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The problem of thrombocytopenia and its management

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The management of treatment-induced thrombocytopenia has become an important health care issue as a result of using intensive chemotherapy in an increasing range of malignant diseases. The problems of using pooled blood products is putting a strain on resources and producing its own medical complications. The advent of cloned cytokines has opened the era of manipulation of the megakaryocyte line and it is hoped that this will mimic the benefits seen in respect of white cell and red cell cytokine control. However, many questions remain to be answered as the complexity of the cytokine targets and interactions are being revealed by current clinical research. A thrombocyte stimulation/maturation product equivalent to granulocyte-colony stimulating factor or erythropoietin has only recently been identified and awaits clinical evaluation.

Introduction

Adequate treatment for malignancy is associated with varying degrees of bone marrow suppression and an increased risk of infection and haemorrhage secondary to neutropenia and thrombocytopenia. Haematological malignancies, in particular the leukaemias, are associated with significant bone marrow involvement at presentation, which further impairs marrow function, so that in association with chemotherapy, severe thrombocytopenia and neutropenia occur.

Prior to the development of adequate support therapy haemorrhage was the immediate cause of death in almost two thirds of patients with acute leukaemia[1] and the therapeutic advances made over the last two decades using high-dose combination chemotherapy would not have been possible without the parallel development of transfusion medicine in supporting patients through periods of marrow hypoplasia.

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In solid tumours, bone marrow involvement is less apparent at presentation than in the haematological malignancies[2,3] and the bone marrow is therefore less compromised by the initial treatment. Problems relating to bone marrow failure are thus less evident at presentation and during conventional chemotherapy. However, micro-metastatic disease is known to occur and does affect long-term survival,[4] so there is increasing interest in escalating the dose intensity of cytostatic drugs and in the use of combination chemotherapy.

Unfortunately, the effect of such combinations is to enhance myelotoxicity,[5] with the serious consequence of significant neutropenia and infection and risk of haemorrhage, and this may lead to dose reduction or delay leading to decrease in chemotherapy dose intensity and an adverse effect on treatment outcome.

The ability to shorten the duration and depth of disease-related or chemotherapy-induced neutropenia and thrombocytopenia may reduce the incidence and severity of infections and bleeding complications. It may also lead to enhanced ability to deliver drug regimens as planned without the need for dose modification as a result of myelotoxicity. It is in this context that the use of haematopoietic growth factors may prove beneficial in both reducing the toxicity of and enhancing the response to chemotherapy.

Haemorrhage and the use of platelet support

Prior to the development of adequate transfusion therapy, haemorrhage was the immediate cause of death in almost two thirds of patients with acute leukaemia.[1] Although the development of supportive transfusion strategies has improved over the last three decades, total haemorrhagic mortality in chemotherapy-treated patients with acute leukaemia still varies between 7% and 23%.[6,7] Causes of

this complication are multifactorial, involving the coagulation system, vessel wall integrity and platelet function. However, thrombocytopenia is the most important factor since the frequency of haemorrhage increases directly with a fall in platelet count,[8] with a substantial increase in haemorrhagic episodes at counts below 20×10^9/l.

With the development of aggressive chemotherapeutic regimes for malignancy, the common practice of transfusing platelets when the count is below this level has increased demand enormously, exposing patients to large numbers of donors and increased risks of allo-immunisation.[9] This has been seen particularly in the acute leukaemias, where remission rates have improved by the intensification of induction chemotherapy that has been achieved because of more effective supporting care.[10,11]

The value of regular prophylactic platelet transfusions has been studied in an autopsy series and in randomised trials and their efficiency in reducing serious haemorrhage by maintaining a higher platelet count [12–16] has been proven, with a reduction in fatal haemorrhage in acute leukaemia trials currently from those reported before widespread platelet use.[17]

Despite this, the level at which platelet transfusion should be instigated remains uncertain and the influence of allo-immunisation on effective platelet replacement and its development, following intensive exposure, is poorly understood.

