

GIL: a red cell antigen of very high frequency

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A new high-frequency red cell antigen has been identified and named GIL. GIL differs from all high-frequency antigens included in the International Society of Blood Transfusion classification. There is very little family information and GIL has not been shown to be an inherited character. Five women with anti-GIL have been found. All had been pregnant at least twice. Red blood cells of two of the babies gave positive direct antiglobulin tests, but there were no clinical signs of hemolytic disease. Anti-GIL may have been responsible for a hemolytic transfusion reaction and results of monocyte monolayer assays of two of the anti-GIL suggested a potential to cause destruction of transfused GIL+ RBCs. *Immunohematology* 1998;14:49-52.

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Antibodies to high-frequency red blood cell (RBC) antigens can be very important in transfusion medicine. Some are capable of causing severe immediate or delayed hemolytic transfusion reactions or hemolytic disease of the fetus and newborn (HDN). Such antibodies create a particular problem, as compatible blood is often very difficult to find. Many antibodies to high-frequency RBC antigens have not been shown to be clinically significant but still cause considerable problems in blood transfusion immunohematology reference laboratories, as they require identification. This procedure is time consuming, requires extensive testing, and may delay the provision of blood for transfusion.

The International Society for Blood Transfusion (ISBT) recognizes 12 antigens of very high frequency that have not been shown to belong to any blood group system or collection: Vel, Lan, At^a, Jr^a, Ok^a, JMh, Emm, AnWj, Duclos, PEL, ABTI, and MAM.¹⁻³ Many other high-frequency antigens, which do not fill the criteria necessary for recognition by the ISBT, are known by immunohematology reference laboratories. One such antigen is GIL. Anti-GIL was first identified in 1980^{4,5} and several other GIL-individuals have been found since that time.

Materials and Methods

Serologic methods

Standard serologic tests were used involving both nor-

mal-ionic-strength saline and low-ionic-strength saline, although the methods used differed in the various laboratories involved in the investigation. Antiglobulin tests were read in the presence of anti-human IgG or polyspecific anti-human globulin after three or four washes of the sensitized RBCs. For a two-stage complement test, RBCs were sensitized in the appropriate antiserum, mixed with 4mg/mL EDTA, washed, incubated for 20 minutes in fresh inert human AB serum, and washed again, and then the test was read after addition of anti-human C4+C3. Eluates were prepared from sensitized RBCs with an acid-elution kit (Elu-kit II, Gamma Biologicals, Houston, TX). RBCs were treated with 4% ficin and with papain, trypsin, α -chymotrypsin, and dithiothreitol (DTT), as described previously.⁶⁻⁸ IgG subclassing was performed by a capillary method.⁶

Monocyte monolayer assay

Monocyte monolayer assays to predict the clinical significance of antibodies were performed by previously described methods.^{6,9} Briefly, RBCs sensitized with the appropriate antibody were incubated with peripheral blood monocytes in tissue culture chamber slides. After fixation and staining, the slides were examined microscopically and the percentage of reactive monocytes was determined.

Immunoblotting

Immunoblotting was carried out as described previously,¹⁰ with membranes prepared in either the presence or absence of the reducing agent 2-mercaptoethanol and with an eluate prepared from GIL+ RBCs sensitized with the Gil antibody.

Results

Propositi

Five propositi have been ascertained through the presence of an antibody to an unidentified high-frequency RBC antigen, subsequently shown to be anti-GIL.

Case 1. Gil is a group A white American woman with no history of blood transfusion. The RBCs of her first child had a weakly positive direct antiglobulin test (DAT), but no antibody could be eluted and there was no clinical sign of HDN. The RBCs of her second baby, despite reacting with her antibody, had a negative DAT. The parents of Gil are not consanguineous and her three siblings are GIL+.

