CURRENT TOPICS

Haemopoietic colony stimulating factors for preterm neonates

Robert Carr, Neena Modi

Bacterial and fungal sepsis is a major cause of morbidity and mortality in neonates. Infection rates are high in infants treated in intensive care units, with the highest rates, of around 30%, occurring in extremely immature preterm neonates.¹⁻³ A survey of neonatal infection at Yale University, ongoing since 1928,⁴ has documented a decline in neonatal septic deaths commensurate with the establishment of neonatal intensive care units and the liberal use of increasingly effective antibiotics. Mortality from sepsis declined steadily until the early 1980s, but since then it has remained constant at near 15%. This plateau of mortality most likely reflects the poor host defences of immature, preterm neonates.⁵

Neutrophil leucocytes are central to the defences against bacterial infection,⁶ and in neonates both neutrophil production and function are immature. Neutropenia, defined as a neutrophil count below the normal range for neonates established by Monroe,⁷ occurs in up to 35% of preterm neonates⁸⁻⁹ and in 50% of all infants born to mothers with pregnancy induced hypertension.¹⁰ The development of sepsis together with neutropenia carries a high mortality of 39%, and two out of every three septic infants whose neutrophils fall below $0.5 \times 10^9/l$ will die.⁹

Kinetics of neutrophil production

It is impossible to measure directly the total neutrophil cell mass in a human neonate. There is a good deal of circumstantial evidence to suggest that neonatal bone marrow has a reduced capacity to produce neutrophils in adequate numbers.¹¹ Neutrophils develop from multipotent haemopoietic stem cells through lineage-committed progenitors (granulocyte-macrophage colony forming units, CFU-GM). These give rise to a proliferative pool, identified morphologically in the bone marrow as promyelocytes and myelocytes, and a storage pool comprising metamyelocytes, bands, and segmented neutrophils, before being released into the circulation. Mature neutrophils circulate with a peripheral blood half life of 6.3 hours before migrating into the extravascular tissues where they undergo apoptosis.¹² In response to infection, adults release marrow storage pool neutrophils into the circulation, while increasing the proliferative rate of committed progeni-

tors to achieve a sustainable neutrophil leucocytosis.¹¹⁻¹³ During sepsis, neutrophil turnover increases from a steady state of $1.6 \times 10^9/kg/day$ to $5.0 \times 10^9/kg/day$.

What is known of the kinetics of neutrophil production early in development largely comes from studies in rats. These have shown that, in comparison to adult animals, newborn rats have a total body pool of CFU-GM less than 10% of the CFU-GM/g body weight of adults; less than 25% of CFU-GM from neonates are in the resting phase of the cell cycle (G_0), compared with > 75% of the progenitors being in G_0 in adults.¹¹⁻¹⁴⁻¹⁵ The absolute neutrophil cell mass per gram of body weight in the newborn rat is only one quarter that of adult animals. During the first four weeks of postnatal life, rodent neutrophil cell mass increases to adult levels, with a corresponding increase in the proportion of quiescent CFU-GM.¹⁶ Studies in healthy human neonates born at or near term show a similar pattern of near maximal CFU-GM proliferation rate¹⁷ compared with the large pool of CFU-GM in G_0 observed in adults.¹⁸

The consequence of this immature pattern of granulopoiesis is that, in the face of overwhelming bacterial sepsis, the neonate has an inadequate reserve of preformed neutrophils and inadequate reserve production capacity, both of which are necessary to mount a rapid and sustained neutrophil leucocytosis. Thus when preterm neonates develop Gram negative or group B streptococcal sepsis, they frequently become neutropenic. When this is associated with marrow neutrophil storage pool depletion, mortality is high.¹⁹⁻²⁰ The lethal consequence of this inability to mount a rapid and effective neutrophil response is further emphasised by the most recent update from the Yale survey, which found that 72% of septic deaths occur within two days of a positive blood culture, despite appropriate antibiotic treatment.⁵

Neutrophil bactericidal function

The most consistent neutrophil functional defects reported in term neonates are abnormalities of adhesion to vascular endothelium and migration. Phagocytosis and bacterial killing seem to be normal in healthy term infants, but tend to become defective during clinical

Department of
Haematology, United
Medical and Dental
School of Guy's and St
Thomas's,
St Thomas's Hospital,
Lambeth Palace Road,
London SE1 7EH
R Carr

Neonatal Paediatrics,
Royal Postgraduate
Medical School,
Hammersmith
Hospital,
London
N Modi

Correspondence to:
Dr R Carr.

stress.²¹⁻²² In preterm neonates of less than 32 weeks gestation there are additional functional defects. Phagocytosis of *Escherichia coli* by neutrophils from uninfected, clinically stable preterm neonates is less efficient than that by cells from term babies and there is similar impaired phagocytosis of larger *Candida* cells.²³⁻²⁴ Preterm neonates have a smaller population of metabolically active cells in assays of the respiratory burst.²⁵ Infants born earlier than 28 weeks also tend to have reduced peak chemiluminescence (reflecting the bactericidal activity of the respiratory burst),²⁶ but this seems to be more related to the clinical complications of prematurity rather than immaturity itself.²⁷⁻²⁸ Septic preterm neonates seem unable to develop the enhanced oxidative metabolism that occurs in the neutrophils of infected adults.²⁹⁻³⁰ There have been very few studies examining postnatal maturation of neutrophil function in preterm neonates, but we have shown that the abnormalities of chemotaxis, phagocytosis, and cell membrane complement (CR3) and Fcγ (FCRIII) receptor expression persist for three to four weeks after birth before 32 weeks of gestation²³⁻³¹⁻³³ compared with the quite rapid postnatal maturation of chemotaxis observed in term babies.³⁴⁻³⁵

