A report of an anti-H antibody with a wide thermal range in a group A_1 blood donor

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Introduction

Over the last few decades immunohaematology laboratories have developed ever more sensitive methods for typing red blood cells and detecting irregular antibodies. The introduction of solid phase technology[1,2] in the mid 1980s, and of column agglutination technology (CAT)[3,4] at the beginning of the 1990s, as well as the use of new and alternative potentiating media (polyethylene glycol, PEG)[5] and more sensitive panels with red blood cells suspended at 0.8% in potentiating solutions (B-LISS), have improved the ability of tests to identify weak antibodies, otherwise not readily detected by traditional tests in the liquid phase in test-tubes.

It is, therefore, possible that in routine daily practice, samples can give positive results for irregular antibodies detectable only when the serum is tested with CAT or in solid phase antibody screens. The interpretation of these cases is, however, often difficult and a definitive identification is not always achieved, particularly when the antibodies react with all the red cells in the panels. Although such immunohaematological situations may, sometimes, be caused by weak, cold antibodies of no clinical relevance, in other cases they can be caused by high titre, low avidity (HTLA) antibodies, or very weak low titre antibodies, which, ideally, should be identified in order to enable correct management of transfusions or to monitor foetal risk during a pregnancy.

The case we report here concerns an unusual pan-agglutinating IgG antibody with anti-H specificity in the serum of a young blood donor, who had never received transfusions.

Materials and methods

Blood group typing, the search for irregular antibodies and the identification of the specificity were carried out using BioVue® cards and test red blood cell panels at 0.8%[6] from Ortho Clinical Diagnostic (Raritan, NJ, USA). Polyspecific and monospecific human anti-immunoglobulin sera, group-specific lectins, rabbit erythrocyte stroma (RESt) and the typing sera used in our laboratory are supplied by Ortho and Immucor (Gamma Diagnostic, TX, USA). Reagent panels were prepared with red blood cells from random donors, cord blood cells, and cells with specific phenotypes, selected from our archive of rare blood cells. Dithiothreitol (DTT, Acros, NJ, USA) treatment of the red blood cells and serum, the search for cold cryoagglutinins and antibody absorption were carried out according to the procedures indicated in the AABB Technical Manual and with the methods described by the manufacturing companies.

Case report

Our immunohaematology laboratory currently carries out red blood cell typing and antibody screens for new donors attending our Transfusion Service. During these routine procedures, the plasma of a young male donor donating blood for the first time was found to be positive in the indirect antiglobulin test with the cells used in the antibody screen. The donor was recalled a few days later to give a new sample and verify that he had not, as previously stated, ever received a blood transfusion. Repeat testing gave the same serological results, confirming the presence in his serum of a weak alloantibody reacting with all the normal cells in the screening tests and antibody identification panels used routinely.

Results

Extended antigen typing of the donor's red blood cells gave the following results: A_1, D+, C+, c+, E-, e+, C^w-, K-, k+, Kp(a+ b+), Fy(a+ b+), Jk(a+ b-), Le(a- b-), MMSS, P_1+, Lu(a+ b+). The Surgiscreen red blood cells agglutinated in CAT with a 2+ score in the indirect antiglobulin test as

they did all the red blood cells of the commercial identification panels tested (Panel C and Panel B). Autologous red blood cells, resuspended at 0.8% with potentiating media of the test panels, were not agglutinated by the donor's serum. Since a panel comprising selected group O red blood cells with the same antigenic profile as that of the donor was pan-reactive, investigations were directed towards a possible specific alloantibody for a high frequency antigen. The antibody did not directly agglutinate the red blood cells in isotonic saline solution, at any temperature, in liquid phase tests, but had a wide thermal range of reactivity in column agglutination, with positivity for the human antiglobulin test being maximum at 4 °C (Table I).

Table I - Study of the temperature of maximum reactivity

Surgiscreen Batch 8SS357				AHG	
N.	Donor ID	Group	CAT 4°C	CAT 20°C	CAT 37°C
1	112790	O	**4+**	**3+**	**2+**
2	118219	O	**4+**	**3+**	**2+**
3	111106	O	**4+**	**3+**	**2+**

At this temperature, the panel of selected fresh red blood cells agglutinated (score 4+) with all group O donor red blood cells; the intensity of the reactions decreased with cord blood cells (score 2+), while no agglutination was seen with red blood cells of the same group (Table II).

Table II - Selected 9-cell panel

N.	Donor ID	Group		AHG	
			CAT 4°C	CAT 20°C	CAT 37°C
1	Donor 1	O	**4+**	**3+**	**2+**
2	Donor 2	O	**4+**	**3+**	**2+**
3	Donor 1	A_1	**0**	**0**	**0**
4	Donor 2	A_1	**0**	**0**	**0**
5	Donor 3	A_1	**0**	**0**	**0**
6	Cord 1	O	**2+**	**1+**	±
7	Cord 2	O	**2+**	**1+**	±
8	Cord 3	A	±	**0**	**0**
9	Rh_{null}	O	**4+**	**3+**	**2+**

Pretreating the red blood cells with proteolytic enzymes (ficin) and DTT 0.2 M did not change the intensity of the reactions substantially. Serum treated with DTT 0.1 M was still reactive, demonstrating the presence of an IgG class antibody component, confirmed also by the negative outcome of the search for cold and complete antibodies at room temperature.

Collectively, the immunohaematological investigations indicated an anti-H antibody reactive at a broad range of temperatures, excluding the possibility of the more common anti-IH in that there was no decrease in the reactivity with rare adult O_i red blood cells (Table III).

Table III - O red blood cells vs adult O_i red blood cells

	1:1	1:2	1:4	1:8	1:16	1:32
Adult OI RBC	3+	3+	2+	1+	+/-	0
Adult O_i RBC	3+	3+	2+	1+	+/-	0

The search for antibodies carried out on the serum after absorption of antibodies by RESt was negative and excluded the presence of further warm alloantibodies.

Discussion

The H antigen is the precursor of the A and B antigens and is strongly expressed on the surface of group O and A_2 red blood cells, whereas it is less strongly expressed on groups A_1 and B red blood cells since more of it is converted into the respective antigens.

Clinically significant anti-H antibodies, able to induce rapid haemolysis of incompatible blood cells, are well recognized in rare Bombay or para-Bombay subjects without the H antigen, and such patients must be transfused with blood selected from donors lacking the H substance[7,8]. Pregnant women with this phenotype must be monitored with particular care because of the risk of haemolytic disease of the newborn[9,10]. In subjects with a normal A_1 or A_1B red blood cell phenotype, particularly pregnant women with group A_1 blood, weak IgM cold antibodies with anti-H specificity are not rare, although less common than antibodies with anti-IH specificity; however, these weak antibodies, which react at low temperatures, do not cross the placental barrier and are not clinically significant[11].

In the blood donor described here, we found an apparently natural IgG anti-H antibody with a wide thermal range, also reacting at 37 °C and, therefore, able to interfere with pre-transfusional investigations. This finding does not prejudice our donor's suitability to donate blood, even if the plasma donated is destined for industrial processing; however, the presence of this antibody must be considered for the eventual donor need transfusions in the future and group A_1 units of red cell concentrates must be selected for cross-matching tests.

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