Observations on the haemopoietic response to critical illness

R J Amos, M Deane, C Ferguson, G Jeffries, C J Hinds, J A L Amess

Abstract

Peripheral blood cytopenias are common in patients receiving intensive care, particularly in those with multiple organ failure. To assess the contribution of bone marrow hypoplasia in such patients 44 bone marrow samples from 24 patients under intensive care were studied by standard morphological techniques and by the granulocyte-macrophage colony forming cell (GM-CFC) assay. Frequently observed morphological abnormalities in the bone marrow included the following: (i) a reduction in overall cellularity in seven patients, with a progressive decrease in most patients studied sequentially; (ii) an increase in the number of actively phagocytic macrophages; and (iii) a disruption of normal bone marrow architecture with the accumulation of intercellular hyaluronic acid glycosaminoglycan. Mean GM-CFC growth was significantly reduced when compared with that in a group of normal controls. In four of five patients studied sequentially GM-CFC growth became subnormal in association with a reduction in bone marrow cellularity. Inhibitory serum factors were not identified. These morphological abnormalities are similar to the changes observed in gelatinous degeneration of the bone marrow.

In both situations disruption of the haemopoietic microenvironment, with the accumulation of hyaluronic acid proteoglycan, may be an important factor in the inhibition of haemopoietic progenitor cell growth. The proliferation of macrophages, by the release of a variety of cytokines or reactive oxygen intermediates, may also be implicated in impaired haemopoiesis and the development of disordered erythropoiesis.

Persistent or recurrent sepsis, often associated with multiple organ failure, is a common clinical finding in patients receiving intensive care.¹ The haematological response in this situation often seems to be inadequate; anaemia is almost universal and variable degrees of thrombocytopenia are common. The neutrophil count may be inappropriately low and overt neutropenia may supervene. It is clearly very difficult in such complex situations to define causative factors; several

elements may be involved in the development of anaemia, including blood loss, the "anaemia of chronic disorder,"^{2,3} and haemolysis.⁴ The presence of thrombocytopenia and neutropenia is often explained on the basis of an increase in the peripheral consumption of mature cells due, for example, to infection or disseminated intravascular coagulation. From previous observations, however, it is our impression that bone marrow hypoplasia and the subsequent failure of blood cell production is an important additional factor in the development of these cytopenias.

Of particular interest is the possible association between multiple organ failure and impaired haemopoiesis. The syndrome of sequential/progressive multiple organ failure came to prominence in the 1970s when it became possible to resuscitate and support patients with previously lethal critical illness.⁵⁻⁸ No generally agreed definition of this clinical syndrome is available but it is characterised by a hypermetabolic state, usually in association with infection, together with failure of the lungs, kidneys, heart and circulation, liver and gastrointestinal tract.⁸ Considerable interest has recently been focused on a group of peptide mediators derived from activated monocytes and lymphocytes, such as interleukin 1 (IL-1), interleukin 2 (IL-2), and tumour necrosis factor (TNF), which may be implicated in the pathogenesis of multiple organ failure.⁸⁻¹⁰ It has been suggested that continued, uncontrolled production of these mediators of acute inflammation, in response, for example, to overwhelming infection or tissue necrosis, may be one of the mechanisms underlying damage to vital organs.⁸ These peptides also have a wide range of effects on the haemopoietic system, including the mediation of acute changes in iron metabolism,² direct effects on haemopoietic progenitor cell proliferation,¹¹⁻¹³ regulation of haemopoietic colony stimulating factor gene expression,¹⁴⁻¹⁷ and modulation of the response of haemopoietic progenitor cells to colony stimulating factors.¹⁸

Methods

Observations were made on 24 patients (18 men and six women, mean age 52 years, range 18-78 years) admitted to the intensive care unit (ICU) at this hospital. Seventeen died, giving a mortality of 64%. Brief clinical details are provided in table 1. Patients with primary haematological disorders and those

Department of
Haematology,
St Bartholomew's
Hospital,
West Smithfield,
London EC1
R J Amos
M Deane
J A L Amess
Intensive Care Unit
C Ferguson
G Jeffries
C J Hinds

Correspondence to:
Dr R J Amos.