Case 2. Boi is a group O white French woman. Her antibody was found following a hemolytic transfusion reaction, which occurred during surgery in a small hospital. Her RBCs had a positive DAT with anti-IgG, but no eluate was prepared. Soon after the transfusion reaction, her anti-GIL had a titer of 1,024, which decreased to a titer of 2 within 3 months. RBCs of her two siblings and six children reacted with her antibody. No symptoms of HDN were reported for any of her children. Boi RBCs did not react with the Gil serum by an antiglobulin test and two adsorptions of the Gil serum with Boi RBCs did not remove the antibody, whereas two adsorptions with GIL+ RBCs removed all antibody. However, a sample of Boi RBCs taken 13 years later was found to be weakly reactive with the Gil serum and with an eluate prepared from GIL+ cells after sensitization with the Hun serum (see Case 3). The Boi serum (with added soluble A substance) did not react with Gil RBCs.

Case 3. Hun, a group O white German woman living in the United States, presented postpartum with an antibody to a high-frequency antigen in her serum. RBCs of the baby had a 3+ DAT, but there was no sign of HDN. Nine years later, the patient presented again postpartum (third pregnancy) with an antibody to a high-frequency antigen, but the baby was unaffected. Hun RBCs did not react with the Gil serum, and an eluate containing the antibody of Hun did not react with Gil RBCs.

Case 4. Gou, a group A white woman, presented with a pelvic infection and possible abdominal mass. She had a history of four pregnancies and no transfusions. The Gou serum did not react with Gil RBCs, and Gou RBCs did not react with the Gil serum, but the Gou serum reacted weakly with Hun RBCs.

Case 5. Mil, a group O white American woman, presented with bradycardia when she was 78 years old. She had received 2 units of packed RBCs 5 weeks earlier when no antibody was detected. She had been pregnant once. Her RBCs did not react with the Gil serum and her antibody adsorbed and eluted from GIL+ RBCs did not react with GIL- RBCs from Gil and Gou; two adsorptions with GIL- RBCs did not remove the antibody from the Mil serum. In addition to anti-

GIL, her serum contained anti-P₁.

Adsorption tests of the five anti-GIL with selected GIL+ cells revealed no additional blood group specificities, with the exception of anti-P₁ in the Mil serum.

Serologic characteristics of GIL antigen

GIL has been shown to differ from the following high-frequency antigens, either by reactivity of Gil RBCs with the antibodies to those antigens or by the reaction of the Gil serum with RBCs lacking those antigens: U; En^a; EnKT; 'N'; EnEP; ENEH; Hr₀; Hr; Rh29; Hr^B; Nou; Sec; Dav; MAR; Lu^b; Lu3; Lu4; Lu5; Lu6; Lu7; Lu8; Lu11; Lu12; Lu13; Lu16; Lu17; Lu20; k; Kp^b; Ku; Js^b; K11; K12; K13; K14; K16; K18; K19; Km; K22; Tou; Fy3; Fy5; Jk3; Di^b; Wr^b; Yt^a; Sc1; Sc3; Gy^a; Hy; Jo^a; Co^a; Co3; LW^a; LW^{ab}; Ch1; Ch2; Ch3; Ch4; Ch5; Ch6; Rg1; Rg2; H; Kx; Ge2; Ge3; Ge4; Cr^a; Tc^a; Dr^a; Es^a; IFC; WES^b; UMC; Kn^a; McC^a; Sl^a; Yk^a; In^b; Cs^a; I; i; Er^a; P; LKE; Vel; Lan; At^a; Jr^a; Ok^a; JMH; Emm; AnWj; Duclos; PEL; ABTI; and MAM.

GIL- RBCs did not react with antibodies to any of the following low-frequency antigens that have not been shown to belong to any blood group system: By; Bx^a; Ti^a; Jr^a; To^a; Pt^a; or Je^a. Boi RBCs were found to be Rd+, but Gil and Hun RBCs were subsequently shown to be Rd-. Gou and Mil RBCs were not tested.

Expression of GIL on RBCs was enhanced by treatment of the cells with papain, ficin, α -chymotrypsin, or trypsin, and was not affected by DTT treatment. Anti-GIL reacted slightly less strongly with cord red cells than with red cells from adults.

