

Autoimmune Hemolytic Anemia Predominantly Associated with IgA Anti-E and Anti-c

A patient with warm autoimmune hemolytic anemia (AIHA) due to predominance of immunoglobulin A (IgA) with an Rh specificity, considered to be the first case in Korea, is described. A 13-yr-old male patient with severe hemolytic anemia showed a weak reactivity (1+) in the direct antiglobulin test (DAT) by using anti-IgG antiglobulin reagent. This finding, however, could not fully explain the patient's severe AIHA. When anti-IgA reagent was used for the DAT, strong reactivity (4+) was observed and free anti-E and anti-c autoantibodies were also detected by anti-IgA and anti-IgG reagents. The patient's hemoglobin began to rise with the administration of steroids. Because RBCs coated with multiple types of immunoglobulins are associated with more severe hemolysis than those only with IgG, the DATs using anti-IgA and other reagents are needed for the correct diagnosis when the result of DAT is not compatible with patient's clinical manifestations.

Key Words : Anemia, Hemolytic, Autoimmune; Direct Antiglobulin Test; Anti-IgA

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INTRODUCTION

A positive direct antiglobulin test (DAT) is an important finding in the diagnosis of autoimmune hemolytic anemia (AIHA). However, red blood cell antibodies of the immunoglobulin A (IgA) class are not common and most of the laboratories use only anti-human IgG and C3d reagents for routine DAT. Therefore, the detection of IgA autoantibodies as a causative antibody for AIHA is difficult.

Warm autoantibodies are usually nonspecific. They may lack reactivity with Rh_{null} cells (1), or rarely are specific for one or more of the common Rh phenotypes. However, IgA autoantibodies that have specificities against antigens within the Rh system, such as anti-C (2), anti-e (3), anti-Ce+anti-e (4), anti-E (5), and autoanti-Gerbich (6) have been reported.

As far as we can determine, there has been no case report on the warm AIHA associated with IgA that have specificities against E and c antigens in Korea. We report here a case of a severe AIHA associated with a strong reactivity by anti-IgA and weak reactivity by anti-IgG reagent in the DAT, and free anti-E and anti-c autoantibodies of IgA and IgG classes in the serum.

CASE REPORT

A 13-yr-old male patient was admitted with a 2-day history of fever, nausea, jaundice, and fatigue. Two weeks prior to admission, he experienced flu-like symptoms. Physical

examination revealed an acutely ill appearance with pallor, icteric sclerae, and hepatomegaly (5 cm) below the costal margin, but no splenomegaly. He had no history of anemia, jaundice or blood transfusion.

The laboratory data were as follows. The hemoglobin was 5.3 g/dL, hematocrit 14.8%, mean corpuscular volume 123.2 fL and the leukocyte count was 20,500/ μ L with myelocyte 2%, metamyelocyte 2%, band neutrophil 1%, segmented neutrophil 65%, lymphocyte 25%, monocyte 4%, eosinophil 1%, and 14 nucleated RBCs per 100 leukocytes. The platelet count was 460,000/ μ L and the corrected reticulocyte count was 21.3%. The peripheral blood smear revealed severe spherocytosis, polychromasia and RBC agglutination (Fig. 1). An increased osmotic fragility was observed and the bone marrow revealed erythroid hyperplasia. Total protein and albumin were 7.2 g/dL and 4.6 g/dL, total bilirubin/direct bilirubin 8.3/1.1 mg/dL, lactate dehydrogenase 1,710 U/L, and the haptoglobin was undetectable (\leq 5.8 mg/dL). The urine hemoglobin was positive (3+). The cold agglutinin titer was 1:64 and Mycoplasma antibody was negative.

