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HLA-DQB1*0201 phenotype and severe primary RhD immunization

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Summary

Purpose of Investigation: Although post-delivery administration of anti-D immunoglobulin (anti-D Ig) and antenatal prophylaxis the residual prevalence of RH alloimmunization is due to an incomplete application of the guidelines or to clinical conditions that foster mechanisms of immunization. *Materials and Methods:* The authors report a case of devastating RhD alloimmunization in a secundipara, group 0 Rh negative, without potentially immunizing events. *Results:* Anti-D antibodies were screened at 6/2 and at 13/4 weeks and were found negative. Fetal echocardiography at 25/0 weeks showed dilated right cardiac sections and a mild pericardial effusion. Titer of anti-D antibodies was (1:1024) with negative Kleihauer-Betke. At 27/0 weeks she had an increase of anti-D antibody titer (1:8192), peak systolic velocity in the middle cerebral artery suggested severe fetal anemia. A positive HLA-DQB1 allele *0201 was found, and she gave birth to an infant with severe immune-mediated hemolysis (HB: 3.8 g/dl) who was discharged 62 days after birth due to perinatal complications. *Conclusion:* HLA-DQB1*0201 phenotyping can improve the surveillance and treatment of non-sensitized D negative patients.

Key words: Anti-D immunoglobulin (anti-D Ig); Rh negative; Phenotyping.

Introduction

The 2011 Royal College of Obstetricians and Gynaecologists (RCOG) Green-top Guideline for anti-D immunoglobulin prophylaxis includes standard clinical algorithms for surveillance and treatment of non-sensitized rhesus D (RhD)-negative patients [1]. The development of anti-D antibodies usually arises as a result of fetomaternal hemorrhage (FMH) occurring either late in pregnancy or in the postpartum in a RhD-negative woman with an RhD-positive fetus. Post-delivery administration of anti-D immunoglobulin (anti-D Ig) and antenatal prophylaxis in late pregnancy or after sensitizing events decreased the rate of sensitization down from 0.17% to 0.28% [2]. The residual prevalence of RH alloimmunization is due to an incomplete application of the aforementioned guidelines or to clinical conditions that foster peculiar mechanisms of immunization. One case of fast and devastating Rh D alloimmunization prompted us to consider possible implementations of the current guidelines that can be considered in future studies.

Materials and Methods

A 37-year-old, group 0 Rh negative, secundipara who denied abortions, transfusion therapy or other potentially immunizing events was referred to the present high-risk pregnancy Unit at 14 and 1/7 weeks of gestation due to previous adrenalectomy performed for Cushing syndrome. According to the official documentation provided, the patient received 300 μg of anti-D IgG 24 hours after the previous birth of a growth restricted D+ newborn delivered by emergency cesarean section at 28 weeks of gestation. Anti-D antibodies were screened by two independent laboratories at 6 and 2/7 and at 13/4 weeks using different methods and found negative. Immediately after genetic amniocentesis performed at 17 and 6/7 weeks, the patient received 300 μg of anti-D immunoglobulins. At 21 and 4/7 weeks no ultrasound detectable abnormalities of fetal organs were found. A 75-gram, two-hour oral glucose tolerance test excluded gestational diabetes. Fetal echocardiography at 25/0 weeks did not reveal structural cardiac defects, arrhythmias and myocardial dysfunction, but showed dilated right cardiac sections (cardiothoracic ratio 0.7) and a mild pericardial effusion. At 25 and 3/7 weeks a complete maternal serum evaluation excluded infection as a possible cause of cardiomegaly; due to the high titer of anti-D antibodies (1:1024), a Kleihauer-Betke test was immediately performed and scored negative. Doppler evaluation of the peak systolic velocity in the middle cerebral artery (MCA-PSV) suggested a moderate fetal anemia (48 cm/s, i.e. 1.4 multiples of the median for gestational age). A repeated anti-D titer and a new MCA-PSV evaluation were scheduled in two weeks. At 27/0 weeks, the patient was admitted to hospital for intensive fetal monitoring, due to an enormously increased anti-D antibody titer (1:8192) and to a MCA-PSV predictive of severe fetal anemia (65 cm/s, i.e. 1.8 multiples of the median). The presence of the HLA-DQB1 allele *0201 was found positive. The patient refused diagnostic cordocentesis and putative intra-uterine transfusion. At 27 and 2/7 weeks of gestation, a 1,075-gram male infant was delivered by emergency cesarean section due to persistent severe fetal tachycardia. The Apgar score was 3 at one minute and 5 at five minutes. Severe immune-mediated hemolysis was detected soon after birth (Hb 3.8 g/dL; hematocrit 15%) and treated with a double-volume (180 mL/kg) exchange transfusion using fresh 0 negative, leukoreduced, and irradiated red blood cells. The newborn experienced hyaline membrane disease despite corticosteroid prophylaxis and was finally discharged 62 days after birth.

