Naturally occurring anti-E red-cell antibodies in a 3-month old infant

Naravna anti-E eritrocitna protitelesa, prisotna pri trimesečnem otroku

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Abstract

Background: Before the transfusion of the red blood cells containing blood components is applied, pre-transfusion testing such as ABO, RhD and Kell blood grouping with a negative cross-match is obligatory. This can be reduced to blood grouping only in infants less than 4 months of age, provided that the initial antibody screening test is negative. Only a few cases of antibody formation in such young infants have been described, the majority of them have resulted from exposure to allogeneic transfusions.

Case report: During the pre-transfusion testing, naturally occurring anti-E red blood cell antibodies were detected in a 3-month old male infant, who underwent surgery for atresia of the extra hepatic bile ducts followed by a bacterial infection.

All immunohaematological tests were performed using the commercial gel column agglutination method, screening cells, RBC panel and enzyme-modified cells according to the manufacturer's instructions.

The passive transfer of anti-E antibodies with blood components and the transfer of the antibodies from the mother during pregnancy and also through lactation were excluded. Immunisation due to exposure to E antigen was ruled out by confirming the E-negativity of all the red blood cell units the infant had received and also by confirming the E-negativity of the mother.

Conclusion: Although the naturally occurring anti-E red-cell antibodies are usually found in adults, we report here a case of a 3-month old infant, who had developed naturally occurring anti-E red cell antibodies.

Izvleček

Izhodišča: Pred transfuzijo komponent krvi, ki vsebujejo eritrocite, je obvezno predtransfuzijsko testiranje v obliki določitve krvnih skupin ABO, RhD in Kell ter navzkrižnega preskusa. Pri otrocih, mlajših od 4 mesecev, lahko navzkrižni preskus opustimo, pod pogojem, da smo izključili prisotnost eritrocitnih protiteles s presejalnim testiranjem. Otroci, mlajši od 4 mesecev, namreč zelo redko tvorijo eritrocitna protitelesa, če pa nastanejo, so večinoma posledica alogenih transfuzij.

Prikaz primera: Ob predtransfuzijskem testiranju smo odkrili eritrocitna protitelesa anti-E pri 3-mesečnem otroku, ki je bil operiran zaradi atrezije žolčevodov in je ob tem prebolel bakterijsko okužbo.

Vsi imunohematološki testi so bili izvedeni z gelsko tehniko, s komercialno dostopnimi presejalnimi celicami, z eritrocitnimi panelnimi celicami in z encimsko modificiranimi celicami. Vse teste smo izvedli v skladu z navodili proizvajalca.

Izključili smo pasivni prenos eritrocitnih protiteles anti–E s krvnimi komponentami ter prenos protiteles iz matere bodisi med nosečnostjo bodisi preko dojenja. Imunizacijo zaradi izpostavitve tujemu antigenu E smo izključili s potrditvijo E-negativnosti vseh eritrocitnih komponent krvi, ki jih je otrok prejel, kakor tudi s potrditvijo E-negativnosti matere.

Zaključki: Čeprav naravno prisotna eritrocitna protitelesa anti-E običajno najdemo le pri odraslih osebah, v našem primeru poročamo o nastanku naravno prisotnih eritrocitnih protiteles anti-E pri komaj 3 mesece starem otroku.

Background

According to the current national standards it is our policy to transfuse ABO, RhD and Kell antigen- compatible red blood cells (RBCs), based on the negative cross-match. An incompatibility in other RBC antigenic systems can lead to an immunologic response in the recipient, followed by the formation of allogeneic RBC alloantibodies. It is generally believed that any red cell alloantibody formation, other than anti-A and –B, in infants less than 4 months old is extremely rare.[1,2]

Only few cases of RBC antibodies (Abs) formation in such young infants have been described. The majority of them have resulted from exposure to allogeneic transfusions, such as a case of an anti-c antibody in a 7-week old child, who had been transfused during surgery 6 weeks previously[3]; anti-Lu^b in a 2 month-old infant, who had been transfused 1 month previously[3]; IgG anti-E in an infant aged 11 weeks, who had received 31 transfusions in the previous 6 weeks;[4] or a case of anti-K antibodies in a 12-week old prematurely born infant, who had been given 28 RBCs transfusions in his first 2 months of life.[5]