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Table 1 Brief clinical details and peripheral blood data of 24 patients studied

Case No	Clinical details	Exposure to nitrous oxide	Outcome	Mean (range) peripheral blood findings		
				Haemoglobin (g/dl)	Neutrophils ($\times 10^9/l$)	Platelets ($\times 10^9/l$)
1 M/58	Cardiac arrest	No	D	14.6 (13.4–16.2)	14.0 (9.3–16.0)	169 (121–296)
2 M/71	Aortic aneurysm repair	Yes	D	9.6 (8.6–10.9)	14.3 (5.2–19.7)	250 (130–444)
3 F/70	CAVBG/AVR	Yes	D	10.7 (6.5–13.2)	11.6 (3.1–29.0)	57 (16–128)
4 M/19	Multiple injuries	Yes	A	12.1 (10.5–13.9)	13.0 (5.5–25.7)	181 (103–380)
5 F/22	Head injury/IC surgery	Yes	A	10.1 (6.8–12.3)	11.2 (8.6–15.7)	244 (107–446)
6 M/32	Septicaemia/debridement	Yes	A	10.5 (8.3–11.4)	10.9 (5.6–11.4)	242 (17–467)
7 M/56	CAVBG	Yes	D	12.1 (10.0–18.0)	14.6 (7.6–28.3)	66 (11–210)
8 M/29	Proctocolectomy	Yes	A	11.3 (9.0–16.6)	11.7 (5.9–45.5)	393 (239–529)
9 M/50	Head injury/subdural	Yes	A	10.6 (9.4–11.2)	8.0 (5.6–11.6)	266 (108–544)
10 M/23	Stab wound	Yes	A	10.5 (8.3–12.6)	14.3 (8.0–22.0)	337 (106–545)
11 M/68	Subdural haematoma	Yes	D	10.7 (8.5–15.6)	6.7 (6.3–9.6)	174 (124–249)
12 M/34	Pancreatitis	No	D	12.1 (8.3–15.2)	14.6 (3.4–26.2)	111 (47–242)
13 M/70	Aortic aneurysm	Yes	D	11.3 (9.7–12.5)	11.7 (5.1–14.4)	46 (16–95)
14 F/39	Abdominal sepsis	No	A	10.0 (9.8–10.2)	15.0 (13.5–16.5)	556 (498–614)
15 F/67	Laparotomy/septicaemia	Yes	D	9.9 (7.7–11.7)	6.4 (2.0–12.0)	142 (67–247)
16 M/62	Septicaemia/tracheostomy	Yes	D	9.2 (7.0–14.9)	8.2 (5.2–12.2)	156 (57–167)
17 M/73	Aortic aneurysm	Yes	D	10.2 (9.6–10.8)	12.4 (8.5–15.2)	105 (72–261)
18 M/61	Aortic aneurysm	Yes	D	11.6 (8.4–16.0)	15.8 (3.0–30.0)	79 (16–168)
19 F/52	Septicaemia	No	D	11.6 (7.8–14.5)	11.4 (2.9–34.0)	121 (16–221)
20 F/77	MVR/AVR	Yes	D	9.7 (7.8–12.4)	17.6 (10.0–28.8)	133 (21–228)
21 M/63	Laparotomy/pneumonia	Yes	D	10.2 (7.4–11.6)	11.1 (5.4–21.6)	141 (57–306)
22 M/60	Pancreatitis	No	D	10.2 (9.5–11.7)	15.6 (10.6–18.5)	127 (54–266)
23 M/31	Cardiac arrest	No	D	10.2 (7.6–13.4)	13.0 (8.1–26.7)	451 (221–610)
24 M/74	Bowel perforation	Yes	D	11.3 (8.8–13.0)	8.5 (3.0–11.6)	88 (15–273)

D—Died on the ICU.

A—Discharged alive from the ICU.

CAVBG—Coronary artery vein bypass graft.

AVR—Aortic valve replacement.

MVR—Mitral valve replacement.