Serologic characteristics of three anti-GIL

Anti-GIL did not directly agglutinate GIL+ RBCs but did react with those RBCs by an indirect antiglobulin test with polyspecific anti-human globulin or anti-human IgG. The Boi serum fixed complement as determined by a two-stage complement technique, whereas the Gil serum did not. Anti-GIL in the Gil serum was IgG1; that in the Hun serum was IgG1 plus IgG2. Anti-GIL was not inhibited by human serum, saliva, Lewis-substance, milk, urine, or pigeon protein.

Frequency of GIL

The Gil plasma diluted 1:3 was used by a microtiter method to screen RBCs from 6,300 predominantly white blood donors and diluted 1:2 by a tube method to screen RBCs from 2,062 black donors in Milwaukee, WI. The Gil plasma, diluted 1:2 was used to screen red cells from 2,760 donors in Atlanta, GA, using an Olympus PK-7100 dextran method. RBCs from 101 Asian, 779 black, and 198 Hispanic donors in Jacksonville, FL, were screened

with anti-GIL. In Connecticut, RBCs from 14,191 donors were screened by a microtiter polybrene method with the Gil serum diluted 1:20. All donors were GIL+, but RBCs from one of the white Milwaukee donors gave a much weaker reaction than all others.

Monocyte monolayer assays

Monocyte monolayer assays (MMA) were carried out with two anti-GIL. With the Gil antibody, the procedure described in *Immunohematology Methods and Procedures*⁶ was used and results of 22 percent and 23 percent, 32 percent and 27 percent were obtained (normal range 0–3%). With the Hun serum, the method of Schanfield et al.⁹ was used and results of 574 percent and 765 percent were obtained, where 100 percent represents the result obtained with a standard IgG1 anti-D. Both sets of results suggest that these antibodies have a potential to cause significant RBC destruction of transfused GIL+ RBCs.

Immunoblotting

No bands were detected by immunoblotting with an anti-GIL eluate derived from the Gil serum, either under reducing or nonreducing conditions.

Discussion

Antibodies in the sera of five women appeared to be detecting a new RBC antigen of high frequency. This antigen is different from all of the high-frequency antigens defined by the ISBT Working Party on Terminology for Red Cell Surface Antigens^{1,2} and has been named GIL. There is a paucity of family information; only the three siblings of the original GIL- proposita were tested with anti-GIL and all were GIL+. Consequently, there is no evidence that GIL is an inherited character and so cannot be included in the ISBT classification. RBCs from 26,391 blood donors from the United States (23,449 white, 2,841 black, and 101 Asian) were tested with anti-GIL and all were GIL+.

A few anomalous results suggest that all five anti-GIL may not be of identical specificity. Boi RBCs may have reacted very weakly with the Gil and Hun sera, although this was not noticed in the original testing. The Gou serum reacted very weakly with Hun RBCs. This kind of heterogeneity among antibodies to high-frequency antigens of similar specificity has been seen with other high-frequency antigens; for example, PEL.¹¹ Boi RBCs were positive for the low-frequency antigen Rd (700015), which is probably associated with the Scianna system,^{12,13} but RBCs of the two other GIL- women (Gil and Hun) tested with anti-Rd were Rd-.

There is no direct evidence that anti-GIL is clinically significant. Monocyte monolayer assays were performed with two examples of anti-GIL and the levels of phagocytosis were high enough to suggest that the antibody had the potential to cause destruction of transfused incompatible RBCs. One woman, subsequently found to have anti-GIL, had a hemolytic transfusion reaction, but no details regarding the severity of this reaction are available and there is no proof that anti-GIL was responsible. RBCs from neonates of two of the women with anti-GIL gave positive DAT reactions, but there was no evidence of HDN.

The GIL antigen is not destroyed by treatment of the RBCs with proteolytic enzymes. Immunoblotting with anti-GIL against membranes derived from GIL+ RBCs produced no bands. Consequently, very little is known about the immunochemistry of the GIL antigen.

We will attempt to find further examples of GIL- individuals and anti-GIL. More family investigations might demonstrate inheritance of the GIL- phenotype, permitting GIL to join the 901 Series of high-frequency antigens in the ISBT classification of RBC cell-surface antigens.

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