Recent studies still indicate that the incidence of severe haemorrhage is 16% in younger adults receiving treatment for acute leukaemia but may be over 30% in the elderly.[18] The data suggest that the incidence of haemorrhage, although diminished, remains an important complication of remission induction therapy for acute leukaemia and following severe thrombocytopenia in other cancers. The failure of adequate supportive care with patients dying of a combination of haemorrhage and infection may be seen in between 20% and 30% in any leukaemia population undergoing intensive induction therapy.[19–22]

Despite this failure rate recent studies have shown that the minimal platelet count of 20×10^9/l which has been accepted for prophylactic platelet transfusion following curative chemotherapy is high, and the NIH Consensus Development Conference on 'Platelet Transfusion Therapy' in 1986 [23] recommended that levels less than 20×10^9/l might be safely used in some patients. The adoption of more restrictive platelet transfusion strategies has been in part for economic reasons, but also to reduce platelet allo-immunisation with subsequent reduced efficacy.[24,25] The use of less intensive replacement regimes has shown that maintenance of lower platelet counts has not been accompanied by severe haemorrhage and also has the advantage of reducing the number of donors to whom a patient is exposed.[26,27]

Clinical studies have demonstrated that the use of leucocyte-poor red blood cell and platelet concentrates, prepared by filtration, significantly reduces the incidence of HLA allo-immunisation with refractoriness to random platelet transfusions.[28–36]

The percentage who still develop allo-immunisation may be due to the low level of leucocyte contamination ($< 10–50 \times 10^6$) found in leucocyte-depleted platelet concentrate, or may be due to the fact that a percentage of female patients may already be allo-immunised due to previous pregnancies.[33] Although filtration of all blood products to patients receiving intensive chemotherapy will reduce the incidence of allo-immunisation and also lower the risk of transfusion-transmitted diseases such as cytomegalovirus and hepatitis C, there are considerable economic implications to filtering all products.

Following allo-immunisation, transfusion of HLA-matched platelets may lead to improved platelet survival [34–36] but there remains a percentage of patients with refractory thrombocytopenia despite all therapeutic endeavours. This may be seen following transfusion of up to 40% of all HLA-matched platelet units, which will fail to provide adequate increments in these refractory patients.[37]

Non-immune factors are being recognised as important in an increasing number of patients.[38] These include fever, infection, drugs that may interfere with platelet function (including antibiotics), the presence of coagulation disorders, heparin therapy and anatomical lesions that lead to increased bleeding potential.

In summary it can be seen that haemorrhage has always been a major association of haematological malignancy and intensive chemotherapy and that although this can be mitigated to a great extent by the use of platelet concentrates, these are not successful in all patients and are associated with allo-immunisation and transmission of infection. In addition, in a proportion of patients with thrombocytopenia, non-immune factors are now known to be important causes of refractoriness to platelet concentrate transfusion. Therefore, the use of haemopoietic growth factors that are able to stimulate early platelet recovery and reduce the platelet nadir achieved in these patients may substantially alter the response to cytotoxic chemotherapy.

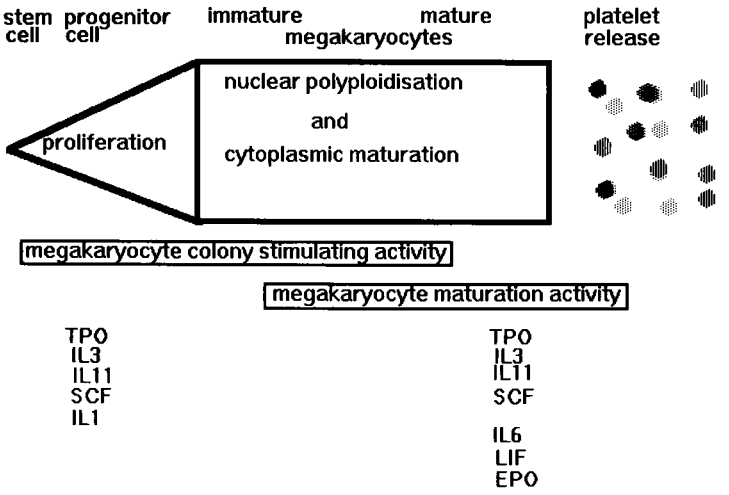

Figure 1. Megakaryocyte maturation and cytokine activity.

Review of megakaryocytopoiesis

Platelets originate from pleuripotent haemopoietic stem cells. Regulation of megakaryocytopoiesis has recently been defined and a hierarchy of megakaryocyte progenitor cells identified. These are defined by time of appearance and size of megakaryocyte colonies appearing in *in vitro* semi-solid culture systems in response to growth factor stimulation.[39] Megakaryocytes are non-dividing cells derived from committed proliferating progenitors designated as burst-forming unit megakaryocytes (BFU-MK) and colony-forming unit megakaryocytes (CFU-MK). The former are the most primitive form, producing the latter which is considered to be a more differentiated progenitor cell. As differentiation proceeds the cells no longer undergo mitosis but acquire the ability of endoreduplication which results in polyploid megakaryocytes. Accompanying or following polyploidy is cytoplastic maturation comprising platelet-specific protein synthesis, granular synthesis and platelet delineation. As a final event, platelets are produced by a poorly defined process that has been thought to be due to physical fragmentation of the megakaryocyte.[40]