Table 2 Bone marrow findings in 24 patients studied

Case No	Cellularity	Erythropoiesis	Macrophages	Intercellular material	Fat atrophy	GM-CFC/ 10 ⁵ cells
1.1	NC	NC MB +	+			—
1.2	NC	NC MB	+			—
1.3	NC	NC MB	++	+		146
1.4	NC	NC MB	++	++		30
1.5	NC	NC MB	++	++	++	48
2.1	NC	RED MB +	+	+		—
2.2	NC	NC	++	++	+	—
2.3	NC/RED	NC	++			—
2.4	RED	NC	++	++	+	—
3.1	NC/RED	NC	+	+		70
3.2	NC/RED	NC	++			41
4.1	NC	NC MB	+			16
4.2	RED	RED	++	+	+	120
5.1	RED	RED MB	+	+	+	—
5.2	RED	RED	+	++	+	—
6.1	NC	NC MB	+	+	+	—
6.2	NC/RED	NC MB	+	++	++	—
6.3	NC/RED	NC MB	++	++	++	86
7.1	NC/RED	RED	++	+		—
7.2	RED	RED	+++	+	+	—
8	NC	NC	+			4
9.1	NC	NC MB	+	+		—
9.2	NC	NC MB	++	++		97
10.1	NC	NC	+	+		—
10.2	NC	NC	++	+		—
10.3	NC	NC	+++	+	++	—
11.1	RED	NC MB	+	+		83
11.2	RED	RED	++++	+		26
12	NC	RED	+			—
13	RED	NC MB	++	+		25
14	NC	RED	+			66
15	NC	RED	+			30
16	NC	RED MB	+++			74
17	NC	RED	++			35
18	NC	RED	++			34
19	NC	RED	++			18
20	RED	RED	+			41
21.1	NC	RED				—
21.2	NC	NC	++			—
22	NC	NC	+++			—
23.1	NC	RED	++	+		—
23.2	NC	RED	++	+		90
24.1	NC	RED	++			223
24.2	NC	RED MB	+++	+		22

Trephine biopsies were performed in patients 1–11. In patients studied on more than one occasion the bone marrows are numbered consecutively. Semiquantitative estimates (indicated by +) are provided for dyserythropoiesis (under erythropoiesis), macrophage numbers, intercellular material and fat atrophy.

NC—Normocellular.

RED—Reduced cellularity.

NC/RED—Areas of both normal and reduced cellularity.

MB—Megaloblastic.

Figure 1 Bone marrow trephine biopsy specimen (case 7.2) showing early morphological changes with areas of focal hypoplasia, loss of haemopoietic cells, and the accumulation of intercellular material (haematoxylin and eosin).

who had received cytotoxic chemotherapy or radiotherapy were excluded. Eighteen patients were admitted within 24 hours of surgery and anaesthesia with nitrous oxide. None of the patients was exposed to nitrous oxide after admission to the Unit during the period of study. All patients received parenteral folate supplementation during admission (1 mg/day). They remained in the ICU for a mean of 20 days (range seven to 49 days).

The study was approved by the local ethical committee. Whenever possible, informed consent for participation in the study was obtained from the patients; otherwise it was obtained from their next of kin.

BONE MARROW MORPHOLOGY

Forty four bone marrow samples were obtained from the 24 patients in the study; 28 samples from 11 patients included a bone marrow trephine biopsy specimen. Eleven patients were studied on only one occasion, on average nine days after admission (range one to 30 days); 13 patients were studied sequentially, on two or more occasions, every three days for periods of up to 14 days.

All samples were processed using standard techniques. Aspirate smears were fixed in methanol and stained by May-Gruñwald-Giemsa and Perls's reaction for iron. Trephine biopsy specimens were decalcified,

Figure 2 Central area of fig 1, enlarged, showing disruption and loss of normal fat spaces.

Figure 3 Bone marrow trephine biopsy specimen (case 2.4) showing an extensive area of marrow hypoplasia. Hyaluronic acid glycosaminoglycan is present in the spaces between the fat cells (haematoxylin and eosin).

paraffin wax embedded, sectioned and stained with haematoxylin and eosin and by silver impregnation for reticulin. Selected trephine biopsy specimens were studied by additional histochemical techniques including periodic acid Schiff (PAS) (neutral polysaccharides), PAS with diastase (glycogen), alcian blue at pH 2.5 +/– hyaluronidase (hyaluronic acid) and alcian blue at pH 1.0 (sulphated mucopolysaccharides). Bone marrow slides were reviewed independently by three observers (RJA, JALA, and MD) and the findings collated.