Naturally occurring Abs, which are not a consequence of allogeneic exposure, are usually found in adults but rarely in children. Only few reports of naturally occurring Abs in children are available. Some examples are: an anti-Jk^a found in a 7-month old boy with orchitis, epididymitis and Escherichia coli urinary tract infection[6], an anti-Jk^a found in 9-month old female twins during a routine pre-surgical evaluation[7] and an anti-K antibody in a 20-day infant with Escherichia coli enterocolitis.[8] Several studies have described the role of bacterial components in the production of naturally occurring Abs directed against non-ABO red cell antigens, most of them reporting a possible association between infection and naturally occurring antibodies against Kell or Kidd blood group antigens.[6,9-12] Some natural Abs are spontaneous, i. e. they are generated without exposure to the antigens, such as anti-E and other antibodies of the Rh system, antibodies to low-incidence antigens, anti-Lu^a, -Di^a and -Xg^a as well as anti-HLA Abs.[3]

Because of the immature immunological status of infants, AABB standards for blood banks and transfusion services allow reduced pre-transfusion serologic testing for infants less than 4 months of age. For them, initial patient pre-transfusion testing must include ABO and RhD typing as well as the screening for unexpected red cell Abs using either plasma or serum from the infant or mother. During hospitalisation, cross-match compatibility testing and repeated ABO and RhD typing may be omitted as long as all of the following criteria are met: the Abs screen is negative, the transfused RBCs are blood group O, ABO-identical or ABO-compatible; and the transfused RBCs are either RhD-negative or RhD identical to the patient's.[13] Repeated type-and-screen testing, as required for adults and children older than 4 months, is unnecessary in this population and merely contributes to iatrogenic and significant blood loss.[14]

We are unaware of previously published reports of naturally occurring anti-E Abs at such an early age as in the following case of our 3-month old male infant.

Case report

A 70-day old male infant, born after 39 weeks of gestation as the second child to a 27 year-old mother after ongoing normal pregnancy, was admitted to the surgical department of the University Medical Centre Ljubljana for a planned surgery of atresia of the extra hepatic bile ducts. Because of pre-existing anaemia and blood loss during the surgery, he had received two units of RBCs at the time of the first surgery (d. 72). Eight days later (d. 80), the infant was re-operated on due to dehiscence of the operating wound. On the next day (d. 81) he received a part of the 3rd unit of RBCs and his first unit of fresh frozen plasma (FFP). He received two more units of FFP on days 82 and 91 because of inadequate blood coagulation.

The drugs metronidazole and gentamycin were given prophylactically from the day of the first surgery (d. 72) onwards. Vancomycin was added two days after the second

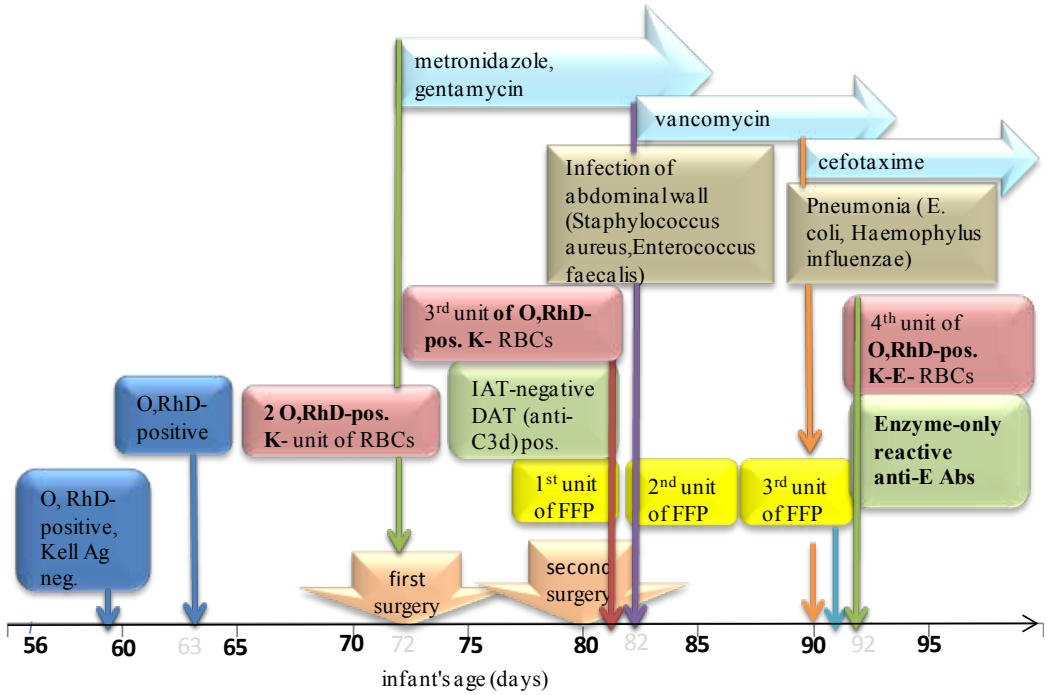

Figure 1: The chronology of events.
(RBC: red blood cell, Abs: antibodies, FFP: fresh frozen plasma, E. coli: Escherichia coli, IAT: indirect antiglobulin (Commbs) test, DAT: direct antiglobulin (Commbs) test)

