Granulocyte colony stimulating factor treatment for alloimmune neonatal neutropenia

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Abstract

Granulocyte colony stimulating factor (G-CSF) treatment was successfully used in three preterm infants with alloimmune neonatal neutropenia (AINN). Two infants had persistent neutropenia despite treatment with intravenous immunoglobulin and random donor granulocyte transfusions for presumed sepsis. Neutrophil counts returned to normal with G-CSF treatment; the response was least convincing in one infant with fulminant necrotising enterocolitis.

It is suggested that treatment with G-CSF be considered early for the treatment of infants with AINN.

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Management of severe alloimmune neonatal neutropenia (AINN) may be difficult. Typically the neutropenia lasts for one to 12 weeks but it may persist.¹ Most infants have only minor skin infections, but deaths with severe infections have been reported. Neutropenia results from neutrophil destruction by transplacentally acquired maternal neutrophil specific immunoglobulin G (IgG) antibodies directed against paternally derived antigens.² Various treatments, including corticosteroids, intravenous immunoglobulin, and exchange or leucocyte transfusions are ineffective or produce only transient neutrophil increments.³ Otherwise healthy infants often have to stay in hospital for long periods.⁴ We describe three infants with AINN, one with fulminant necrotising enterocolitis (NEC), who were treated successfully with granulocyte colony-stimulating factor (G-CSF).

Case reports

The three case histories are summarised in table 1. Briefly, three male premature infants with respiratory distress at birth were investigated for sepsis and found to have leucopenia and severe neutropenia. Maternal investigations were performed and a detailed clinical history was taken to exclude factors related to maternal disease as a cause of neonatal neutropenia. Pre-eclampsia, drugs, and viral infections could not be implicated. The complete blood counts were normal.

The three infants were treated for presumed sepsis, although blood and gastric aspirate cultures subsequently proved sterile. All received

antibiotics and intravenous immunoglobulin (Sandoglobulin, Sandoz), and random donor granulocyte transfusions were also given to cases 1 and 2 as shown (table 1) (fig 1). These treatments failed to produce a sustained increment in absolute neutrophil count in cases 1 and 2. Confirmation of AINN was obtained by day 6 in all infants (table 1). Case 1 developed fulminant necrotising enterocolitis (NEC) on day 7 and required ventilatory support; an intestinal perforation developed two days later and the infant underwent a laparotomy and an ileostomy. The three infants received a course of daily subcutaneous injections of G-CSF (Filgrastim) (table 1) (fig 1). In all three infants the absolute neutrophil count returned to normal within 48 hours of starting treatment, although there was considerable variation in the magnitude of the increment. On day 42 case 1 developed a mild transient neutropenia ($1.3 \times 10^9/l$) in association with a further episode of sepsis due to coagulase negative *Staphylococcus aureus* infection. The circulating neutrophil antibody was no longer detectable at this stage.

Serological findings

Sera were screened for neutrophil antibodies by standard granulocyte agglutination and immunofluorescence tests. In each case a neutrophil specific antibody was detected in both maternal and umbilical cord serum which reacted with paternal and the infant's recovery neutrophils but not maternal neutrophils. In case 1 no recognised antibody specificity could be defined despite testing with extensive neutrophil panels, suggesting a possible new antigen. Anti-NA2 was implicated in both cases 2 and 3. Serological and DNA based neutrophil typing in these cases confirmed that the mothers were negative for NA2 and that the fathers and infants were NA2 positive.

Discussion

The three infants were investigated and treated for presumed sepsis because of prematurity, respiratory distress, and severe neutropenia. Two of these infants had been treated before with both intravenous immunoglobulin and random donor granulocyte transfusions which had failed to produce a sustained increase in neutrophil count. In all three infants the absolute neutrophil count returned to normal with G-CSF treatment, although there was considerable variation in the magnitude of the neutrophil increment. There was an indisputable neutrophil response in case 2. A prompt neutrophil response was also seen in the third

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Table 1 Clinical characteristics at birth and haematological and serological data in three infants with alloimmune neonatal neutropenia

	Case 1	Case 2	Case 3
Sex	M	M	M
Gestation (weeks)	30	30	26
Birthweight (g)	1470	1371	955
Mode of delivery	VD	CS	VD
Clinical	RDS	RDS	RDS
Perinatal factors	PROM 6 days		PROM 8 weeks Placenta praevia
Initial white cell count ($\times 10^9/l$)	2.6	3.16	2.68
Initial absolute neutrophil count ($\times 10^9/l$)	0.26	0.05	0.03
AINN			
Day of diagnosis	6	5	2
Antigen involved	Unrecognised	NA2	NA2

VD = vaginal delivery; CS = caesarean delivery; RDS = respiratory distress syndrome; PROM = premature rupture of membranes; AINN = alloimmune neonatal neutropenia

case, although there was a three day overlap between intravenous immunoglobulin and G-CSF treatment and an effect due to intravenous immunoglobulin cannot be excluded. The neutrophil response in the first infant was less convincing. The latter infant had fulminant necrotising enterocolitis with intestinal perforation and had had surgery before G-CSF was administered. The absolute neutrophil count vacillated around $2 \times 10^9/l$ during the course of treatment, possibly as a result of ongoing severe sepsis. All infants tolerated the drug well.

