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CASE REPORT

Alloimmune Neonatal Neutropenia Due to Anti-HNA-2a Alloimmunization with Severe and Prolonged Neutropenia but Mild Clinical Course: Two Case Reports

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Alloimmunization to granulocyte-specific antigens can occur during pregnancy. Maternal IgG can cross the placenta and result in neonatal neutropenia. The clinical course of alloimmune neonatal neutropenia is usually self-limiting with only mild infection. However, in severe cases complicated with bacterial sepsis it is a potentially life-threatening disorder. The effect of intravenous (IV) immunoglobulin, prophylactic antibiotic therapy, and recombinant human granulocyte-colony stimulating factor is variable and may prove useful in some cases. Two cases of alloimmune neonatal neutropenia due to anti HNA-2a alloimmunization in two siblings are reported. The first neonate was administered IV gammaglobulins to increase the blood neutrophil count, at a standard dosage (0.4 g/kg body weight) for 5 days without response. The second neonate did not receive specific therapy for blood neutrophil count increase. Neutropenia persisted for 2 and 6 months, respectively. The choice and efficacy of specific therapy for neutrophil count increase in the management of alloimmune neonatal neutropenia have not yet been fully defined and require additional evaluation in the majority of cases.

Key Words: Alloimmune neonatal neutropenia, Human neutrophil antigen (HNA-2a), Alloimmunization, Neutropenia, Neonate, IV immunoglobulins, Prophylactic antibiotics.

Introduction

Alloimmune neonatal neutropenia (ANN) is an uncommon but potentially life-threatening disorder of the neonate (1). ANN is the result of maternal alloimmunization to granulocyte antigens. The passive transfer of maternal neutrophil-specific antibodies and subsequent sensitization of fetal neutrophils can result in severe neutropenia in the neonate. The pathogenesis of the disease is similar to alloimmune neonatal thrombocytopenia in which alloimmunization to platelet-specific antigens occurs during pregnancy (2).

The incidence of ANN has been estimated at 1/1000–6000 live births and accounts for 1.5% of all neonatal intensive care unit admissions (2,3). ANN should be suspected in a newborn with isolated neutropenia (<1500 cells/μL). The course of pregnancy is uneventful and the mother has normal granulocyte count and no clinical history of frequent bacterial infections. The clinical course is usually self-limiting with only mild infection (4). On differential diagnosis, severe congenital neutropenia (Kostmann's syndrome), an autosomal recessive disorder characterized histopathologically by early stage maturation arrest of myeloid differentiation, should be considered (5). Demonstration of alloantibodies against granulocyte-specific antigen shared by neonatal and paternal granulocytes in the maternal serum is essential in the diagnosis of ANN (6). Antibodies to granulocyte-specific antigens HNA-1a and HNA-1b have been most commonly reported to cause ANN (5). Anti-HNA-2a, and antibodies to receptor Fc gamma III (CD16) if mother is a HNA-null phenotype, are rarely involved in neonatal neutropenia (7).

The treatment usually includes antibiotics, intravenous (IV) gammaglobulins, and recombinant human granulocyte colony-stimulating factor (rh G-CSF); however, with variable success (8–10). In severe cases of ANN complicated with bacterial sepsis, therapy with rh G-CSF along with

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antibiotics is indicated (11). In contrast to this, there are several literature reports of inconsistent response to rh G-CSF when used in the treatment of alloimmune and autoimmune neutropenia caused by anti-HNA-2a (NB1) antibodies (12,13).

We report on two cases of ANN due to anti-HNA-2a alloimmunization in two siblings.

Case Reports

Case Report 1

The first case is a female newborn weighing 3540 g at birth and born from the first, uncomplicated pregnancy to a healthy 29-year-old mother at the 40th week of gestation. Severe neutropenia (170 neutrophils/μL) with normal findings of other laboratory tests was detected on the first day of the newborn's life. Although there were no clinical symptoms of perinatal infection, antibiotic therapy (ampicillin and gentamicin) was administered for 1 week. Bacteriological testing showed no infection either (blood culture, cerebrospinal fluid culture, and urine culture were sterile).