A blood count, including examination of a peripheral blood film, was performed on a

daily basis. Serum vitamin B₁₂ and serum and red cell folate were estimated by microbiological assay before starting treatment with prophylactic folic acid. Other investigations were performed as clinically indicated.

BONE MARROW CULTURE STUDIES

Bone marrow culture studies were performed on 17 patients; in 12 patients on one occasion and in five patients sequentially.

Myeloid progenitor cells were assayed in the granulocyte-macrophage colony forming cell assay (GM-CFC).¹⁹ Briefly, bone marrow cells were collected into 10 ml Hanks's balan-

Figure 4 Bone marrow aspirate (case 11.2) showing increased numbers of macrophages with foamy cytoplasm; some contain necrotic cellular debris (May-Grünwald Giemsa).

Figure 5 GM-CFC growth in 31 haematologically normal subjects and in 17 patients from the ICU.

ced salt solution (HBSS) (Flow Laboratories) containing 50 units of preservative free heparin. A mononuclear cell enriched fraction was prepared by density gradient centrifugation over Histopaque (density 1.077; Sigma) and 10^5 mononuclear cells were cultured in 2 ml 0.3% agar in Alpha medium (Gibco) containing 10% fetal calf serum, 10% human AB serum, and 5% placental conditioned medium²⁰ as a source of colony stimulating activity. Cultures were incubated at 37°C in a fully humidified atmosphere of 5% carbon dioxide in air and colonies counted on day 10. All assays were performed in duplicate.

Using the method described, bone marrow cultures from 31 haematologically normal subjects gave a mean GM-CFC growth of 108 colonies/ 10^5 mononuclear cells (range 40–215; SD 44). Results were compared using Student's *t* test.

Results

PERIPHERAL BLOOD HAEMATOLOGY

Peripheral blood findings are summarised in table 1. Anaemia was almost universal. A number of red cell abnormalities were commonly observed, including rouleaux formation, target cells, burr cells and irregularly contracted cells, together with occasional red cell fragments and spherocytes. When tested, the direct antiglobulin test was uniformly negative.

Overt neutropenia (neutrophils $<2.5 \times 10^9/l$) only developed in one patient, but in six patients the neutrophil count was $<3.5 \times 10^9/l$ in the context of severe infection. Characteristically, peripheral blood neutrophils showed toxic granulation and cytoplasmic vacuolation. Immature granulocytes were often present.

Thrombocytopenia (platelets $<140 \times 10^9/l$) developed in 21 (83%) patients usually in association with massive blood transfusion or documented disseminated intravascular coagulation.

BONE MARROW MORPHOLOGY

Bone marrow morphological findings are summarised in table 2 and shown in figs 1–4.

Cellularity

A hypocellular bone marrow was found in seven (29%) patients at some point during admission to the ICU. Of the 13 patients whose bone marrow was studied sequentially, a progressive reduction in bone marrow cellularity was noted in 10 (77%). Trephine biopsy specimens showed both a diffuse reduction in cellularity and focal, intertrabecular areas of hypoplasia, usually in association with stromal changes and a disruption of normal bone marrow architecture.

Erythropoiesis

Although megakaryocyte numbers and granulopoiesis seemed to be normal in most patients, erythropoiesis was reduced in 16 (66%) patients at some point during admission to the ICU. Dyserythropoiesis, such as binu-

cleate erythroblasts, abnormal nuclear configurations, cytoplasmic vacuolation and cytoplasmic bridging was also observed in 17 (71%) patients. Megaloblastic change was noted in 10 (42%) patients.

Megaloblastic erythropoiesis was observed during the first week of admission in seven patients (cases 1, 2, 4–6, 11, and 13), six of whom had been admitted following anaesthesia with nitrous oxide. In the six patients in whom follow up studies were available erythropoiesis became normoblastic in four (cases 2, 4, 5, and 11) and in these, the original megaloblastic change is likely to have been related to the inactivation of vitamin B₁₂ by nitrous oxide.²¹ In two patients, however (cases 1 and 6), megaloblastic change persisted for periods of up to 18 days and, in addition, a further three patients were identified (cases 9, 16, and 24) in whom megaloblastic change developed seven to 20 days after admission. These five patients with unexplained megaloblastic change all had normal pre-treatment serum vitamin B₁₂, serum and red cell folate concentrations, and they had all received prophylactic folate supplements.