surgery (d. 82) because of an infection of the abdominal wall. Staphylococcus aureus and Enterococcus faecalis were isolated from the wound. The metronidazole and gentamycin were discontinued after 14 days (d.85), but the infant was still receiving vancomycin for eight days (d.93). At the age of 90 days, the infant's lung function worsened. Escherichia coli (E. coli) and Haemophilus influenzae were isolated from the aspirate of the trachea and cefotaxime was added to his therapy. The blood cultures were negative throughout the hospitalisation period.

At the age of 92 days, on the 20th day after the first surgery, the infant was planned to receive a part of the 4th unit of RBCs due to anaemia. Our immunohaematology laboratory received his blood sample and performed a routine crossmatch compatibility test, which was positive in the enzyme technique only. By means of antibody specification, an enzyme-only reactive anti-E alloantibody was discovered in the serum of the infant, and therefore an E-negative unit of RBCs with a negative crossmatch compatibility test was administered to the infant.

After that, no additional transfusions of RBCs or FFP were required. Fifteen days later (d.107), the infant was moved to the paediatric hospital.

From the 5th day after the last surgery (d.85), the infant was continuously fed with a combination of mother's expressed breast milk and milk formula. See Figure 1 for the chronology of events.

In testing procedures we used blood specimens with the addition of ethylenediaminetetraacetic acid (EDTA). All pretransfusion tests (blood group ABO, RhD, Kell and E-antigen typing, crossmatch compatibility testing, direct and indirect antiglobulin tests [DAT, IAT], Ab identification) were performed using commercially available "Micro Typing Cards" (DiaMed AG, Cressier, Morat, Switzerland). For Ab screening and Ab identification we used commercially available screening cells "DiaCell I, II, III", papainized "DiaCell I-P, II-P, III-P", RBC panel "DiaPanel" and papainized "DiaPanel-P" (DiaMed AG, Cressier, Morat, Switzerland). The infant's and the donor's RBC suspensions were made in "Modified LISS for red-cell suspensions" (Diamed AG, Cressier, Morat, Switzerland). Where needed, a "Modified Bromelin" solution (Diamed AG, Cressier, Morat, Switzerland) was used. Ab screenings, identification tests and crossmatch compatibility tests were conducted with a 15-minute incubation at 37°C. All tests were performed according to the manufacturer's instructions.

At the age of 59 days, the infant's ABO, RhD and Kell blood groups were determined for the first time as O, RhD-positive, Kell-negative. On the 63rd day, the blood group was reconfirmed.

The first three units of RBCs were randomly chosen O, RhD-positive and Kell-negative units; two were transfused on the 72nd and one on the 81st day. They were transfused with compatible (negative) crossmatch compatibility tests and no adverse reactions were observed. When DAT anti-C3d became weakly positive (d.81), the infant had been already receiving, among other therapy, antibiotics for several days. The specification of DAT- causing antibodies by means of an antibody elution procedure was not performed. At the infant's age of 92 days, an enzyme-only reactive anti-E Ab was discovered in the serum of the infant during routine crossmatch compatibility testing before the transfusion of the 4th RBC unit. The infant was phenotyped as E-negative and an E-negative unit of RBC with a compatible crossmatch compatibility test was prepared for the infant.

On the next day (d. 93), the testing was repeated from the infant's new blood sample in order to exclude misidentification of the initial sample. The results were the same as from the previous sample. The Ab identification showed the presence of enzyme-only reactive anti-E RBC antibodies. The titration of anti-E RBC Abs was performed on gel cards using two enzyme-modified cells. We used homozygous E-positive RBCs with the addition of a bromelin solution and homozygous E-positive RBCs pre-treated with papain. In both techniques, the titre was 8.

All archived samples of the blood components the infant had received were re-screened for the presence of enzyme-only reactive anti-E Abs. The look-back procedure revealed that all units contained no Abs. The stored samples of all three RBC units the infant had received were retested for E antigen and were all E-negative.

We obtained a blood sample of the mother, who was already known to have anti-M Abs present in her serum. During pregnancy, the titres of anti-M Abs were monitored. The presence of anti-E Abs in the mother's serum was ruled-out using enzyme-modified red cells and IAT. The mother was also phenotyped as E-negative.

