Determination of IgG1 and IgG3 Subclasses of Red Blood Cell Antibodies: An Important Tool for Predicting Immune-Mediated Hemolysis

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Background

Red blood cell (RBC) antibodies of the IgG class can lead to hemolysis in patients depending on many factors, including the ability to activate the complement cascade, the Fc glycosylation, the antigen specificity (in the case of alloantibodies), and the antibody avidity as well as the IgG subclass. IgGs, particularly IgG1 and IgG3, are recognized by phagocytic cells through Fc receptors, found on various endothelial cells. Therefore, IgG1 and IgG3-coated RBCs are quickly recognized for phagocytosis, leading to a greater potential for RBC destruction.

Laboratory tools that can easily evaluate the amount and subclass of IgGs coated on RBCs may help predict the risk of hemolysis in cases of autoimmune hemolytic anemia due to IgG (a "warm type"), in cases of incompatible transfusions (such as in the risk of delayed hemolytic transfusion reactions), and in cases of hemolytic disease of the fetus and newborn.

Various methods such as conventional tube technique (CTT), column agglutination technology (CAT), or flow cytometry (FC) can be used for the detection of immunoglobulins and/or complement fragments bound to RBCs. Compared to CTT, CAT is easier to perform and read, requiring less sample and reagent volumes. FC is the most sensitive method but requires specific skills and materials not readily available to all blood transfusion laboratories.

Study Objectives

Primary

To correlate the presence of IgG1 and IgG3 subclasses with the risk of hemolysis in the context of RBC alloantibodies and autoantibodies.

Secondary

1. To compare the frequency of IgG1 and IgG3 subclasses in patients and blood donors presenting with RBC autoantibodies
2. To compare the *in vitro* behavior of IgG antibodies of IgG1 and IgG3 subclasses versus non-IgG1 and non-IgG3 subclasses
3. To evaluate the frequency of IgG1 and IgG3 subclasses in cases of incompatible transfusions and hemolytic disease of the fetus and newborn

Methods

Patient and Donor Recruitment and Testing

Blood donors and patients presenting with positive polyspecific direct antiglobulin test (DAT) (Bio-Rad, Cressier, LISS/Coombs card, ref. 004017) were recruited for a 2-month study at a tertiary hospital. The included samples were tested with acid elution and only the eluates that reacted with the panel or reverse RBCs were included in the study.

All selected donors and patients were further tested with monospecific DAT gel cards (Bio-Rad, Cressier, DC Screening I card with anti-IgG, -IgM, -IgA, -C3c, -C3d, ref. 004857), DAT IgG-dilution (Bio-Rad, Cressier, ref. 004033), which indicates the amount of IgG bound to RBCs, and DAT IgG1/IgG3 (Bio-Rad, Cressier ref. 004043), which further differentiates the IgG1 and IgG3 subclasses.

A monocyte monolayer assay (MMA) was performed in select cases with RBC IgG autoantibodies and the results were expressed in terms of monocyte index (MI).

Statistical Comparisons

Patients and donors presenting with IgG autoantibodies were compared for frequency of IgG1 and IgG3 using the chi-square test. IgG1 and/or IgG3 were compared to non-IgG1 and non-IgG3 groups using the Fisher's exact test for the categorical variables, and Mann-Whitney test for the continuous variables. In all comparisons, a p-value less than 0.05 was considered significant.

Results

Description of the Studied Population

A total of 42 patients presented positive monospecific DAT indicating IgG and were included in the study. Of these, 4 (9.5%) were newborns (NB) and tests were performed as part of the mother/NB routine. The other patients (n=38) presented with a positive DAT. The patient eluates revealed either irregular antibodies with the same specificity as detected in irregular antibody screening and identification, suggesting recent incompatible transfusions (n=9, 21.4%), or IgGs with panagglutination (n=29, 69%).

A total of 68 donors presenting a positive polyspecific DAT were initially selected for the study and of these, 27 confirmed positive monospecific DAT with IgG and were included in the study.

Newborn (NB) Patients

Of the included NBs (n=4), the following antibodies were identified in the eluate: anti-D (n=1), anti-C (n=1), anti-A (n=1), and anti-B (n=1). None of the NBs presented with unexpected jaundice to justify bilirubin quantification. Phototherapy was also not indicated for any of them. IgG1 and IgG3 were not detected in all cases and C3d was also absent on the RBC membrane.

