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Blood Group Antigens on Fetal Red Cells Obtained by Umbilical Vein Puncture under Ultrasound Guidance: A Rapid Hemagglutination Test To Check for Contamination with Maternal Blood

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ABSTRACT. Seventy-two fetal blood samples obtained by umbilical vein puncture under ultrasound guidance for prenatal diagnosis and monitoring purposes were tested for the expression of 38 blood group antigens. The gestational age at sampling ranged from 18 to 34 wk with 76% of fetuses between 20 and 25 wk. Compared to adult red blood cells, the following antigens showed either abnormally decreased frequency, or significantly reduced expression: A, B, A_1, H, Lu^a, Lu^b, Le^a, Le^b, P_1, P, and I. Selected anti-I and anti-i cold agglutinins, active at room temperature, were used at appropriate dilution, in a rapid slide or spin test to check for possible mixture of the sample with maternal blood and were shown to detect contaminations up to 5%. The test has proved particularly helpful for immediate and in process assessment of foetal origin of the sampled blood. (*Pediatr Res* 20:1082–1084, 1986)

Abbreviations

BG, blood group
RBC, red blood cells

Fetal BG studies have so far been performed on cord samples taken in cases of therapeutic abortion either in occasional circumstances (1) or in series such as those reported by Cleghorn (unpublished observations cited in Reference 1), Toivanen and Hirvonen (2), and Mackenzie *et al.* (3). These studies have already highlighted the fact that the antigenic make-up of the adult RBC membrane is not achieved at the same developmental stage. Some antigens such as rhesus which is determined by protein structures are well developed as early as the 6th gestational wk, while others such as A, B, H, Lewis, I, which are determined by immunodominant sugars and require simple or multiple transferase enzymes for their development, are not fully expressed at birth.

The current availability of numerous fetal blood samples collected *in utero* in pregnant women for diagnosis and monitoring purposes led us to design an extensive study of BG antigens of these fetuses on a systematic basis under standardized conditions in order to supplement the already available data on the development of BG markers in man. As a byproduct of this investigation we studied whether the appropriate use of antisera to antigens I and i which are among the specific markers of respectively adult and fetal RBC could prove of any appreciable help to check for accidental contamination of the fetal sample by blood aspirated from maternal vessels.

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MATERIALS AND METHODS

Fetal blood was obtained in 72 pregnant women for prenatal diagnosis and monitoring purposes. Two ml of blood were aspirated from the umbilical vein at the placental cord insertion using a 20-gauge needle guided by ultrasound as described previously (4). The gestational age at sampling is shown in Figure 1 where 55 fetuses (76.6%) were 20 to 25 wk, 4 (5.5%) 18 to 20 wk, and 13 (18%) 26 to 34 wk.

RBC were frozen in liquid nitrogen immediately after sampling. The samples could thus be thawed simultaneously and tested with the same reagents under homogenous and standardized conditions.

Seventy-two cord blood samples taken at delivery from full-term babies were processed in a similar way but were tested only for antigens showing immature development on fetal RBC (Fig. 2).

RBC phenotyping was performed using 38 antisera of different specificities and appropriate positive and negative adult RBC controls by conventional hemagglutination techniques (saline, indirect Coombs' on protease treated or untreated RBC according to antisera). Antigenic expression was graded according to the strength of agglutination obtained by undiluted antiserum and by 1:2 titrating dilutions thereof. The following agglutination scores were used throughout the study: 10: one or several large agglutinates, no or few free cells; 8: large agglutinates in a sea of small clumps and some free cells; 5: small agglutinates of approximately 20 cells visible macroscopically; 2: small agglutinates visible only microscopically; 0: no visible agglutination.

Titration score of each RBC sample was developed by summing the scores obtained at each dilution of the reagent. Scores below 70% of the adult controls run in parallel were considered to reflect weak reactivity and immature development of the antigen.

Since antigens I and i are easily accessible and clear-cut markers of adult and fetal RBC, following preliminary tests two sera with potent anti-I and anti-i antibodies from patients with chronic cold agglutinin disease were selected. These were appropriately diluted with serum from group AB donors in order to get rid of anti-A and -B antibodies simultaneously present and to obtain clear-cut positive and negative reactions at room temperature with adult and cord RBC. The reagents were then divided into 0.5-ml aliquots and kept frozen at −30° C until use.