Abnormal cells

A moderate increase in the number of mature plasma cells (up to 10%) was noted in 13 (54%) patients. An almost universal observation in 22 (92%) patients was a variable and sometimes striking increase in the number of foamy, actively phagocytic macrophages. Many of the macrophages contained phagocytosed red cells, granulocytes or platelets. They occurred singly or in clumps and in the latter case were sometimes associated with fibrillary, pink-staining material. There was a progressive increase in the number of macrophages in 11 of the 13 (85%) patients studied sequentially.

Stromal changes

Stromal changes were most clearly appreciated in the 11 patients in whom trephine biopsy specimens were obtained. Typically, there was a localised disruption of normal bone marrow architecture, usually associated with the accumulation of intercellular ground substance together with varying degrees of fat atrophy and loss of haemopoietic cells. In some patients bone marrow necrosis (one out of 11) and focal, fibrinous exudates (six out of 11) were also identified. A mild, diffuse increase in reticulin was noted in five of 11 patients.

A focal increase in pink-staining ground substance was seen in 10 of 11 patients and there was a progressive increase in the seven patients with follow up trephine biopsies. The material was associated with varying degrees of fat atrophy and loss of haemopoietic cells. In marrow aspirates it was identified in 13 (54%) patients as pink-staining, fibrillary material associated with bone marrow fragments or clumps of macrophages. The material stained weakly with PAS; pre-digestion with diastase had no effect. It also stained with alcian blue, much more strongly at pH 2.5 than at pH 1.0; staining was prevented by pretreatment with

Figure 6 GM-CFC growth in five patients studied sequentially after admission to the ICU. The broken line indicates the lower limit of colony growth in 31 haematologically normal subjects.
 Δ case 1 cardiac arrest, bronchopneumonia; died
 ● case 3 coronary artery vein bypass graft, aortic valve replacement; died
 ○ case 4 multiple injuries; survived
 □ case 11 subdural haematoma; died
 ■ case 24 bowel perforation; died

hyaluronidase. Negative results were obtained with Congo red and silver impregnation. The histochemical reactions suggest that the extra-cellular material consists of acidic mucopolysaccharides, predominantly hyaluronic acid—that is, hyaluronic acid glycosaminoglycan, one of the proteoglycans widely distributed in the extra-cellular environment.²²

BONE MARROW CULTURE

Bone marrow was cultured from 17 patients. The mean GM-CFC growth was 67 colonies/ 10^5 mononuclear cells (range 4–222). This compares with results from a group of normal subjects where mean GM-CFC growth was 108 colonies/ 10^5 mononuclear cells (range 40–215) (fig 5). The difference between the two groups was significant ($p < 0.01$). In six (38%) patients colony growth was less than the range found in normal subjects.

In five patients GM-CFC assays were performed on more than one occasion. In four of five patients GM-CFC growth decreased and became subnormal during admission to the ICU; in each case this was associated morphologically with a reduction in bone marrow cellularity (fig 6). All four patients died.

The addition of autologous serum to cultures of patients' bone marrow produced no inhibition of colony growth, and similarly, the addition of patients' serum to cultures of bone marrow from normal subjects did not inhibit colony growth. There was therefore no evidence of any serum factors inhibitory to haemopoietic progenitor cell growth.

Discussion

We have described a constellation of morphological abnormalities in the bone marrow of a group of patients with critical illness. In essence they consist of: (1) hypoplasia, which was also reflected by a reduction in haemopoietic progenitor cell growth *in vitro*; (2) disruption of normal bone marrow architecture, with the accumulation of abnormal inter-cellular material, identified as hyaluronic acid glycosaminoglycan, variable degrees of fat atrophy, fibrinous exudates and bone marrow necrosis; and (3) proliferation of actively phagocytic macrophages. A reduction in erythropoiesis was also almost universal and was often associated with dyserythropoietic and megaloblastic change, the latter apparently unrelated to vitamin B₁₂ or folate deficiency. In a proportion of patients, particularly in those who developed multiple organ failure and who subsequently died, these changes seemed to have been progressive.