Colony stimulating factors

Granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) are naturally occurring proteins that regulate haematopoiesis by stimulating the proliferation and differentiation of myeloid progenitor cells.³⁶ Since they were cloned in 1986 and 1985, respectively,³⁷ and became commercially available in pharmacological quantities, these two growth factors have become part of standard clinical treatment in adult and general paediatric medicine for stimulating neutrophil production to correct disease related or iatrogenic neutropenia.³⁸ Both are in routine use for shortening the period of neutropenia following chemotherapy for solid tumours and leukaemia, and after bone marrow transplantation. G-CSF, in particular, has been effective in correcting neutropenia associated with the congenital neutropenias, Kostmann's syndrome, and cyclical neutropenia.³⁹

The cellular targets and activities of G-CSF and GM-CSF are different. The effect of G-CSF is restricted to the neutrophilic granulocyte lineage, whose committed progenitors it stimulates to proliferate and differentiate, which shortens their transit time through the marrow and enhances the survival of the mature cells in the circulation. GM-CSF is an earlier and more widely acting cytokine, which induces growth and differentiation of multilineage haematopoietic progenitors as well as progenitors committed to both the granulocyte and macrophage lineage.⁴⁰⁻⁴¹ GM-CSF also has a much greater effect on the bactericidal functions of mature neutrophils than G-CSF⁴²⁻⁴³ as well as functionally enhancing monocytes, which are unaffected by G-CSF.⁴⁴⁻⁴⁵ The effects of GM-CSF on mature phagocyte bactericidal function occur at lower concentrations than are

necessary for induction of progenitor proliferation. Most of these functional effects are indirect and require secondary stimuli, such as chemotactic factors or immune sera, to trigger the full enhanced response. This indirect, or priming, nature of the GM-CSF effect may be physiologically useful to the host, in that endogenous release of GM-CSF would then only result in activation of neutrophils/monocytes at sites of injury and inflammation, where the secondary stimuli are concentrated.⁴⁶ The normal physiological interplay between G- and GM-CSF remains to be fully elucidated. One possible model is that G-CSF and GM-CSF are both elaborated during acute infections, but that G-CSF circulates and stimulates neutrophil proliferation and maturation, while GM-CSF remains localised at the site of infection to help retain and activate arriving effector cells.⁴⁶

In current clinical practice G-CSF is more commonly used to shorten the period to recovery from chemotherapy induced neutropenia. However, recent interest has focused on the potential clinical benefit of the functional enhancement induced by GM-CSF, particularly for the treatment of invasive fungal infections.⁴⁵⁻⁴⁷⁻⁴⁹

Colony stimulating factor physiology in neonates

The potential for G- and GM-CSF to reduce the incidence and severity of infection in preterm neonates is supported by the nature of the abnormalities displayed by the immature phagocyte immune system, the efficacy of growth factors at enhancing neutrophil and monocyte immunity in adults, and the in vitro and animal experiments that have specifically examined these cytokines in relation to neonate immune function.

Both G- and GM-CSF are present in measurable concentrations in the cord blood of both full term and preterm neonates.⁵⁰⁻⁵¹ In one study,⁵² G-CSF concentrations measured in the immediate postnatal period correlated with gestational age and values were higher in infants with clinical signs of infection, suggesting an appropriate physiological response. However, several in vitro studies have shown reduced production of both G-CSF⁵³⁻⁵⁴ and GM-CSF⁵⁵⁻⁵⁶ by stimulated mononuclear cells isolated from term neonate cord blood, compared with production by adult cells. The neonatal cells accumulate less G- and GM-CSF mRNA, which may be secondary to an alteration in the mRNA post-transcriptional stability.⁵⁷ Mononuclear cells from preterm infants produce even less G-CSF than term neonates.⁵³⁻⁵⁸ G-CSF production does not increase appropriately in response to experimental sepsis in newborn rats.⁵⁹ Nor did it increase in response to neutropenia in a small series of neutropenic human neonates, who had serum G-CSF values no different from those of non-neutropenic controls.⁵³ This is in contrast to the high circulating G-CSF concentrations seen in septic or neutropenic adults. The weight of evidence suggests, therefore, that colony stimulating factor production does not

increase appropriately in response to physiological stimuli in the newborn and that this deficiency is more severe in premature infants.