The DAT was weakly reactive (1+), and anti-E and anti-c antibodies were detected in the antibody identification test using polyspecific AHG reagents by gel column (DiaMed-ID, Cressier/Morat, Switzerland). However, the finding of weakly reactive DAT could not fully explain the patient's severe hemolytic anemia. When an anti-IgA reagent (Dia Med-ID, Cressier/Morat, Switzerland) was used, the strongly reactive (4+) DAT was observed. Free anti-E and anti-c autoantibodies were also detected in the serum by the tube

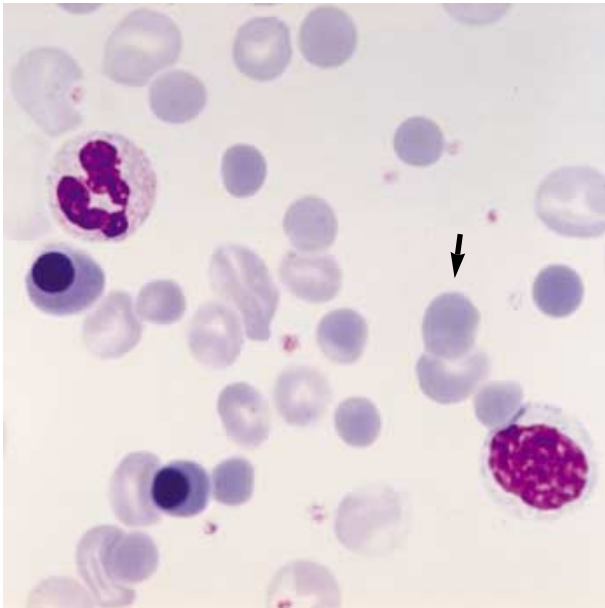


Fig. 1. Peripheral blood smear (Wright stain, ×1,000) shows spherocytosis, polychromasia, red blood cell micro-agglutination (arrow) and two erythroblasts.



Fig. 2. The results of direct antiglobulin test by gel column (Dia Med-ID, Cressier/Morat, Switzerland) shows weak reactivity (1+) in anti-IgG, strong reactivity (4+) in anti-IgA and no reactivities in anti-IgM, anti-C3c and anti-C3d.

Table 1. Reactivity of antibody identification tests (antihuman globulin phase) in serum and eluates

	Rh-hr					Kell		Duffy		Kidd		Lewis		P	MNS				Luth		Anti-IgG by gel column ^a		Anti-IgA by tube test ^b			
	D	C	E	c	e	C ^w	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a		Le ^b	P1	M	N	S	s	Lu ^a	Lu ^b	Serum	Eluates	Serum	Eluates
1	+	+	-	-	+	+	-	+	+	+	+	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-
2	+	+	-	-	+	-	-	+	+	-	+	+	+	-	-	-	+	-	+	-	+	-	-	-	-	-
3	+	-	+	+	-	-	-	+	-	+	+	-	+	-	+	+	-	+	+	-	+	2+	2+	2+	+	+
4	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	+	-	-	+	±	±	±	±	±
5	-	-	+	+	+	-	+	+	-	+	+	+	-	-	+	+	+	+	-	+	+	2+	2+	2+	2+	2+
6	-	-	-	+	+	-	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	2+	ND ^c	+	+	+
7	-	-	-	+	+	-	-	+	+	-	+	+	-	-	-	+	+	-	+	+	+	+	+	2+	2+	+
8	+	-	-	+	+	-	-	+	-	-	+	-	-	-	+	-	+	+	-	-	+	2+	2+	2+	±	±
9	-	-	-	+	+	-	-	+	-	-	+	-	-	-	+	+	+	-	+	+	+	2+	2+	2+	2+	+
10	+	-	+	+	+	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	-	2+	2+	2+	2+	+
11	-	-	-	+	+	-	-	+	+	-	+	-	-	+	+	-	+	-	+	-	+	2+	2+	2+	2+	±
	Autocontrol																				+	+	+	+		

Panel cells: ID-DiaPanel (DiaMed-ID, Cressier/Morat, Switzerland).

Anti-IgG by gel column^a: DiaMed-ID, Cressier/Morat, Switzerland; Anti-IgA by tube test^b: monospecific Coombs sera anti-IgA, Biotest AG, Germany; ND^c: Eluate could not be reacted with panel cells, however, eluates showed reactivities against screening RBCs containing E and c antigens using anti-IgG reagent.

method only in antiglobulin phase with anti-IgA (Monospecific Coombs sera Anti-IgA, Biotest AG, Germany) and panel cells (DiaMed-ID, Cressier/Morat, Switzerland) (Table 1). DATs, however, were negative by the reagents (DiaMed-ID, Cressier/Morat, Switzerland) of anti-IgM, anti-C3d and anti-C3c (Fig. 2). The patient's Rh subgroup type was CcDEe with pre-transfusion specimen. IgA and IgG eluates showed reactivities only against RBCs containing E and c antigens (Table 1).