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Results and Discussion

The reported kinetics of fast antibodies production cannot be ascribed to a secondary immune response boosted by amniocentesis but should rather be attributed to severe primary anti-D immunization; indeed, the patient denied previous abortions, transfusion therapy, or other potentially immunizing events and had no evidence of anti-D antibodies in two separate evaluations with different methods (indirect antiglobulin test and low affinity IgM antibody-sensitive automated hemagglutination, respectively).

Theoretical hypotheses to explain this fast-proceeding immune response include: 1) the occurrence of concealed large/repeated bleeds that exceeded the protection granted by 300 μg of anti-D IgG, 2) genetic/epigenetic host factors that either created a hyper-responsiveness to an aliquot of fetal Rh+ erythrocytes that accessed maternal circulation early in pregnancy or alternatively disrupted the mechanisms of anti-D mediated immune suppression.

The notion that fetal blood volume between 16-20 weeks is as low as 10-30 ml [3] ruled out the possibility that an acute post-amniocentesis transplacental hemorrhage might have outweighed the neutralizing ability of the administered anti-D IgG [4]; similarily, the negative Kleihauer-Betke test excluded that repeated hemorrhages led to a D antigen concentration exceeding that of the administered antibody [5].

The precocious small transplacental hemorrhage (up to 0.05 mL) that occasionally occurs in the first trimester [6, 7] might have triggered Rh immunization in this patient, due to a peculiar combination of genetic (presence of the HLA-DQB 1*0201 allele) [8] and epigenetic conditions (rebound immunity triggered by previous adrenalectomy) [9].

D alloimmunization is not correlated to any specific HLA type, because the large Rh protein easily fits into a given MHC pocket [10]; however, the presence of the HLA-DQB 1*0201 may influence T cells networks and modify T-cell receptors repertoire [11] or reflect linkage disequilibrium with positive modulators of B lymphocytes and dendritic cells [12] that foster germinal center reaction and antigen-driven production of high-affinity plasma cells [13].

Also, the likelihood of developing different blood group antibodies (including anti D) is increased in several conditions of non-infectious inflammation including impaired immunity, autoimmune disorders, and rebound immunity [9, 10, 14]. Similarly, in animal models, viral-like inflammation shifts intra-splenic red blood cells uptake from macrophages to dendritic cells, increasing the immunogenic stimulation of antigen-specific naive $CD4^+$ T cells [15].

The present authors cannot exclude the intriguing possibility that they recorded a possible failure of post-amniocentesis immunoprophylaxis due to the interference of the reported genetic/epigenetic combination with various mechanisms of anti-D Ig immune protection, including red blood cells preferential clearance by splenic red pulp macrophages [12, 16] or down-regulation of B cells activity [17, 18]. Based on the aforementioned considerations, the present authors suggest that screening for the HLA-DQB 1*0201 allele may implement the current guidelines of surveillance and treatment of non-sensitized Rh-negative patients [1, 2]. Indeed, in the allele positive population, the possible development of a fast and devastating antibody response may prompt a more intensive surveillance than that classically adopted (a single routine 28-week anti-D test after a negative screening obtained at the beginning of pregnancy). Also, in these patients, a putative condition of hyper-responsiveness to the D antigen may benefit from anti-D Ig administration by the end of the first trimester (in addition to those scheduled at 28 weeks or after overt sensitizing events). The present authors note that the additional cost of HLA-DQB 1*0201 testing offered to all RH negative patients might be favorably counterbalanced by outdistancing the standard clinical surveillance of the allele negative anti-D immunized patients. Interestingly, they provide the unreported evidence [19, 20] that cardiomegaly may represent the first sign of an impending severe fetal anemia and propose that an exhaustive evaluation of a dilated fetal heart should include the screening of anti-D Ig. In conclusion, HLA DQB1*0201 phenotyping does not only help to predict the probability of severe hemolytic disease of the newborn in anti-D immunized patients as previously reported [8], but can possibly improve the surveillance and treatment of non-sensitized D negative patients; the benefits of such universal screening must to be validated in future trials.