Adult and cord RBC were mixed at increasing proportions as shown in Table 2. A 5% v/v suspension in saline of these mixtures was added to an equal volume of the antiserum at room temperature either on a slide or in a test tube and centrifuged at 1000 cpm for 30 s. Reactions were read macroscopically and microscopically and inspected for the presence of mixed field pattern of agglutination.

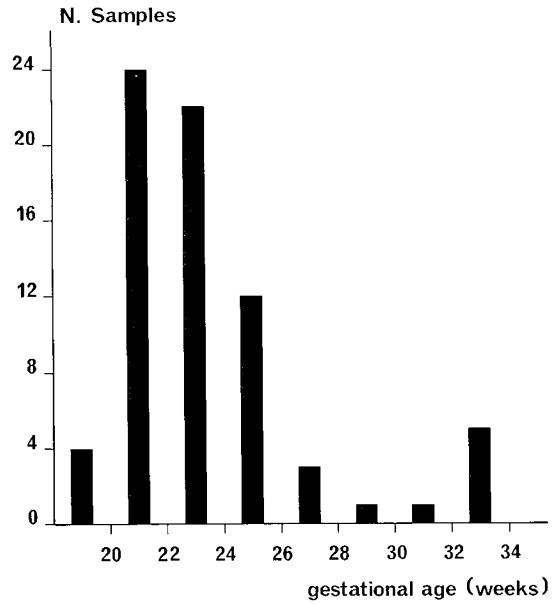

Fig. 1. Gestational age of fetuses at blood sampling.

RESULTS

The panel of reagents used for blood grouping included antisera to the following 38 BG antigens. Polymorphic antigens: A, A_1, B, D, C, C^w, c, E, e, K, k, Kp^a, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, s, Lu^a, Lu^b, Le^a, Le^b, P_1, Xg^a. Monomorphic antigens: H, Rh17, Kp^b, Js^b, Fy^3, Jk^3, P, I, i, Ve^a, Ge^a, Emma.

The strength of reaction and frequency of these antigens were identical to adult controls except for the following: A, A_1, B, H, Le^a, Le^b, Lu^a, Lu^b, P_1, P, I, i. These antigens were tested for among the 72 cord blood samples from full-term babies. The results are shown in Figure 2. It may be seen that the above antigens are either unexpressed, underrepresented, or weakly developed in fetal RBC. The findings in cord blood samples indicate, as expected, an intermediate developmental stage between fetal and adult conditions. The expression of antigen H is known to vary according to the ABO group in adults. Table 1 provides detailed evidence on the immaturity of this antigen in fetal RBC.

Using the selected anti-I and anti-i reagents described in "Materials and methods" against various proportions of adult and cord blood, as indicated in Table 2, a mixed field pattern of agglutination was observed even in 95%/5% mixtures. Contaminations as small as 5% could therefore be identified by simple inspection.

DISCUSSION

The present study conducted in the same laboratory under homogenous and standardized conditions confirms the following