However, while considering the potential efficacy of therapeutic colony stimulating factors, an important finding has been that the number and affinity of both G-CSF and GM-CSF receptors on cord blood neutrophils are identical to those of adult cells.⁵⁴⁻⁵⁵ In vitro functional experiments have confirmed that GM-CSF can prime term and preterm neonate neutrophils for enhanced chemotactic and respiratory burst responses to appropriate stimuli.²⁵⁻⁶⁰⁻⁶¹ Similarly, granulocyte and granulocyte-macrophage committed progenitors isolated from neonate blood samples respond well to the proliferative stimuli of exogenous G- and GM-CSF, respectively, in vitro.⁶² In our laboratory, progenitors from preterm neonates (median gestation 31 weeks) were at least as responsive to GM-CSF as those from adults.⁶³ It has also been shown that the in vitro proliferative response to G-CSF is independent of the infant's endogenous plasma G-CSF concentration.⁶²

Colony stimulating factor treatment in newborn rats

Further encouragement to attempt to enhance the ability of human neonates to resist infection using colony stimulating factors has come from studies in rodents. Following an initial study showing that a single dose of either G-CSF or GM-CSF given to 1 day old newborn rats could induce a significant neutrophilia,⁶⁴ Cairo's group administered recombinant human G-CSF (RrHu G-CSF, Amgen), 5 µg/kg/day intraperitoneally, to newborn rats for seven consecutive days. By the seventh day the absolute peripheral blood neutrophil count had increased by 750% and the marrow neutrophil storage pool by 100% over untreated animals. The marrow proliferative pool remained unchanged, showing that there was no depletion of early myeloid progenitors. They were then infected with a lethal dose of type III group B streptococci (GBS) and randomly allocated to receive antibiotics 24 hours later. Seventy-two hours after infection all the animals pretreated with G-CSF and given antibiotics survived, whereas only 50% given antibiotics without G-CSF survived. G-CSF on its own did not protect against septic death.⁶⁵ Subsequent studies have shown that a single dose of G-CSF given immediately before inoculation with GBS could similarly enhance survival (91% survival: G-CSF with antibiotics; 28% survival: antibiotics alone). However, if the G-CSF was given 12-18 hours after GBS infection, it had no beneficial effect.⁶⁶

GM-CSF can also increase neutrophil production and reduce mortality in experimentally infected newborn rats. Cairo's group administered recombinant murine GM-CSF (rmGM-CSF, Immunex, 4×10^7 units/mg protein) at a dose of 75 µg/kg/day for seven days before inoculating with GBS on day 8. Mice receiving rmGM-CSF had a 75% increase in circulating neutrophils and a 50% increase in the marrow neutrophil storage pool by day 7, compared

with control animals. But the GM-CSF group had the same mortality as the control group (57% vs 59%), both groups having also received antibiotics.⁶⁷ In two other studies, where much lower doses of GM-CSF were given, there was improved survival. Frenck⁶⁸ gave a single dose of rmGM-CSF before inoculating newborn rats with *Staphylococcus aureus* 6 hours later. Titrating the dose over a wide range, rats given rmGM-CSF (Immunex, 4×10^7 units/mg protein) at a dose of 0.03 µg/kg had 52% survival at three days compared with the control group survival of 10%. But at higher doses, survival declined (29% at 10 µg/kg). The increased survival was entirely due to GM-CSF, as no antibiotics were given. At these low doses there was a transient (50%) increase in the neutrophil count at 6 hours, but over the subsequent 36 hours there was no increase in the neutrophil count, marrow neutrophil storage, or proliferative pools compared with untreated animals. This suggests that the enhanced survival achieved by the single dose of GM-CSF was entirely due to bactericidal functional priming. Similar results were found by Wheeler⁶⁹ when intraperitoneal rmGM-CSF (Sandoz, 6.2×10^7 units/mg protein) at a dose of 0.2 µg/kg was given 7-19 hours after intraperitoneal inoculation with GBS. At this optimal dose, mortality was reduced to 37% (compared with 67% in controls), again without antibiotics. There was no induced neutrophilia, but neutrophils recovered from the peritoneum 3.5 hours after GM-CSF administration showed significantly enhanced respiratory burst activity. These data suggest that the beneficial effect of GM-CSF is related to enhanced neutrophil/monocyte function. Higher doses, which increase neutrophil numbers, may in fact be detrimental through overactivating the cells, making them hyperadherent and interfering with migration into infected sites.⁷⁰⁻⁷¹

Colony stimulating factor therapy in human neonates

The first documented human neonate to receive a therapeutic colony stimulating factor was a 654g infant born at 30 weeks gestation, to a mother with severe pregnancy induced hypertension. After birth he remained persistently neutropenic and had five episodes of septicemia (including one with *S aureus* and three with GBS). At four weeks he started G-CSF, rapidly established a normal neutrophil count, and had no more septic episodes.⁷² Since then, Christensen and Cairo have reported two phase I/II studies of CSF treatment in newborn infants with suspected sepsis. These showed that both G- and GM-CSF could effectively increase the neutrophil count, without evidence of toxicity.⁷³⁻⁷⁴ In the first study, 35 infants of 26-41 weeks gestation were given G-CSF (Amgen) within 72 hours of birth. G-CSF was administered by one hour intravenous infusion for three consecutive days at a dose of 1, 5, or 10 µg/kg/day, or 5 µg/kg or 10 µg/kg/12 hourly. A significant increase in neutrophil count was seen at 48 hours with 5 µg/kg/day and 10 µg/kg/day, but no additional

increase was seen with the 12 hourly dosing schedule. Gestation did not seem to affect the response. All infants had tibial bone marrow aspirates at 72 hours, which showed a dose dependent increase in the neutrophil storage pool (metamyelocytes, bands, and segmented neutrophils), but no significant change in the number or morphology of early committed granulocyte progenitors.⁷³

In the GM-CSF study, 15 infants of 24-33 weeks gestation were given GM-CSF (Immunex) by two hour intravenous infusion for seven consecutive days at doses of 5 µg/kg/day, 5 µg/kg/12 hourly or 10 µg/kg/day, starting within 72 hours of birth. All doses produced a significant neutrophilia, which persisted for five days after the last dose, as well as a monocytosis. The 5 µg/kg/day dose also caused a significant rise in platelet count, compared with placebo treated controls.⁷⁴ In both studies there was evidence of *in vivo* neutrophil activation, as indicated by an increase in the expression of the neutrophil adhesion molecule C3bi (CR3, CD11b) on the cell membrane. In neither study could clinical benefit be assessed as there were few positive blood cultures.