References

[1] Royal College of Obstetricians and Gynaecologists: "Rhesus D Prophylaxis, The Use of Anti-D Immunoglobulin for (Green-top 22)". April 27, 2011. Available at: http://www.rcog.org.uk/womens-health/clinical-guidance/use-anti-d-immunoglobulin-rh-prophylaxis-green-top-22

[2] Qureshi H., Massey E., Kirwan D., Davies T., Robson S., White J. *et al.*: "BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn". *Transfus. Med.*, 2014, *24*, 8.

[3] Reali G.: *"*Inadequate prophylaxis in anti-D immunisation". *Blood Transfus.,* 2008, *6*, 169.

[4] Wylie B.J., D'Alton M.E.: "Fetomaternal hemorrhage". *Obstet. Gynecol.*, 2010, *115*, 1039.

[5] Contreras M., Mollisin P.L.: "Rh immunization facilitated by passively-admnistered anti-Rh?". *Br. J. Haematol".,* 1983, *53*, 153.

[6] Bowman J.M., Pollock J.M., Penston L.E.: "Fetomaternal transplacental hemorrhage, during pregnancy and after delivery". *Vox. Sang.*, 1986*, 51*, 117.

[7] Jakobowicz R., Williams L., Silberman F.: "Immunization of Rh negative volunteers by repeated injections of very small amounts of Rh positive blood". *Vox Sang*, 1972, *23*, 376.

[8] Hilden J.O., Gottvall T., Lindblom B.: "HLA phenotypes and severe Rh(D) immunization". *Tissue Antigens*, 1995, *46*, 313.

[9] Da Mota F., Murray C., Ezzat S.: "Overt immune dysfunction after Cushing's syndrome remission: a consecutive case series and review

of the literature". *J Clin. Endocrinol. Metab.*, 2011*, 96*, E1670.
[10] Zimring J.C., Hendrickson J.E.: "The role of inflammation in alloimmunization to antigens on trasfused red blood cells". *Curr. Opin. Hematol.*, 2008, *15*, 631.
[11] Altmann D.M., Sansom D., Marsh S.G.: "What is the basis for HLA-DQ associations with autoimmune disease?" *Immunol. Today*, 1991, *12*, 267.
[12] Ryder A.B., Zimring J.C., Hendrickson J.E.: "Factors Influencing RBC Alloimmunitazion: Lessons Learned from Murine Models". *Transfus. Med. Hemother.,* 2014*, 41*, 406.
[13] McHeyzer-Williams M.G.: "B cells as effectors". *Curr. Opin. Immunol.*, 2003*, 15*, 354.
[14] Gehrie E.A., Tormey C.A.: "The Influence of Clinical and Biological Factors on Transfusion-Associated Non ABO Antigen Alloimmunization: Responders, Hyper-Responders,and Non-Responders". *Transfus. Med. Hemother.*, 2014*, 41*, 420.
[15] Hendrickson J.E., Chadwick T.E., Roback J.D., Hillyer C.D., Zimring J.C.: "Inflammation enhances consumption and presentation of transfused RBC antigens by dendritic cells". *Blood*, 2007, *110*, 2736.
[16] Kumpel B.M.: "On the immunologic basis of Rh immune globulin (anti-D) prophylaxis". *Transfusion*, 2006, *46*, 1652.
[17] Brinc D., Le-Tien H., Crow Ar., Freedman J., Lazarus Ah.: "IgG-mediated immunosuppression is not dependent on erythrocyte clearance or immunological evasion: implications for the mechanism of ant-D in the prevention of haemolytic disease of the newborn?". *Br. J. Haematol.*, 2007, *139*, 275.
[18] Brinc D., Denomme G.A., Lazarus A.H.: "Mechanisms of anti-D action in the prevention of hemolytic disease of the fetus and newborn: what can we learn from rodent models?" *Curr. Opin. Hematol.*, 2009*, 16*, 488.
[19] Wuttikonsammakit P., Uerpairojkit B., Tanawattanacharoen S.: "Causes and consequences of 93 fetuses with cardiomegaly in a tertiary center in Thailand". *Arch. Gynecol. Obstet.,* 2011, *283*, 701.
[20] Bigras J.L., Suda K., Dahdah N.S., Fouron J.C.: "Cardiovascular evaluation of fetal anemia due to alloimmunization". *Fetal. Diagn. Ther.,*, 2008, *24*, 197.

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