

Case Report

Alloimmune neonatal neutropenia and thrombocytopenia associated with maternal anti HNA-1a, HPA-3b and HLA antibodies

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The incidence of alloimmune neonatal neutropenia combined with neonatal alloimmune thrombocytopenia is very low. We report a case of a neonate who suffered severe neutropenia and thrombocytopenia with widespread petechial spots. The presence of alloantibodies in mother's and patient's sera was analyzed by lymphocytotoxicity test, agglutination test, granulocyte indirect immunofluorescence test, platelet immunofluorescence test (PIFT) and solid phase enzyme-linked immunosorbent assay. Human neutrophil antigens (HNA) and human platelet antigen (HPA) genotypes were tested by polymerase chain reaction analyses. The mother's and patient's sera reacted with neutrophils and lymphocytes of the father. PIFT revealed the presence of IgG anti-platelet antibodies in the patient's serum but the test was negative in the maternal serum. Analyses of HNA-1 and HPA genotypes of the family revealed maternal-neonatal HNA-1a and HPA-3b mismatch. The study of the mother's and patient's sera showed the presence of anti HNA1a, HPA-3b and HLA antibodies specific for HLA-A3 and HLA-B38 antigens. These results suggest that the transplacental passage of maternal HNA-1a, HPA-3b and HLA alloantibodies caused neutropenia and thrombocytopenia in this patient.

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The maternal-fetal transport across the placenta induces the sensitization of the mother to fetal inherited paternal antigens, mainly human leucocyte antigens (HLAs). This sensitization in normal pregnancy is not harmful (1), but when these alloantibodies cross the placenta there is a risk to the newborn to develop severe cytopenias (2).

Alloimmune neonatal neutropenia (ANN) is an uncommon but potentially life-threatening disorder of the newborn. It is caused by maternal sensitization to fetal neutrophil antigens (3). The human neutrophil antigens (HNA) most commonly involved in ANN are HNA-1a, HNA-1b and HNA-2a, which are located on FcγRIIb (CD16) of neutrophils, a receptor for complexed IgG.

Neonatal alloimmune thrombocytopenia (NAIT) results from fetomaternal platelet

antigen incompatibility, the dominant human platelet antigens (HPAs) are HPA-1-a, HPA-3-a and HPA-5-a (4). The incidence of NAIT is about 1.5 per 1000 live births. Death or neurologic impairment may occur in up to 25% of the cases. Although HLA antibodies occur in up to one third of the pregnancies, the fetus is usually protected and HLA antigens have only rarely been implicated as cause of ANN or NAIT (5, 6). We report here a case of ANN and NAIT resulting from the coexistence of maternal HNA-1a, HPA-3b and HLA alloantibodies.

Case report

A 4000 g male infant was born to a 35-yr-old, gravida 4, para 3 (abortion 1) mother. The pregnancy and delivery were uncomplicated.

Apgar scores were 9 and 10 at 1 and 5 min, respectively. The infant appeared to be healthy and physical examination revealed no abnormalities. At 26 h after birth, widespread petechial spots were noted and laboratory examinations showed severe neutropenia and thrombocytopenia: white cells count 3700/ μ L, neutrophils count 550/ μ L, and the platelet count was 75,000/ μ L. A bone marrow (BM) aspirate showed normal cellularity of all lineages except for rarity of mature neutrophils. A single dose of intravenous immunoglobulin (IVIG) 1 g/kg was administered at day 3 without benefit, moreover, a decrease in the platelet (7900/ μ L) and absolute neutrophil counts (48/ μ L) were observed, as shown in Fig. 1. From day 9 the platelet count showed a progressive increase towards restoring their normal levels, whereas the absolute neutrophil count was 51/ μ L. From day 22 to 28 the infant received G-CSF, 5 mg/kg/day. On day 29 the umbilical cord remained attached and still had neutropenia (195/ μ L). On day 43 the absolute neutrophil count was 2550/ μ L, whereas on day 86 the absolute neutrophil count decreased to 1140/ μ L.