There was no clear association between the presence or severity of these changes and peripheral blood variables, an observation which is perhaps not surprising in view of the complex, interacting factors that determine the peripheral blood count in such patients. These morphological abnormalities, however, do probably limit the capacity of the bone marrow to respond appropriately. Indeed, we have direct evidence *in vitro* of impaired haemopoietic progenitor cell proliferation.

There are some similarities between the abnormalities we have noted and the changes seen in gelatinous degeneration of the bone marrow.^{23,24} In this condition bone marrow hypoplasia, fat atrophy, and the accumulation of acidic mucopolysaccharide ground substance, identified as predominantly hyaluronic acid,^{24,25} develop in patients chronically malnourished for prolonged periods of time. It has been observed in tuberculosis and other long standing infections,²³ disseminated malignancy,^{23,24} anorexia nervosa^{25–30} and in one patient maintained on chronic intravenous nutrition.³¹ In the latter two situations gelatinous degeneration has been associated with peripheral blood cytopenias or pancytopenia. Interestingly, in some patients refeeding and clinical improvement have been associated with resolution of the bone marrow abnormalities or the peripheral blood cytopenias.^{27,30} It has been emphasised that gelatinous degeneration is a feature of conditions characterised by energy or carbohydrate deprivation and is not a feature of protein malnutrition.²⁶ These considerations may have some relevance to the abnormalities noted in patients receiving intensive care, but a striking dissimilarity is the rapidity with which the changes we have described develop and progress.

Whatever the precise association is between the abnormalities in ICU patients and in gelatinous degeneration it is clear that in both situations major disruption of the haemopoietic microenvironment occurs. This is reflected particularly by changes in the extra-cellular matrix, which may be directly implicated in the development of impaired haemopoiesis. There is now considerable evidence that the bone marrow microenvironment has an important role in haemopoietic cell regulation and differentiation.^{32,33} Of particular importance are the proteoglycans of the extra-cellular matrix^{34,35} which may regulate haemopoietic activity by influencing the diffusion of large macromolecules, including haemopoietic growth factors,²² by direct binding of haemopoietic growth factors^{36,37} and by effects on cell adhesion and detachment.³⁸ In rabbit bone marrow hyaluronic acid is only a minor component (16%) of the proteoglycans in the extra-cellular matrix.³⁹ An increase in the quantity of hyaluronic acid, because of its large molecular domain, may contribute to the inhibition of haemopoietic activity by restricting the diffusion of haemopoietic growth factors²² or by preventing the adhesion of haemopoietic progenitor cells to stromal components.⁴⁰

The accumulation of actively phagocytic macrophages was a further striking abnormality in many of the patients studied. Macrophages have emerged as a key element in current concepts about the pathogenesis of multiple organ failure,^{41,42} and their presence in the bone marrow may have important implications for haemopoiesis. Macrophages produce a wide variety of secretory products,⁴³ including the cytokines IL-1, IL-2, and TNF, together with reactive oxygen intermediates. TNF, in addition to its possible role in the

pathogenesis of multiple organ failure,⁴² also inhibits haemopoietic progenitor cell growth in vitro^{44,45} and may contribute to the anaemia of malaria by promoting dyserythropoiesis and erythrophagocytosis,⁴⁶ both of which were features commonly observed in this study. Reactive oxygen intermediates also inhibit haemopoietic progenitor cell growth in vitro, both in short⁴⁷ and long term⁴⁸ bone marrow culture systems, and may therefore be a further factor implicated in the development of impaired haemopoiesis. Finally, it is of interest that oxygen radicals, released by stimulated human macrophages, have been shown directly to damage DNA in cultured mammalian cells.⁴⁹ Furthermore, megaloblastic change is a feature of erythroid colonies grown in vitro in the presence of monocytes.⁵⁰ It may be, therefore, that the megaloblastic change noted in some of our patients is also related to macrophage proliferation and DNA damage by released reactive oxygen intermediates.

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