Serological Delayed Hemolytic Transfusion Reaction (DHTR)

Nine patients had positive DAT and irregular antibodies on the RBC membrane: anti-Jk^a (n=1), anti-Jk^b (n=1), anti-Fy^a (n=3), anti-E (n=2), anti-Fy^a,-c (n=1), and anti-c,-E (n=1). Antibody identification was requested as pre-transfusion testing for a new RBC transfusion and none of the patients had been recently transfused in the reference center, suggesting incompatible transfusions in other health services and serological transfusion reactions. None of the involved antibodies were IgG1 or IgG3. Only one case (anti-Jk^b) had concomitant C3b on the RBC membrane.

Patients with IgG Autoantibodies

Of the 29 patients presenting with autoantibodies of IgG class, 15 (51.7%) presented with either IgG1 or IgG3 and 14 (48.3%) had neither IgG1 nor IgG3. C3d was concomitant in 7 (46.7%) patients with IgG1 or IgG3 and in 3 (21.4%) patients without IgG1 or IgG3. Among the 27 blood donors presenting with IgG autoantibodies, 26 (96.3%) did not present with either IgG1 or IgG3. A striking statistical significance was observed when the frequency of IgG1 and IgG3 was compared between donors and patients ($p<0.001$, Table 1). The presence of IgG1 or IgG3 subclasses of autoantibodies exhibited sensitivity of 51.7%, specificity of 96.3%, positive predictive value (VPP) of 93%, and negative predictive value of 65% for distinguishing donors and patients with IgG positive DAT.

MMA was performed in 12 cases.The difference between IgG1 or IgG3 versus non-IgG1 or IgG3 resulting in a positive (>5%) monocyte index (MI) was 83.3% versus 33.3%. The p-value was 0.07, reflecting the restricted sample size.

Table 1. Comparison of the presence of IgG1 and IgG3 in a clinical scenario

	IgG1 and/or IgG3	Not IgG1 nor IgG3	p-value
Subject classification			
Patients with auto-IgG (n=29)	15	14	<0.001
Donors with auto-IgG (n=27)	1	26	
MMA result (n=12)			
MI>5%	5	2	0.07
MI<5%	1	4	

The concomitant presence of C3d on the RBC membrane did not significantly differ between autoantibodies of IgG1 or IgG3 subclasses and non-IgG1 and non-IgG3 (Table 2). Also, the presence of irregular antibodies in the samples presenting with IgG1 or IgG3 autoantibodies did not statistically differ from non-IgG1 and non-IgG3 autoantibodies (Table 2). However, IgG1 or IgG3 autoantibodies were associated with significantly higher agglutination scores on DAT (p=0.01, Table 2).

Table 2. Comparison of immunohematological findings between IgG1 or IgG3 autoantibodies and non-IgG1 and non-IgG3 autoantibodies

	IgG1 and/or IgG3	Not IgG1 nor IgG3	p-value
Presence of complement			
C3d+ (n=10)	7	3	0.1
C3d- (n=16)	5	11	
Associated alloantibodies			
With alloantibodies (n=14)	7	7	0.22
Without alloantibodies (n=9)	2	7	
Agglutination intensity (n=29)	211.5*	139.5*	0.01174

*Sum of DAT reaction strengths between each group.

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Highlights of the Study

- The presence of antibodies of non-IgG1 and non-IgG3 subclasses was associated with benign cases of serological transfusion reactions and hemolytic disease of the fetus and newborns.
- The frequency of IgG1 or IgG3 autoantibodies was much higher among patients than donors. This suggests that the identification of non-IgG1 and non-IgG3 autoantibodies points to a non-pathological immune context for autoantibody development.
- IgG1 or IgG3 autoantibodies showed a higher prediction for hemolysis, as shown by *in vitro* functional tests.

Clinical Conclusions

- The determination of the subclass of IgG might be a helpful tool in laboratory practices, because the absence of IgG1 and IgG3 can rule out overt hemolysis and clinical anemia.
- Donors with positive DAT and autoantibodies of non-IgG1 and non-IgG3 subclasses do not need specific follow-up. On the contrary, donors with IgG1 or IgG3 autoantibodies (uncommon) should be referred to clinical counselling.

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