On the day of admission, two units of crossmatching com-

patible and E and c antigen negative packed RBCs were administered. However, the hemoglobin level dropped further to 4.9 g/dL and this low level was maintained until the administration of steroids on the 5th hospital day when we could diagnose IgA autoantibodies of AIHA as a cause of the disease. Before steroid treatment was started, ceftriaxone, roxithromycin and Gabexate mesylate were administered for control of suspicious Mycoplasma infection and disseminated intravascular coagulation. Relief of jaundice followed and the hemoglobin rose to 8.4 g/dL. He was discharged on the

12th hospital day. The steroid was tapered off and was discontinued two weeks later. Three weeks after the discharge, his hemoglobin was elevated to 11.3 g/dL, mean corpuscular volume decreased to 97.3 fL and the corrected reticulocyte count decreased to 2.1%, but the cold agglutinin titer remained at 1:128. The DAT was still weakly reactive by using anti-IgG reagent (1+) and not done by using anti-IgA reagent, but the free antibodies were not detected in the serum by using anti-IgG and anti-IgA antiglobulin reagent.

DISCUSSION

The initial diagnosis of this patient with warm AIHA was not easy. Hereditary spherocytosis was seriously considered because of severe spherocytosis and increased osmotic fragility. Since positive DATs with reactivities '1+' or less can be observed in apparently normal subjects, the finding of weakly positive DAT by anti-IgG reagent could not explain the patient's severe intravascular hemolysis. Thus, it was necessary to use other means to detect responsible autoantibodies.

IgA autoantibodies in many patients have been reported to be idiopathic (2) contrary to this case. IgA autoantibodies of the RBCs are usually warm-reacting and the clinical course of the patients with such autoantibodies is similar to that of patients with IgG antibodies (4). Whether the warm-reacting IgA autoantibodies can activate the complement system of their own is controversial. Several studies (2, 7) have shown that IgA antibodies coating RBCs may activate the complements and complement components (C3) on the RBCs, resulting in severe intravascular hemolysis, but not in other reports (3, 5) as in our case. Salama et al. (8) reported that the DAT was strongly reactive for IgA (weakly reactive for IgG) and negative for complement as in our case. They presented the circumstantial evidence for the involvement of reactive hemolysis, i.e., C3-independent binding of the cytolytic C5b-9 complement complex to bystander RBCs inducing intravascular hemolysis. IgA is also able to activate complements by the alternative pathway (9).

IgA and IgM were usually found in association with IgG. Although only IgA was found in 0.8-2.0% of AIHA (10), the 14% of IgA autoantibodies was reported in the warm-type AIHA, such as IgG+IgA (5.8%), and IgG+IgM+IgA (8%) using sensitive enzyme-treated DAT (2). The presence of more than one type of antibodies on RBCs, even when undetected by agglutination methods, has been reported to be the major cause of hemolysis along with other factors, such as the quantity of bound IgG, the IgG subclass pattern, and complement (2, 11). IgA acts synergistically with other immunoglobulins, usually IgG. Therefore, to predict the prognosis of patients with AIHA, it is important to evaluate not only the reactivity of DAT by anti-IgG reagent but also the existence of other classes of immunoglobulin as a causative autoantibody. In our case, the patient had IgA- and IgG-coat-

ing RBCs and showed severe intravascular hemolysis.

The patients with multiple classes of immunoglobulins coated RBCs were reported to respond less successfully to steroids, and most of them was revealed to have no underlying disease (11). However, the present patient had AIHA of an acute transient type, which is known to be more frequent among young children and responds well to therapy (12).

The diagnosis of the AIHA can be overlooked when the routine DAT using polyspecific reagent containing anti-IgG and anti-C3d is negative or weakly positive. Therefore, the DAT using anti-IgA or other reagents is needed when the results of the DAT are not compatible with patient's clinical manifestations. Immediate steroid therapy must be started when the warm AIHA is suspected, even if the routine DAT shows weak reactivity, since severe hemolysis may occur when the small amount of detectable immunoglobulins act synergistically with undetected immunoglobulin classes as in the present case.

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