In an uncontrolled British study,⁷⁵ 12 critically ill, neutropenic neonates with presumed sepsis (seven confirmed) were given G-CSF 5-10 µg/kg/day. In all infants the neutrophil count increased, even though all but one had greatly increased endogenous G-CSF concentrations before treatment. Six of these infants survived.

Potential colony stimulating factor toxicity in neonates

Neonatal paediatricians have been slow to introduce the haemopoietic colony stimulating factors into clinical practice. Even though their extensive use in adults has shown them to be safe drugs when used in appropriate therapeutic doses,⁷⁶ there is understandable anxiety about toxicity from these powerful modulators of granulopoiesis and phagocyte function on the neonate's developing immune system. Some of this anxiety stems from early reports of a "first dose" effect with GM-CSF when high doses given intravenously caused transient pulmonary sequestration of leucocytes and the induction of various cytokine cascades via neutrophil and monocyte activation.⁷⁷ Such toxicity is avoided and the therapeutic effect enhanced by giving G- and GM-CSF by slow intravenous infusion or by the subcutaneous route.⁷⁸ At doses ≤ 10 µg/kg and when high peak blood concentrations are avoided by slow infusion or subcutaneous administration, the only adverse effects commonly encountered are mild fever and occasional bone pain.⁷⁶ Their perceived safety in adults is best exemplified by the growing practice of giving G-CSF to healthy haemopoietic stem cell transplant donors, in whom safety is a paramount concern, to allow harvesting of multipotent haemopoietic stem cells from the peripheral blood, rather than subject them to the discomfort and risks of a general anaesthetic for a conventional marrow harvest.⁷⁹

There are specific anxieties related to giving therapeutic colony stimulating factors to neonates: GM-CSF, in particular, could exacerbate acute and chronic lung injury through its powerful activating effects on monocytes and neutrophils. Their stimulation of granulopoiesis could result in a "lineage steal" effect, with a reduction in erythropoiesis and thrombopoiesis. Such an association has been observed in some neonatal erythropoietin (Epo) studies when infants given Epo developed neutropenia.⁸⁰⁻⁸² However, neutropenia has not developed during other studies.⁸³⁻⁸⁴ Thrombocytopenia has been observed in some septic infants given G-CSF, but this might well have been a consequence of the sepsis itself.⁷⁵ GM-CSF, on the other hand, tends to increase the platelet count, through its known, but mild, effect on megakaryopoiesis.⁷⁴⁻⁸⁵ An additional anxiety, that exposing newborn babies to G-CSF in infancy may predispose to leukaemia in later life, arises from the experience in Kostmann's syndrome. This congenital neutropenia can be corrected by long term treatment with G-CSF, but small numbers of children have developed leukaemia on treatment.⁸⁶ However, there is evidence that Kostmann's syndrome is in itself a pre-leukaemic condition and the development of leukaemia may relate to the structural abnormality of the neutrophils and their G-CSF receptor, as well as the longer survival achieved by preventing early neutropenia-related septic deaths.⁸⁷⁻⁸⁸

The evidence to date from clinical trials is that short term administration of G-CSF or GM-CSF to neonates undergoing intensive care is safe with, in particular, no evidence of pulmonary or haematological toxicity.⁷³⁻⁷⁴ Even more encouraging is that 21 of the infants enrolled in the G-CSF pilot study have now been followed up at two years and had normal haematological, immunological, and neurological development.⁸⁹

Future directions

What, then, is the current status of colony stimulating factor treatment for neonates at high risk of sepsis? There is good theoretical evidence for its beneficial use, supported by carefully conducted experiments with infected newborn rats. In human newborn infants the treatment can raise the peripheral blood neutrophil/monocyte count, even in the presence of Gram negative sepsis. Above all, its use seems to be safe in the short term, with no long term sequelae.

However, much work now needs to be done to establish how best to use them to achieve the greatest effect. Should colony stimulating factors be used as an adjunct to antibiotics in acute life threatening sepsis or would greater benefit be achieved by prophylactic use over several weeks, in an attempt to accelerate the maturation of phagocyte immunity and so prevent infection? Which colony stimulating factor is more appropriate in which circumstances? And what doses should be used? Selecting the correct dose of GM-CSF may be particularly important in the light of the rodent model

experience where higher doses seem to afford less protection against death.⁶⁸

In the presence of so much uncertainty, there is a strong argument that the use of these powerful biological response modifiers should be restricted to randomised clinical trials. These should be designed to address these questions with both laboratory and clinical endpoints. A small number of such trials are already underway in the USA and the UK. However, others have argued that when faced with a critically ill, septic, and neutropenic infant, given the evidence to date and high mortality with conventional antibiotic treatment, it would be unethical to withhold G-CSF, even in the context of a randomised trial. Though we have some sympathy with this view, we feel that these concerns can and should be taken into account through careful trial design. G-CSF is already entering into use in neonatal intensive care units in an uncontrolled way and, unless this research is actively promoted, we may lose the opportunity to obtain firm evidence of efficacy.

