Resistance to Recombinant Human Granulocyte Colony-Stimulating Factor in Neonatal Alloimmune Neutropenia Associated With Anti–Human Neutrophil Antigen-2a (NB1) Antibodies

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ABSTRACT. Neonatal alloimmune neutropenia is the neutrophil counterpart of the erythrocyte disorder of hemolytic disease of the newborn. Fetal neutrophil antigens, which are inherited from the father but foreign to the pregnant mother, provoke the formation of maternal antibodies, which, on transplacental passage, cause fetal/neonatal neutropenia. Because infants with this disorder are at a higher risk of infection, recombinant hematopoietic growth factors, such as recombinant human granulocyte colony-stimulating factor, have been tried, with generally good results, to treat those with severe and prolonged neutropenia. We report a neonate who had neonatal alloimmune neutropenia associated with antibodies directed against human neutrophil antigen-2a (NB1) and initially failed to respond to even very high doses of recombinant human granulocyte colony-stimulating factor but eventually had a therapeutic response. Pediatrics 2002;109(4). URL: http://www.pediatrics.org/cgi/content/full/109/4/e64; neonate, alloimmune, neutropenia, growth factors, treatment.

ABBREVIATIONS. GBS, group B streptococci; WCC, white blood cell count; ANC, absolute neutrophil count; CBC, complete blood count; rhG-CSF, recombinant human granulocyte colony-stimulating factor; HNA, human neutrophil antigen; NAN, neonatal alloimmune neutropenia.

Alloimmune neutropenia occurs when a mother becomes sensitized to a foreign antigen of paternal origin that is present on the neutrophils of her fetus. Specific immunoglobulin G antibody directed against this antigen is then formed. The antigen systems most commonly involved are NA1, NA2, NB1, and NC1. The transplacental passage of antibody into the fetal circulation involves are NA1, NA2, NB1, and NC1. The transplacental passage of antibody into the fetal circulation results in neutropenia, and, as such, is a self-limited condition in the neonate. Generally, the duration of the neutropenia is a few weeks or a few months. We report a unique case of an infant with severe, prolonged neutropenia initially refractory to recombinant human granulocyte colony-stimulating factor (rhG-CSF) therapy, but who eventually responded to an unusually higher dose.

CASE REPORT

A 3080-g female infant was born at 39 4/7 weeks’ gestation by spontaneous vaginal delivery to a 25-year-old gravida 2 para 1 (abortion 1) mother who was O Rh positive; negative for Venereal Disease Research Laboratories, human immunodeficiency virus, and hepatitis B; but positive for group B streptococci (GBS). The pregnancy was otherwise uncomplicated. Rupture of placental membranes occurred 10 hours before delivery. Adequate intrapartum antibiotic prophylaxis for GBS was received. The delivery was complicated by maternal fever (100°F).

Apgar scores were 8 and 9 at 1 and 5 minutes, respectively, and the infant seemed well and was sent to the well-infant nursery. A complete blood count was obtained at 6 hours of age in view of maternal GBS status and pyrexia. The white blood cell count (WCC) was 6300/μL with an absolute neutrophil count (ANC) of 166/μL. A repeat complete blood count (CBC) confirmed the findings (WCC = 3700/μL, ANC = 148/μL). A subsequent CBC showed a WCC of 4900/μL with an ANC of 0. Intravenous ampicillin and gentamicin therapy was initiated after blood cultures were obtained, and rhG-CSF was started at 10 μg/kg/d.

In view of the persistent severe neutropenia, a bone marrow biopsy was performed. It was cellular with normal erythroid and megakaryocytic development but showed arrested development of the myeloid series at the myelocyte stage. There were abundant blasts and promyelocytes, but none of the subsequent stages in granulocyte development could be identified. Granulocyte typing, granulocyte cross-matching, and serum assays to detect neutrophil-specific antibodies were performed at the Neutrophil Serology Reference Laboratory of the American Red Cross (St Paul, MN). The granulocyte agglutination test and granulocyte immunofluorescence assay were positive on both maternal and infant sera. The antineutrophil antibodies were specific for human neutrophil antigen (HNA)-2a (NB1) antigen. The father’s neutrophils were HNA-2a positive; the mother’s were negative.

The rhG-CSF dose was increased to 15 μg/kg/d, but no increase in peripheral neutrophil concentration was observed. The dose was then increased to 20 μg/kg/d given in 2 equally divided doses. The infant also received intravenous immunoglobulin at 400 mg/kg/d for 3 days. Her ANC persisted at 0; therefore, on day 15 of life, the dose of rhG-CSF was doubled to 40 μg/kg/d. After 24 hours, as the ANC was still 0, the dose of rhG-CSF was increased further to 80 μg/kg/d. A CBC on day of life 18 showed a WCC of 84 000/μL with 46% polymorphonuclear cells, 24% bands, and 1% metamyelocytes (ANC = 59 640/μL). The rhG-CSF was held, and additional doses were not given until day 35 of life when the ANC again decreased to 810/μL. rhG-CSF was reinitiated at 10 μg/kg/d with twice-a-week dosing. At 7 weeks of age, the dosing frequency was decreased to once a week. The infant maintained her neutrophil counts above 1000/μL without rhG-CSF after 8 weeks of age. She has subsequently been well and is no longer neutropenic.