Laboratory findings

The flow cytometric analysis of peripheral blood (PB) of the infant at day 6 after birth revealed a very low percentage (1.5%) of neutrophils in the sample, although their immunophenotype and that of the rest of the series (eosinophils, monocytes and lymphocytes) were normal. The analysis of BM at the same day showed a normal distribution of leukocytic populations but with left shift in the myeloid series. The neutrophils represented the 65% of the sample, they showed normal size but low granularity, expressed the cell surface antigens CD45, CD33, CD13 and CD15

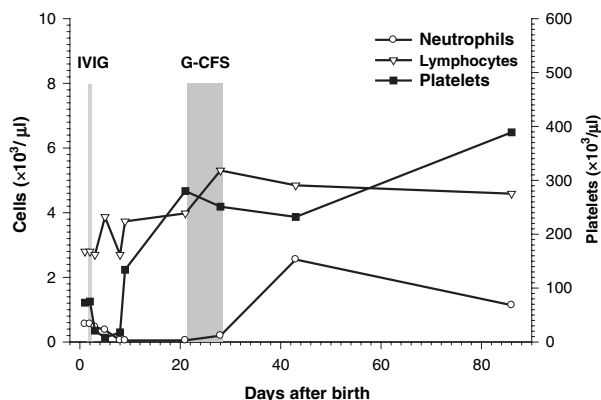


Fig. 1. Absolute neutrophil, platelet and lymphocyte counts of the patient during the first 86 days of life. Each bar represents the period of treatment with IVIG or G-CSF.

but lack of CD16, indicating an immature phenotype. The PB sample obtained at day 86, revealed an increase in the percentage of neutrophils (18%) showing a mature phenotype (CD45+, CD33+, CD13+, CD15+ and CD16) but the count was still low (1140 cells/ μ L).

The platelet immunofluorescence test (PIFT) detected the presence of anti-platelet IgG antibodies in the newborn, but no IgM antibodies. We excluded the presence of autoantibodies from the mother because this test was negative on the mother's platelets.

The presence of HLA antibodies was examined in the mother's and patient's sera by lymphocytotoxicity test (LCT). The results are presented in Table 1. The maternal sera obtained at day 9, 29, and 86 reacted with lymphocytes from the father and patient even when diluted at 1:4. Sera obtained from the patient during the first week of life reacted with lymphocytes from the father, but sera obtained at day 29 and 86 did not react with the father's lymphocytes. These reactions were completely abrogated when mother's and patient's sera were adsorbed with a purified platelets pool. To examine the specificity of HLA antibodies in the maternal serum, the family was HLA class I typed by a standard complement-dependent microcytotoxicity assay and HLA-A3, B38, and Cw5 were suggested as possible alloantigens. The maternal sera were tested against a panel of lymphocytes from 30 HLA-typed donors by LCT but they were

Table 1. Reactivity of the mother's and patient's sera against lymphocytes and neutrophils from mother, father, patient and random donors

	Lymphocytes (LCT)					
Sera	Mother	Father	Patient	Donors (n = 30)		
MS (days 9, 29, 86)	—	+	+	+ 80%		
MS (adsorbed)	—	—	—	+ 18%		
PS (day 6)	—	+	+	nt		
PS (days 29, 86)	—	—	—	nt		
CS	—	—	—	—		
	Neutrophils (GIFT and GAT)					
				HNA-1 phenotype (donors)		
Sera	Mother	Father		a/a	a/b	b/b
MS	—	+	+	+	+	—
MS (adsorbed)	—	+	+	+	+	—
PS	—	+	nt	nt	nt	nt
HLAS	±	±	nt	nt	nt	nt
CS	—	—	—	—	—	—

MS, maternal serum; PS, patient's serum; HLAS, anti-HLA serum; CS, control serum; —, negative; ±, weakly positive; +, positive; nt, not tested.

considered to be polyspecific, reacting with the 80% of HLA-typed donors. On the basis of the flow cytometric and polymerase chain reaction with sequence-specific primers (PCR-SSP) analyses, the HNA-1 genotypes and phenotypes were determined to be HNA-1a/HNA-1b for the patient and father, and HNA-1b/HNA-1b for the mother.