- 1 Hensley OJ, Hart CA, Cooke WI. Serious infection in a neonatal intensive care unit: A two-year survey. *J Hyg Camb* 1985;**95**:289-97.
- 2 de Louvois J. Septicaemia and meningitis in the newborn. In: de Louvois J, Harvey D, eds. *Infection in the newborn*. Chichester: John Wiley & Sons Ltd, 1990:107-15.
- 3 Baley JE, Fanaroff AA. Neonatal infections. In: Sinclair J, Bracken MB, eds. *Effective care of the newborn infant*. Oxford: Oxford University Press, 1992:454-506.
- 4 Freedman RM, Ingram DI, Gross I, Ehrenkranz RA, Warshaw JB, Baltimore RS. A half century of neonatal sepsis at Yale: 1928 to 1978. *Am J Dis Child* 1981;**135**:140-4.
- 5 Gladstone IM, Ehrenkranz RA, Edberg SC, Baltimore RS. A ten-year review of neonatal sepsis and comparison with the previous fifty-year experience. *Pediatr Infect Dis J* 1990;**9**:819-25.
- 6 Gallin JI. Disorders of phagocytic cells. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: Basic principles and clinical correlates*. New York: Raven Press Ltd, 1992:459-74.
- 7 Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. *J Pediatr* 1979;**95**:89-98.
- 8 Baley JE, Stork EK, Warkentin PI, Shurin SB. Neonatal neutropenia. Clinical manifestations, cause and outcome. *Am J Dis Child* 1988;**142**:1161-6.
- 9 Rodwell RL, Taylor KMCD, Tudehope DI, Gray PH. Hematologic scoring system in early diagnosis of sepsis in neutropenic newborns. *Pediatr Infect Dis J* 1993;**12**:372-6.
- 10 Koenig JM, Christensen RD. Incidence, neutrophil kinetics, and natural history of neonatal neutropenia associated with maternal hypertension. *N Engl J Med* 1989;**321**:557-62.
- 11 Christensen RD. Neutrophil kinetics in the fetus and neonate. *Am J Pediatr Hematol Oncol* 1989;**11**:215-23.
- 12 Cartwright GE, Athens JW, Wintrobe MM. The kinetics of granulopoiesis in normal man. *Blood* 1964;**24**:780-803.
- 13 Christensen RD, MacFarlane JL, Taylor NL, Hill HR, Rothstein G. Blood and marrow neutrophils during experimental group B streptococcal infection: quantification of the stem cell, proliferative, storage and circulating pools. *Pediatr Res* 1982;**16**:549-53.
- 14 Christensen RD, Rothstein G. Pre- and postnatal development of granulocytic stem cells in the rat. *Pediatr Res* 1984;**18**:599-602.
- 15 Christensen RD. Developmental changes in pluripotent hematopoietic progenitors. *Early Hum Dev* 1988;**16**:195-205.
- 16 Erdman SH, Christensen RD, Bradley PP, Rothstein G. Supply and release of storage neutrophils. *Biol Neonate* 1982;**41**:132-7.
- 17 Christensen RD, Harper TE, Rothstein G. Granulocyte-macrophage progenitor cells in term and preterm neonates. *J Pediatr* 1986;**109**:1047-51.
- 18 Fauser AA, Messner HA. Proliferative state of human pluripotent hemopoietic progenitors (CFU-GEMM) in normal individuals and under regenerative conditions after bone marrow transplantation. *Blood* 1979;**54**:1197-200.
- 19 Christensen RD, Rothstein G. Exhaustion of mature marrow neutrophils in neonates with sepsis. *J Pediatr* 1980;**96**:316-18.
- 20 Wheeler JG, Chauvenet AR, Johnson CA, Dillard R, Block SM, Boyle R, et al. Neutrophil storage pool depletion in septic, neutropenic neonates. *Pediatr Infect Dis* 1984;**3**:407-9.
- 21 Wilson C. Immunological basis for increased susceptibility of the neonate to infection. *J Pediatr* 1986;**108**:1-12.
- 22 Hill HR. Biochemical, structural, and functional abnormalities of polymorphonuclear leukocytes in the neonate. *Pediatr Res* 1987;**22**:375-82.
- 23 Falconer AE, Carr R, Edwards SW. Impaired neutrophil phagocytosis in preterm neonates: lack of correlation with expression of immunoglobulin or complement receptors. *Biol Neonate* 1995;**68**:264-9.
- 24 Al-Hadithy H, Addison IE, Goldstone AH, Cawley JC, Shaw JC. Defective neutrophil function in low-birth-weight, premature infants. *J Clin Pathol* 1981;**34**:366-70.
- 25 Jaswon MS, Jones MH, Lynch DC. The effects of recombinant human granulocyte-macrophage colony stimulating factor on the neutrophil respiratory burst in the term and preterm infant when studied in whole blood. *Pediatr Res* 1994;**36**:623-7.
- 26 Horan TD, English D, McPherson TA. Association of neutrophil chemiluminescence with microbicidal activity. *Clin Immunol Immunopathol* 1982;**22**:259-69.