The stored infant's blood sample from the age of 81 days (i.e. immediately before the infection started) was re-tested with enzyme-modified RBCs and the antibody screening was non-reactive.

Discussion

Here we report a case of a 3-month old E-negative male infant, who developed anti-E red blood cell antibodies in the absence of obvious antigenic stimuli during his hospitalisation, after he underwent surgical treatment for atresia of the extrahepatic bile duct. The seroconversion took place between the infant's age of 81 and 92 days.

There are several possible ways of an antibody acquiring or *de novo* formation in our infant. The Abs could be transferred passively from the mother during pregnancy and through lactation, or with the blood components the infant had received. The Abs could also be formed during an active immune response to the foreign antigen after transfusion, transplantation or infection.

Passive transfer of anti-E Abs with the transfused blood components was ruled out by re-screening all the blood units the infant had received for the presence of enzyme-reactive anti-E Abs. The absence of anti-E Abs in the mother excluded the possibility of passive transfer of the Abs from the mother to the foetus during pregnancy, as well as passive transfer of the Abs through lactation. The fact that the infant's mother was E-negative excluded the primary immunisation via maternal-foetal haemorrhage. The 3 RBC units the infant had received were ruled out as the source of antigenic stimuli by their confirmed E-negativity. The infant hadn't undergone any kind of transplantation by the time the red-cell Abs was discovered. On the basis of the facts described above, we conclude that the enzyme-only reactive anti-E Abs found in this infant were of natural origin.

Natural anti-E Abs may be frequently found in the serum of adult individuals, who have not been transfused or pregnant.

In other words, anti-E antibodies are often "non-immune" (i. e. non-red cell stimulated) Abs.[15-18] This type of Abs are not truly anti-red cell in nature, but most probably cross-react with a red blood cell-borne structure (hence its apparent specificity).[19]

At the time of the detection of anti-E Abs, the infant had an E. coli infection among others. Several studies have described a possible association between Abs against non-ABO RBC antigens and microbial infection,[6,9-12] but we are unaware of a known association between infection and the formation of naturally occurring anti-E Abs in patients generally regardless of their age. Unfortunately, we did not conduct any test to confirm E. coli as the source of stimuli for the antibody formation as was previously performed in the case of the naturally occurring anti-Kell stimulated by the E. coli enterocolitis in a 20-day-old child.[8] It is known that naturally occurring anti-E Abs are among many that can form spontaneously[3] and the E. coli infection in our case can be just a coincidental finding. Whatever, the fact remains, that the enzyme-only reactive anti-E Abs in our case are naturally occurring.

Many examples of this type of anti-E Abs, as in our case, react only with enzyme-modified E-positive panel red cells.[19] To our knowledge, no large studies regarding the clinical significance of such enzyme-only reactive Abs have been reported. On the contrary, Contreras et al. reported the clinical insignificance of enzyme-only anti-E Abs in two adult subjects.[20]

At present, there is no quick *in vitro* test available that could discriminate between potentially destructive and potentially benign alloantibodies.[19] It is easier and more cost-effective to provide E-negative units for transfusion to the patient with anti-E Abs than to conduct tests to determine which examples are clinically significant and which would be benign *in vivo*.[19] In our particular case, we did not perform the monocyte monolayer, ADCC or other *in vitro* assays to evaluate the potential clinical significance of the Abs, and therefore we recommended that E-negative RBC units should be selected for future transfusions, since there is a possibility that a provision of E-positive RBC could result in haemolysis and an adverse clinical outcome especially if the patient is unstable.

Conclusion

The AABB standards indicate that infants less than 4 months of age do not require a crossmatch compatibility testing, provided that initial Abs screens are negative.[13] Despite the immaturity and inexperience of the neonatal immune system,[21] some infants younger than 4 months, at the time of so called immature immunological status, may form red cell Abs, the majority of them truly as a result of exposure to antigenic stimuli.

We were able to document the presence of the naturally occurring anti-E red-cell Abs in such a young infant thanks to the policy of our immunohaematology laboratory, which considers the crossmatch compatibility test obligatory prior to every transfusion of RBCs, including in infants less than 4 months of age.

Firstly, because there is no quick *in vitro* test available for distinguishing potentially destructive and potentially benign alloantibodies, and secondly, because of the lack of evidence of the clinical significance of naturally occurring Abs, it is in our opinion better to recommend that the antigen-negative units of RBCs are selected for a transfusion in such cases since there is no guarantee that a provision of antigen-positive RBCs would not result in haemolysis and an adverse clinical outcome in an already unstable patient.

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