Our findings substantiate a previous report which demonstrates the value of G-CSF in the treatment of AINN.³ In the latter study G-CSF was used late (at one and two months, respec-

tively) in the clinical course of two infants, in contrast to its early use in the present cases. These findings contrast with a recent report,⁵ which describes disappointing results with G-CSF treatment in AINN due to an anti-HLA antibody. Two of the present cases involved anti-NA2; the third antibody was of unrecognised neutrophil antigen specificity. The success of G-CSF in this disorder may differ according to the antigen involved.⁶

The incidence of AINN is estimated at $< 0.1\%$.^{1,2} However, the prevalence of neutrophil specific antibodies in pregnant women is much higher, ranging from 1.1% to 20%. These antibodies may be against the known granulocyte specific antigens, including NA1, NA2, NB1, ND1, NE1 and RED, or may involve as yet undefined antigens. Severe AINN may occur in the firstborn and in women who have never been transfused.² Usually, subsequent pregnancies are similarly affected.² Neutropenia is often an incidental finding in healthy term infants, in contrast to the three sick premature infants in the present study. This disorder should always be considered as a potential cause of the neutropenia associated with sepsis as it may be a predisposing factor. Early institution of effective treatment is vital for these infants: in a previous study we found a mortality of 61% in infants with neutropenia and documented early onset sepsis.⁷

Granulocyte colony stimulating factor increases circulating neutrophil numbers by stimulating release of neutrophils from the bone marrow and inducing myeloid proliferation.⁸ Gilmore *et al*³ suggest that G-CSF may abrogate neutropenia in AINN in two ways: by increasing neutrophil production relative to destruction; and by down-regulation of antigen expression, thereby rendering the neutrophils less vulnerable to circulating antibodies. These mechanisms are not mutually exclusive. Further studies need to determine the precise mechanisms involved. Our data support a previous report,³ that G-CSF is a safe effective treatment for AINN. It has the potential to abrogate neutropenia and reduce hospital stay in healthy infants, and reduce morbidity and mortality in infected infants. However, success with this treatment may depend on the antigen involved.

Figure 1 Changes in absolute neutrophil count in three infants with AINN, treated variously: intravenous immunoglobulin (0.5 g/kg/day) and/or granulocyte transfusions and G-CSF (5 μ g/kg/dose/day for cases 1 and 2, 10 μ g/kg/dose/day for case 3).

- 1 Bux J, Jung KD, Kauth T, Mueller-Eckhardt C. Serological and clinical aspects of granulocyte antibodies leading to alloimmune neonatal neutropenia. *Transfus Med* 1992; 2:143-9.
- 2 Lalezari P. Alloimmune neonatal neutropenia. In: Engelfriet CP, von dem Borne AEG, eds. *Clinical immunology and allergy*. London: Baillière Tindall, 1987:443-52.
- 3 Gilmore MM, Stroncek DF, Korones DN. Treatment of alloimmune neonatal neutropenia with granulocyte colony-stimulating factor. *J Pediatr* 1994; 125:948-51.
- 4 Rodwell RL, Tudehope DI, O'Regan P, Minchinton R, Waters AH. Alloimmune neonatal neutropenia in Australian aboriginals: an unrecognized disorder? *Transfus Med* 1991; 1:63-7.
- 5 Bedu A, Baumann C, Rohrlach P, Duval M, Fenneteau O, Cartron J. Failure of granulocyte colony-stimulating factor in alloimmune neonatal neutropenia. *J Pediatr* 1995; 127:508.
- 6 Korones DN, Gilmore MM, Stroncek DF. Failure of granulocyte colony-stimulating factor in alloimmune neonatal neutropenia. *J Pediatr* 1995; 127:509.
- 7 Rodwell RL, Taylor MCD, Tudehope DI, Gray PH. Hematologic scoring system in early diagnosis of sepsis in neutropenic newborns. *Pediatr Infect Dis J* 1993; 12:372-6.
- 8 Cairo MS. Cytokines: a new immunotherapy. *Clin Perinatol* 1991;18:343-59.