- 27 Peden DB, Van Dyke K, Ardekani A, Mullett MD, Myerberg DZ, Van Dyke C. Diminished chemiluminescent responses of polymorphonuclear leukocytes in severely and moderately preterm neonates. *J Pediatr* 1987;**111**:904-6.
- 28 Driscoll MS, Thomas VL, Ramamurthy RS, Casto DT. Longitudinal evaluation of polymorphonuclear leukocyte chemiluminescence in premature infants. *J Pediatr* 1990;**116**:429-34.
- 29 Barbour AG, Allred CD, Solberg CO, Hill HR. Chemiluminescence production by granulocytes from patients with active bacterial infection. *J Infect Dis* 1980;**141**:14-26.
- 30 Babior BM. The respiratory burst of phagocytes. *J Clin Invest* 1984;**73**:599-601.
- 31 Carr R, Davies JM. Abnormal FcRIII expression by neutrophils from very preterm neonates. *Blood* 1990;**76**:607-11.
- 32 Carr R, Pumford D, Davies JM. Neutrophil chemotaxis and adhesion in preterm babies. *Arch Dis Child* 1992;**67**:813-17.
- 33 Carr R, Huizinga TWJ, Kleijer M, Davies JM. Changes in plasma FcRIII demonstrate increasing receptor production during late pregnancy and after preterm birth. *Pediatr Res* 1992;**32**:505-8.
- 34 Sacchi F, Rondini G, Mingrat G, Stronati M, Gancia GP, Marsaglia GL, Siccardi AG. Different maturation of neutrophil chemotaxis in term and preterm newborn infants. *J Pediatr* 1982;**101**:273-4.
- 35 Eisenfeld L, Krause PJ, Herson V, Savidakis J, Bannon P, Maderazo E, et al. Longitudinal study of neutrophil adherence and motility. *J Pediatr* 1990;**117**:926-9.
- 36 Cannistra SA, Griffin JD. Regulation of the production and function of granulocytes and monocytes. *Semin Hematol* 1988;**25**:173-88.
- 37 Golde DW, Gasson JC. Hormones that stimulate the growth of blood cells. *Sci Am* 1988;**259**:34-42.
- 38 Davis I, Morstyn G. Clinical uses of growth factors. *Baillière's Clin Haematol* 1992;**5**:753-86.
- 39 Dale DC, Bonilla MA, Davis MW, Nakanishi AM, Hammond WP, Kurtzberg J, et al. A randomized controlled Phase III trial of recombinant human granulocyte colony-stimulating factor (Filgrastim) for treatment of severe chronic neutropenia. *Blood* 1993;**81**:2496-502.
- 40 Moore MAS. The clinical use of colony stimulating factors. *Annu Rev Immunol* 1991;**9**:159-91.
- 41 Nemunaitis J. Granulocyte-macrophage-colony-stimulating factor: a review from preclinical development to clinical application. *Transfusion* 1993;**33**:70-83.
- 42 Sullivan GW, Carper HT, Mandell GL. The effect of three human recombinant hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and interleukin-3) on phagocyte oxidative activity. *Blood* 1993;**81**:1863-70.
- 43 Treweek AT, Aziz KA, Zuzel M. The role of G-CSF in mature neutrophil function is not related to GM-CSF-type cell priming. *J Leuk Biol* 1994;**55**:612-16.
- 44 Cannistra SA, Vellenga E, Groshek P, Rambaldi A, Griffin JD. Human granulocyte-monocyte colony-stimulating factor and interleukin 3 stimulate monocyte cytotoxicity through a tumor necrosis factor-dependent mechanism. *Blood* 1988;**71**:672-6.
- 45 Williams MA, Kelsey SM, Collins PW, Gutteridge CN, Newland AC. Administration of rHuGM-CSF activates monocyte reactive oxygen species secretion and adhesion molecule expression in vivo in patients following high-dose chemotherapy. *Br J Haematol* 1995;**90**:31-40.
- 46 Rapoport AP, Abboud CN, DiPersio JF. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF): receptor biology, signal transduction, and neutrophil activation. *Blood Rev* 1992;**6**:43-57.
- 47 Richardson MD, Brownlie CED, Shankland GS. Enhanced phagocytosis and intracellular killing of *Candida albicans* by GM-CSF-activated human neutrophils. *J Med Vet Mycol* 1992;**30**:433-41.
- 48 Roilides E, Holmes A, Blake C, Venzon D, Pizzo PA, Walsh TJ. Antifungal activity of elutriated human monocytes against *Aspergillus fumigatus* hyphae: enhancement by granulocyte-macrophage colony-stimulating factor and interferon- γ . *J Infect Dis* 1994;**170**:894-9.
- 49 Bodey GP, Anaissie E, Gutterman J, Vadhan-Raj S. Role of granulocyte-macrophage colony-stimulating factor as adjuvant therapy for fungal infection in patients with cancer. *Clin Infect Dis* 1993;**17**:705-7.