DISCUSSION

Neonatal alloimmune neutropenia (NAN) remits when maternal antineutrophil antibodies diminish to a critically low concentration in the neonate’s marrow and blood.1-11 During the period of neutropenia, antibiotic treatment is somewhat controversial. Prophylactic antibiotics may be used until the results of
cultures can be determined (48–72 hours), but after that time, routine antibiotics are not warranted and should be reserved for neonates with severe or culture-proven infections.12

rhG-CSF administration is generally very effective in NAN, rapidly increasing the ANC to levels considered safe.5,13–15. Such treatment is rational, particularly for neonates with very severe and prolonged neutropenia16 when one considers a mortality rate of 5% from overwhelming infections17 and also on extrapolating from observations that neonates with other causes of severe and prolonged neutropenia are indeed at risk for acquiring infections.14 However, as illustrated by the case we report, certain patients with NAN fail to respond to the usual doses of rhG-CSF.

A wide variety of antigenic targets have been identified in NAN, including the HNA-1, HNA-2, and HNA-3 antigen groups, besides NC1, SH, SAR, LAN, LEA, CN1,18–21 and possibly,22 the human leukocyte antigens.2,23,24 In the United States, however, one half of all of the cases of NAN are mediated by antibodies that bind to 1 of 3 antigens: HNA-1a (NA1), HNA-1b (NA2), or HNA-2a (NB1).25

rhG-CSF is known to downregulate the expression of certain neutrophil antigens (eg, antigenic determinants on FcyIIb, such as HNA-1a and -1b),26 thereby rendering the neutrophils less vulnerable to circulating antibodies. Increased levels of soluble FcyII and decreased apoptotic reactions of neutrophils may also lead to adsorption of the antibodies, thereby providing indirect protection to the neutrophils.26,27 rhG-CSF–induced neutrophil activation may also reverse27,28 some of the antibody-mediated qualitative defects that have been recorded even in the absence of neutropenia.28–33

There have been a few reports of inconsistent responses in NAN to rhG-CSF.13–20 Although the reasons are yet to be fully ascertained, this variability has been postulated to be attributable to differences in the relative affinity of antibodies to myeloid precursors versus more mature cells, and the cellular response to rhG-CSF in terms of expression levels of different antigens.13 These factors may be particularly important in immune-mediated neutropenias secondary to antibodies against HNA-2a (NB1). Stroncek et al34 demonstrated that rhG-CSF, unlike the aforementioned downregulating effect on HNA-1a and -1b, may actually increase the neutrophil surface expression of HNA-2a. They showed that administration of rhG-CSF to healthy adult volunteers led to an initial increase in the neutrophil surface expression of HNA-2a, followed by a decrease to pretreatment levels after 4 days and a second peak again after 10 days. More recently, Pocock et al35 reported a case of chronic myeloid leukemia with HNA-2a–mediated autoimmune neutropenia after unrelated stem cell transplantation, where rhG-CSF may have actually resulted in perpetuation of the disorder. Their patient remained profoundly neutropenic despite 3 weeks of rhG-CSF therapy and improved rapidly after cessation of the rhG-CSF treatment. The authors postulated that rhG-CSF–induced upregulation of HNA-2a expression on circulating neutrophils may have increased the potency of the circulating anti–HNA-2a antibodies that were present at that time and also might have increased antibody production. In our case, a similar mechanism in increasing the potency of the antibody is highly probable, although increased antibody production would not be of concern because, in NAN, the antibodies are of maternal origin.

The usual dose of rhG-CSF used in infants with immune-mediated neutropenias is 5 to 10 µg/kg/d.5,13–15 Very often, a 10 µg/kg/d dose is administered for 3 days by intravenous or subcutaneous injection, and then additional doses are titrated to keep the blood neutrophil counts over 1000/µL.16 Usually, the response is evident within 24 to 48 hours, and a total of 2 to 3 weeks of treatment is sufficient.3,36 Our case was unusual in having required a very high dose to achieve an elevation in circulating neutrophil concentration and also in terms of the long latency before a therapeutic response was observed. The eventual response suggests that it is possible to overcome the neutropenia by increasing the rate of neutrophil production high enough to exceed destruction and perhaps by “mopping-up” the antibodies by providing sufficient binding sites. Despite the high doses of rhG-CSF used in our patient, no adverse effects of therapy were observed, which is consistent with the published experience with infants.5,13–15

We speculate that neonates with severe and prolonged NAN do benefit from rhG-CSF and that this benefit outweighs the risks of the short treatment courses and the low doses generally needed. However, we recognize that some patients with NAN, particularly those with antibody directed against HNA-2a, might fail to respond to the usual doses of rhG-CSF. We speculate further that administering rhG-CSF to these refractory patients is useful as a means of accelerating neutrophil production and providing additional binding sites for maternal antibody, thus hastening antibody clearance and speeding recovery. Obviously, such speculations require additional examination.

ACKNOWLEDGMENTS

This work was supported by grants HD-01180 (Dr Calhoun), HL-61798 (Dr Christensen), and HD-42326 (Dr Calhoun) from the National Institutes of Health.

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Pediatrics 2002;109;e64
DOI: 10.1542/peds.109.4.e64
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