would be advised.

In conclusion, the severity of hemolysis after IVIG in this patient is multifactorial. Underlying inflammatory (or hyperphagocytic) conditions, the distribution of vulnerable ABO antigen expression in BMT chimera, Secretor status, concentration of anti-A/B in IVIG, and IVIG dosing are all potential contributors to this process. This is the first report of the use of an MMA to evaluate IVIG-associated hemolysis. The MMA and testing for before and after (proinflammatory) cytokines may prove useful in unraveling the mechanism of hemolysis associated with the administration of IVIG.

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CONFLICTS OF INTEREST

None.

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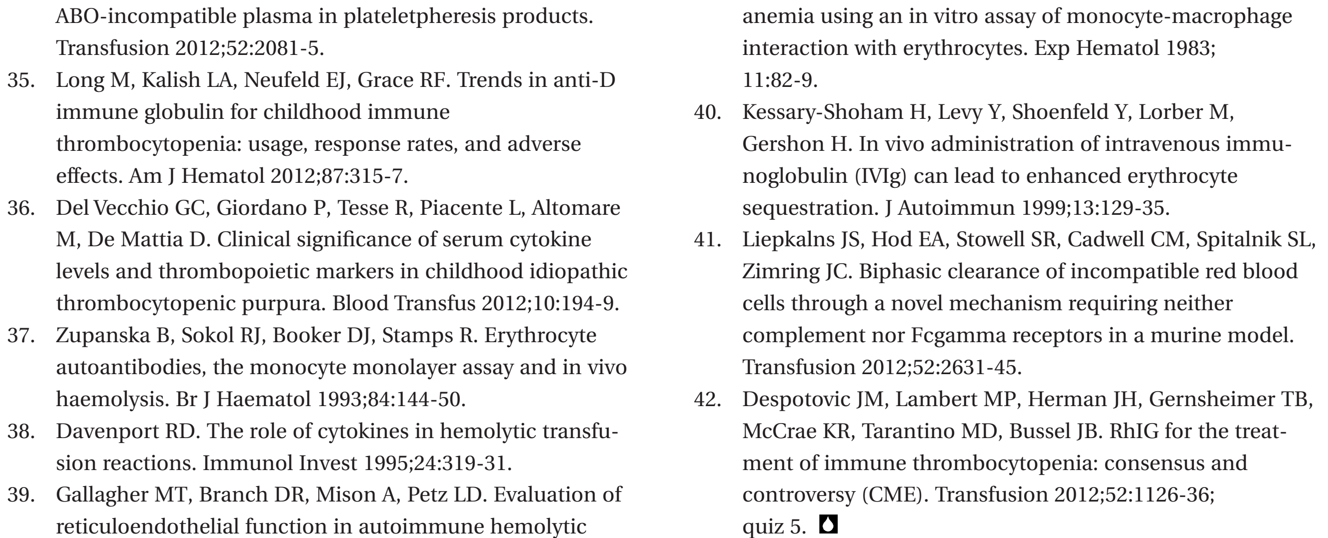

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