- 50 Laver J, Duncan E, Abboud M, Gasparetto C, Sahdev I, Warren D, *et al.* High levels of granulocyte and granulocyte-macrophage colony-stimulating factors in cord blood of normal full-term neonates. *J Pediatr* 1990;**116**:627-32.
- 51 Bailie KEM, Irvine AE, Bridges JM, McClure BG. Granulocyte and granulocyte-macrophage colony-stimulating factors in cord and maternal serum at delivery. *Pediatr Res* 1994;**35**:164-8.
- 52 Gessler P, Kirchmann N, Kientsch-Engel R, Haas N, Lasch P, Kachel W. Serum concentrations of granulocyte colony-stimulating factor in healthy term and preterm neonates and in those with various diseases including bacterial infections. *Blood* 1993;**82**:3177-82.
- 53 Schibler KR, Liechty KW, White WL, Christensen RD. Production of granulocyte colony-stimulating factor *in vitro* by monocytes from preterm and term neonates. *Blood* 1993;**82**:2478-84.
- 54 Cairo MS, Yu Suen, Knoppel E, Dana R, Park L, Clark S, *et al.* Decreased G-CSF and IL-3 production and gene expression from mononuclear cells of newborn infants. *Pediatr Res* 1992;**31**:574-8.
- 55 Cairo MS, Yu Suen, Knoppel E, van de Ven C, Nguyen A, Sender L. Decreased stimulated GM-CSF production and GM-CSF gene expression but normal numbers of GM-CSF receptors in human term newborns compared with adults. *Pediatr Res* 1991;**30**:362-7.
- 56 English BK, Hammond WP, Lewis DB, Brown CB, Wilson CB. Decreased granulocyte-macrophage colony-stimulating factor production by human neonatal blood mononuclear cells and T cells. *Pediatr Res* 1992;**31**:211-16.
- 57 Lee SM, Knoppel E, van de Ven C, Cairo MS. Transcriptional rates of granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, interleukin-3, and macrophage colony-stimulating factor genes in activated cord versus adult mononuclear cells: alteration in cytokine expression may be secondary to posttranscriptional instability. *Pediatr Res* 1993;**34**:560-4.
- 58 Cairo MS. Therapeutic implications of dysregulated colony-stimulating factor expression in neonates. *Blood* 1993;**82**:2269-72.
- 59 Liechty KW, Schibler KR, Ohls RK, Perkins SL, Christensen RD. The failure of newborn mice infected with *Escherichia coli* to accelerate neutrophil production correlates with their failure to increase transcripts for granulocyte colony-stimulating factor and Interleukin-6. *Biol Neonate* 1993;**64**:331-40.
- 60 Cairo MS, van de Ven C, Toy C, Mauss D, Sender L. Recombinant human granulocyte-macrophage colony-stimulating factor primes neonatal granulocytes for enhanced oxidative metabolism and chemotaxis. *Pediatr Res* 1989;**26**:395-9.
- 61 Frenck RW Jr, Buescher ES, Vadhan-Raj S. The effects of recombinant human granulocyte-macrophage colony stimulating factor on *in vitro* cord blood granulocyte function. *Pediatr Res* 1989;**26**:43-8.
- 62 Bedford Russell AR, Davies EG, Gibson FM, Gordon-Smith EC. The *in vitro* effects of granulocyte and granulocyte-macrophage colony-stimulating factor on interleukin-3-dependent proliferation of human neonatal circulating progenitor cells. *Pediatr Res* 1995;**37**:630-3.
- 63 Westwood NB, Chung R, Emmerson AJB, Pearson TC. The *in vitro* effects of stem cell factor, interleukin 3 and granulocyte-macrophage colony stimulating factor on haemopoietic progenitor cells from premature infants. *Br J Haematol* 1994;**86**:468-74.
- 64 Cairo MS, van de Ven C, Mauss D, Kommareddy S, Norris K, Sheikh K, Modanlou H. Modulation of neonatal rat myeloid kinetics resulting in peripheral neutrophilia by single pulse administration of Rh granulocyte-macrophage colony-stimulating factor and Rh granulocyte colony-stimulating factor. *Biol Neonate* 1991;**59**:13-21.
- 65 Cairo MS, Plunkett JM, Mauss D, van de Ven C. Seven-day administration of recombinant human granulocyte colony-stimulating factor to newborn rats: modulation of neonatal neutrophilia, myelopoiesis, and group B Streptococcus sepsis. *Blood* 1990;**76**:1788-94.
- 66 Cairo MS, Mauss D, Kommareddy S, Norris K, van de Ven C, Modanlou H. Prophylactic or simultaneous administration of recombinant human granulocyte colony stimulating factor in the treatment of group B streptococcal sepsis in neonatal rats. *Pediatr Res* 1990;**27**:612-16.
- 67 Cairo MS, Mauss D, Plunkett JM, Gillis S, van de Ven C. Modulation of neonatal myelopoiesis in newborn rats after 7 days' administration of either granulocyte-monocyte colony stimulating factor or interleukin-3. *Pediatr Res* 1991;**29**:504-9.
- 68 Frenck RW, Sarman G, Harper TE, Buescher ES. The ability of recombinant murine granulocyte-macrophage colony-stimulating factor to protect neonatal rats from septic death due to staphylococcus aureus. *J Infect Dis* 1990;**162**:109-14.