Upon diagnosis of ANN due to HNA-2a antibodies (Table 1), therapy with IV gammaglobulins at a standard dosage (0.4 g/kg body weight) was administered for 5 days; however, without response. Two-week corticosteroid therapy was also not successful.

On day 15, mild omphalitis developed, which was treated for 7 days by an antibiotic (ceftriaxone) according to umbilical swab finding. No additional infections were observed during the course of neutropenia. The newborn was discharged from the hospital on day 24 with normal clinical status. Neutropenia persisted for 2 months (Figure 1).

Case Report 2

A male newborn weighing 3700 g was born at term to the same mother 2 years later after a normal pregnancy. On the first day of the newborn's life, neutropenia (123 neutrophils/μL) developed, and the same antibodies that had caused the disorder in the first child were also detected in maternal serum at mid-gestation. Serological analysis confirmed the diagnosis of ANN due to HNA-2a antibodies (Table 1). The newborn had no clinical or laboratory findings indicative of infection in the immediate neonatal period. Mild omphalitis developed on day 5 of the neonate's life and was treated for 1 week with antibiotic therapy according to the umbilical swab antibiotic sensitivity report (ceftriaxone). No specific therapy to increase the blood neutrophil count was administered. The child was discharged from the hospital on day 12, and neutropenia persisted for 6 months, considerably longer than in his older sister (Figure 1). There were no additional infections during the follow-up period.

Serological Analysis

When neonatal alloimmune neutropenia is suspected, serological screening is primarily expected to demonstrate that the immune degradation of neonatal neutrophils is due to the reaction between neutrophil IgG alloantibodies that have passively crossed to the neonate's circulation via placenta to bind to the corresponding antigens present on neonatal neutrophils. A positive direct test for antigranulocyte antibodies is performed with the neonate's granulocytes and positive cross-matching between paternal granulocytes and maternal/neonatal serum point to ANN. Direct and indirect granulocyte immunofluorescence test (GIFT-DT, IT) are most widely used on serological screening. This is followed by determination of antineutrophil antibody specificity in maternal/neonatal serum, mostly performed by use of monoclonal antibody immobilization of granulocyte antigens (MAIGA) and immunoblot (IB) assays. In addition, screening for the presence of HLA class I antibodies that may also be present in maternal/neonatal serum along with anti-HNA antibodies should be performed by microlymphocytotoxicity test (LCT) or enzyme-linked immunoassay (ELISA). In most cases, isolated presence of anti-HLA

Table 1. Results of serological testing

	Test name							
	GIFT DT IT	LIFT IT	GAT IT	MAIGA* CD16R Fc g IIIb	MAIGA* CD177HNA-2a	MAIGA* CD11 b/CD18	IMMUNO-BLOT*	LCT/ELISA, GTI-QUICK ID*
Maternal serum gran/ly	pos/neg	neg	pos	neg	pos	neg	HNA-2a (NB1) pos	neg/neg
Neonate 1 serum gran/ly	pos/pos	neg	neg	neg	pos	neg	nt	nt
Neonate 2 serum	pos/pos	neg	neg	neg	pos	neg	nt	nt
Paternal gran/ly and maternal serum	CM/pos	CM/neg	nt	nt	nt	nt	nt	nt

GIFT, granulocyte immunofluoroescence test; DT, direct test; IT, indirect test; LIFT, lymphocyte immunofluoroescence test; GAT, granulocyte agglutination test; LCT, lymphocytotoxicity test; MAIGA, monoclonal antibody immobilization of granulocyte antigens; ELISA-GTI QUICK ID-kit, enzyme-linked immunoassay, anti-HLA class I antibody identification; CM, cross-match; gran, granuloytes; ly, lymphocytes; R Fc g IIIb, ReceptorFc gamma IIIb; pos, positive; neg, negative; nt, not tested.

*Serological studies on alloantibody identification were done by Professor Bux, Institut für Klinische Immunologie und Transfusionmedizin, Giessen, Germany.