Granulocyte indirect immunofluorescence (GIIFT) and agglutination test (GAT) were used to determine whether the patient's and mother's sera reacted with neutrophils from the father and random donors whose HNA-1 phenotype was determined by flow cytometry (Table 1). The direct neutrophil immunofluorescence test performed on neutrophils from the mother was negative. The GIIFT revealed a serologic incompatibility between mother's serum and the father's neutrophils, this reactivity was also noted after adsorption of HLA antibodies using a purified platelet pool. The mother's serum reacted only with those neutrophils from donors bearing the HNA-1a antigen. Similar results were obtained by the GAT with mother's and patient sera even when diluted at 1:16. These reactivity were maintained when maternal serum was depleted from HLA antibodies using a purified platelet pool (Table 1).

The analysis of HPA genotypes in the family revealed one mismatch in HPA-3 locus between the mother (HPA-3b⁻) and both father and patient (HPA-3b⁺). The study of anti-HPA and anti-HLA class I antibodies in mother's serum by enzyme-linked immunosorbent assay (ELISA; PAK[®]12 Platelet Antibody Screening Kit, GTI, Waukesha, WI, USA) showed the presence of anti-HPA-3b antibodies and confirmed the presence of anti-HLA class I antibodies at higher titer (3:1) compared with anti-HPA-3b antibodies.

Discussion

Maternal alloimmunization is a well-recognized phenomenon caused by maternal incompatibility to fetal alloantigens. It has been reported that only 3% of mothers with an HNA incompatible child develop alloantibodies against the HNA-1a and 1b antigens (7), whereas the reported incidence of NAIT because of HPA incompatibility was of one in 1000 to one in 5000 (4). In contrast, the frequency of HLA antibodies in pregnancy has varied in different series from 7% to 39%, but only in 13% of them do antibodies appear in the corresponding cord blood samples (1). When HLA antibodies cross the placenta only 25 % of babies become severely thrombocytopenic (2).

We described here a case of ANN and NAIT with the coexistence of anti HNA-1a, HPA-3b and HLA antibodies. Whereas neutropenia could mainly be because of anti-HNA-1a antibodies and thrombocytopenia to HPA-3b antibodies, HLA antibodies could exert a synergistic role in both pathologies. Anti HNA-1a antibodies showed a slow clearance kinetics, causing a severe neutropenia for a month which only was overcome by granulocyte colony-stimulating factor (G-CFS) treatment. These antibodies not only affected the PB but also the BM mature neutrophils as demonstrated by flow cytometric analysis, which revealed a left shift in the myeloid series. This finding could be attributed to the destruction of the more mature myeloid forms on which the relevant neutrophil antigen is expressed. This fact is because CD16 is selectively expressed on mature but not immature granulocytes (8) and could explain the lack of CD16 in the BM neutrophils of the patient. Although the kinetics of HPA-3b antibodies was not analyzed in the patient because of the lack of serum availability, it is interesting to remark their lower titer in maternal serum compared with anti HLA class I antibodies, suggesting that anti HLA class I antibodies could exert a more important role in thrombocytopenia. Although the role of anti-HLA antibodies in NAIT is controversial (2), it is tempting to speculate that those anti-HLA antibodies that overcome the placental tissues, which express HLA antigens, could be mainly neutralized by the platelets, thus causing thrombocytopenia.

The G-CSF administration is very effective in ANN, rapidly increasing the absolute neutrophil count to levels considered safe. It is known that G-CSF down-regulate the expression of certain neutrophil antigens such as HNA-1a and HNA-1b (9), thereby rendering the neutrophils less vulnerable to circulating antibodies. In our patient, the treatment with one dose of G-CFS was very effective, rendering a normal count of neutrophils after treatment.

Intravenous immunoglobulin has been used successfully in the treatment of various IgG antibody-mediated hematologic disorders including neonates with immune thrombocytopenia or neutropenia. The mechanism of action of IVIG has been postulated as competitive inhibition of antibody binding or reticuloendothelial blockage (10). In our patient the treatment with IVIG did not appear to be effective, moreover it caused a deeper thrombocytopenia and neutropenia probably because of the presence of alloantibodies in the IVIG.

The incidence of ANN and NAIT is very low in contrast with that expected on the basis of the

high number of maternal-fetal neutrophil and platelet antigen mismatches. This could be due to the fact that many infants who acquire these alloantibodies have mild neutropenia or thrombocytopenia, or that the disease is undetected or undiagnosed. Therefore, as ANN and NAIT pose a risk for the newborn's life, the early detection and characterization of this alloantibodies is very important in order to apply appropriate therapy as soon as possible.

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