- 69 Wheeler JG, Givner LB. Therapeutic use of recombinant human granulocyte-macrophage colony-stimulating factor in neonatal rats with type III group B streptococcal sepsis. *J Infect Dis* 1992;**165**:938-41.
- 70 Addison IE, Johnson B, Devereux S, Goldstone AH, Linch DC. Granulocyte-macrophage colony-stimulating factor may inhibit neutrophil migration *in vivo*. *Clin Exp Immunol* 1989;**76**:149-53.
- 71 Toner GC, Jakubowski AA, Crown JPL, Meisenberg B, Sheridan C, Gabrilove JL. Colony-stimulating factors and neutrophil migration. *Ann Intern Med* 1989;**110**:846-7.
- 72 Roberts RL, Szalc CM, Scates SM, Boyd MT, Soderstrom KM, Davis MW, *et al.* Neutropenia in an extremely premature infant treated with recombinant human granulocyte colony-stimulating factor. *Am J Dis Child* 1991;**145**:808-12.
- 73 Gillan ER, Christensen RD, Yu Suen, Ellis R, van de Ven C, Cairo MS. A randomized, placebo-controlled trial of recombinant human granulocyte colony-stimulating factor administration in newborn infants with presumed sepsis: significant induction of peripheral and bone marrow neutrophilia. *Blood* 1994;**84**:1427-33.
- 74 Cairo MS, Christensen R, Sender LS, Ellis R, Rosenthal J, van de Ven C, *et al.* Results of a phase I/II trial of recombinant human granulocyte-macrophage colony-stimulating factor in very low birthweight neonates: significant induction of circulatory neutrophils, monocytes, platelets, and bone marrow neutrophils. *Blood* 1995;**86**:2509-15.
- 75 Bedford Russell AR, Davies EG, Ball SE, Gordon-Smith E. Granulocyte colony stimulating factor treatment for neonatal neutropenia. *Arch Dis Child* 1995;**72**:F53-F4.
- 76 Miller LL, American Society of Clinical Oncology Recommendations for the use of hematopoietic colony-stimulating factors: Evidence-based clinical practice guidelines. *J Clin Oncol* 1994;**12**:2471-508.
- 77 Lieschke GJ, Cebon J, Morstyn G. Characterisation of the clinical effects after the first dose of bacterially synthesised recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1989;**74**:2634-43.
- 78 Lieschke GJ, Maher D, O'Connor M, Green M, Sheridan W, Rallings M, *et al.* Phase I study of intravenously administered bacterially synthesized granulocyte-macrophage colony-stimulating factor and comparison with subcutaneous administration. *Cancer Res* 1990;**50**:606-14.
- 79 Schmitz N, Dreger P, Suttrop M, Rohwedder EB, Haeflrich T, Löffler H, *et al.* Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). *Blood* 1995;**85**:1666-72.
- 80 Halpérin DS, Wacker P, Lacourt G, Félix M, Babel J-F, Aapro M, *et al.* Effects of recombinant human erythropoietin in infants with the anemia of prematurity: A pilot study. *J Pediatr* 1990;**116**:779-86.
- 81 Ohls RK, Christensen RD. Recombinant erythropoietin compared with erythrocyte transfusion in treatment of the anaemia of prematurity. *J Pediatr* 1991;**119**:781-8.
- 82 Christensen RD, Liechty KW, Koenig JM, Schibler KR, Ohls RK. Administration of erythropoietin to newborn rats results in diminished neutrophil production. *Blood* 1991;**78**:1241-6.
- 83 Emmerson AJB, Coles HJ, Stern CMM, Pearson TC. Double blind trial of recombinant human erythropoietin in preterm infants. *Arch Dis Child* 1993;**68**:291-6.
- 84 Shannon KM, Mentzner WC, Abels RI, Freeman P, Newton N, Thompson D, *et al.* Recombinant human erythropoietin in the anaemia of prematurity: Results of a placebo controlled pilot study. *J Pediatr* 1991;**118**:949-55.
- 85 Aglietta M, Monzeglio C, Sanavio F, Aprà F, Morelli S, Stacchini A, *et al.* *In vivo* effect of human granulocyte-macrophage colony-stimulating factor on megakaryopoiesis. *Blood* 1991;**77**:1191-4.
- 86 Bonilla MA, Dale D, Zeidler C, Last L, Reiter A, Ruggiero M, *et al.* Long-term safety of treatment with recombinant human granulocyte colony-stimulating factor (r-metHuG-CSF) in patients with severe congenital neutropenias. *Br J Haematol* 1994;**88**:723-30.
- 87 Fan Dong, Brynes RK, Tidow N, Welte K, Löwenberg B, Touw IP. Mutations in the gene for the granulocyte colony-stimulating-factor receptor in patients with acute myeloid leukemia preceded by severe congenital neutropenia. *N Engl J Med* 1995;**333**:487-93.
- 88 Naparstek E. Granulocyte colony-stimulating factor, congenital neutropenia, and acute myeloid leukemia. *N Engl J Med* 1995;**333**:516-18.
- 89 Rosenthal J, Healey T, Ellis R, Gillan E, Cairo MS. A two-year follow-up of neonates with presumed sepsis treated with recombinant human granulocyte colony-stimulating factor during the first week of life. *J Pediatr* 1996;**128**:135-7.