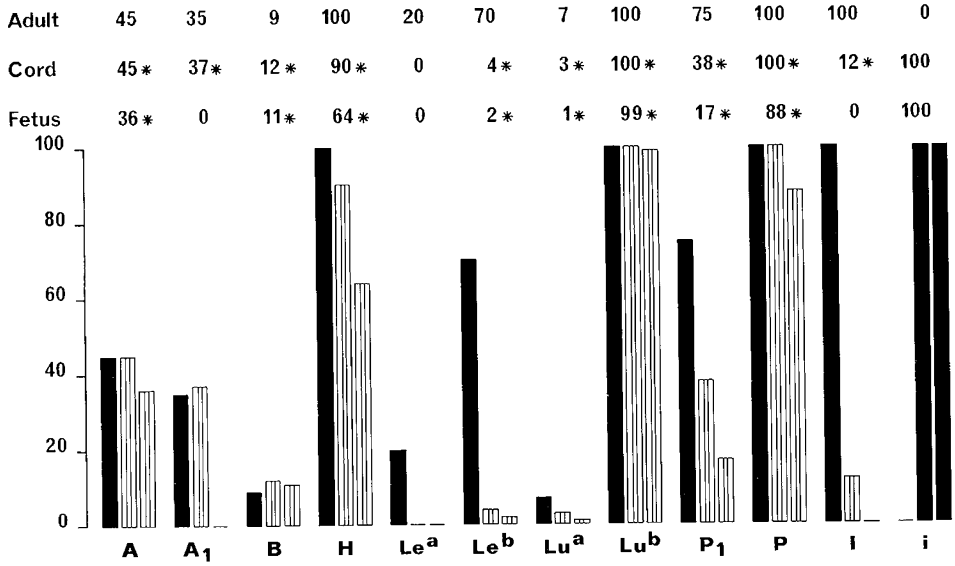

Fig. 2. Frequency and reactivity of blood group antigens identified as showing immaturity at birth. *Asterisk* and *hatched bars* indicate that a variable proportion of these samples exhibited weak reactivity. *Black bars* indicate normal antigenic strength. *Figures* indicate the percentage frequency of antigens and *bars* represent from left to right adult, cord, and fetal bloods.

Table 1. *Percentage values indicating reduced expression of H antigen in fetal samples compared to adults according to ABO blood groups*

	Fetal samples			Adult controls		
	Positive			Positive		
Blood group	Strong	Weak	Negative	Strong	Weak	Negative
O	80	20	0	100	0	0
A	4	28	68	20	80	0
B	0	16	84	0	100	0

Table 2. *Agglutination of mixtures of adult and cord RBC obtained by the selected anti-I and anti-i reagents**

RBC mixture													
% adult	100	95	90	80	70	60	50	40	30	20	10	5	0
% cord	0	5	10	20	30	40	50	60	70	80	90	95	100
Reaction pattern													
Anti-I	P	P	P	MF	MF	MF	MF	MF	MF	MF	MF	MF	N
Anti-i	N	MF	MF	MF	MF	MF	MF	MF	MF	MF	P	P	P

* P, strongly positive; MF, mixed field pattern; N, negative.

observations made in occasional circumstances (1) or in limited series (2, 3) by previous authors.

The antigens D, C, c, E, e, K, k Kp^a, Kp^b, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, s, Xg^a, Ve^a, and Ge^a are fully developed in the second half of pregnancy since their strength and frequency are similar to those found in adults. The presence of IgG antibodies to these antigens in mother triggered by previous pregnancies or blood transfusions may cause hemolytic disease of fetus in the second half of intrauterine life if not earlier.

Antigens I, Le^a, Le^b are not expressed on fetal RBC and antigens A, B, H, P_1, P, Lu^a, and Lu^b show immature development since their strength and frequency are decreased compared to adults.

The new contribution of the present study may be summarized as follows. 1) Antigens Rh17, Jk^3, Fy^3, Js^b, and Emma are fully developed at gestational ages investigated. 2) Antigen P, the high frequency RBC membrane globoside, is absent in 12% and weakly expressed in 10% of samples, which are constantly P_1 negative. 3) Antigen A_1 as determined by the Dolichos Biflorus lectin is not expressed on fetal RBC at gestational ages investigated. This finding is in accordance with the documented immaturity of antigen H, the biochemical substrate for A and A_1 antigens. 4) Investigation of the 11 antigens with weak fetal expression on cord blood samples obtained from 72 full-term babies shows intermediate developmental stages between the observed fetal and adult conditions. 5) Since antigens I and i are specific and reliable markers of adult and fetal RBC, provided appropriately adjusted anti-I and anti-i are used, these reagents represent useful and remarkably easy to handle tools to check, by an immediate spin or slide test, for accidental puncture of maternal vessels during fetal blood sampling for prenatal diagnosis procedures. Indeed, other methods are currently available for identification of fetomaternal blood mixtures which are principally based on differential characteristics of fetal and adult hemoglobins. However, these methods are time consuming and do not yield extemporaneous results as those obtained by anti-I and anti-i reagents implemented in this study.

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