The process of platelet production is regulated by humoral factors which affect the process at multiple cellular levels. The cytokines serve as proliferation factors, amplifying platelet production by expanding the progenitor cell pool,[41,42] and they also act as maturation factors on the more differentiated cells. Following thrombocytopenia, the size and ploidy of megakaryocytes are increased whereas the number of megakaryocyte progenitors and megakaryocytes themselves are not affected. These latter are specifically influenced by the number of progenitors themselves, being influenced by the class of factors designated as megakaryocyte-colony stimulating activity (MK-CSA). There is, of course, considerable overlap in the effects of these growth factors and the hypothesis is not universally accepted. However, the concept of proliferation and maturation factors remains useful for classification and in considering their potential therapeutic role[43] (Figure 1).

Haemopoietic growth factors stimulating platelet production

Recent studies have provided an increasing understanding of the effects of growth factors on thrombopoiesis, *in vitro* and *in vivo*.[39,43,44] These growth factors affect both progenitor cell proliferation and megakaryocyte maturation. They may act directly at the progenitor cell level or paradoxically possess no direct MK-CSA activity but act synergistically with other growth factors.

Several lineage non-specific growth factors are known to exert an impact on megakaryocyte maturation. These include stem cell factor (SCF) C-KIT, interleukin 1 (IL-1), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 11 (IL-11), leukaemia inhibitory factor (LIF), granulocyte macrophage-colony

stimulating factor (GM-CSF), erythropoietin (EPO), and the recently cloned C-Mpl ligand (now known as thrombopoietin, TPO). Of these only TPO is lineage specific. However, the lineage non-specific factors may affect thrombopoiesis by increasing progenitor cell proliferation or accelerating maturation of the megakaryocytes.[45] TPO appears to influence both megakaryocyte numbers and maturation.

It appears that the transition from multi-potential progenitors to megakaryocyte-restricted progenitors may involve the MPL oncogene, which is highly expressed in megakaryocyte lines and on progenitor cells. The MPL ligand has been shown to be specific for the megakaryocyte lineage as its inhibition *in vitro* will decrease megakaryocte differentiation.[46] TPO will undoubtedly be pivotal to our understanding of megakaryocyte development. The influence of other factors is less specific. IL-3 like TPO will stimulate megakaryocytopoiesis, and GM-CSF has a similar activity although to a considerably lesser degree. These observations have been supported by studies using the fusion protein constructed from GM-CSF and IL-3 (PIXY 321) which has quite marked effects on BFU-MK and CFU-MK colony formation. This fusion protein contains the active components of both GM-CSF and IL-3. IL-1 and SCF have no direct effect on early progenitors but will amplify the effect of IL-3. The ability of this latter factor to stimulate proliferation can also be stimulated by SCF, IL-6, LIF and IL-11. These combinations produce not only increased numbers of megakaryocyte colonies, but also larger individual colonies. Thus, although many factors appear to influence thrombopoiesis, either directly or by secondary modulation, their individual value is uncertain because of the interaction of the growth factors that is seen *in vitro* and almost certainly occurs *in vivo*. Clinical trial data using individual compounds is now available but optimal application of growth factors in the thrombocytopenic patient remains equivocal.

Interleukin 3

IL-3 is a glycoprotein produced by T cells and natural killer cells. The gene for IL-3 is located on chromosome 5 close to the GM-CSF gene. IL-3 exerts its main effect in early haemopoiesis and its ability to stimulate all cell lines has been demonstrated in both murine and non-human primate models. Both megakaryocyte progenitors and immature megakaryocytes are stimulated by administration of IL-3. Early studies using escalating doses in patients with advanced cancer marrow failure and severe cytopenias showed a dose-dependent increase in the platelet count and in some the use of platelet transfusions could be discontinued.[47] These observations were confirmed in other phase I/II studies using IL-3 following chemotherapy which demonstrated a dose-dependent recovery of neutrophils, platelets, monocytes and red blood cells.[48–50] Other studies in aplastic anaemia and myelodysplastic syndrome have shown that IL-3 will induce variable improvements in all cell lineages.[51,52] These initial studies suggest that platelet response, although variable, is quite marked in patients with malignancies but appears to a lesser degree in patients with primary marrow failure states or chemotherapy-induced marrow suppression. IL-3 appears to act synergistically with G-CSF and GM-CSF in inducing myeloid colonies and with IL-6 in early progenitor cell assays, and in particular, when used with GM-CSF, appears to have a synergistic stimulatory effect on haemopoiesis. It is likely that the place of IL-3 will be in combinations with other cytokines. At the time of writing, definitive clinical data that might indicate more exactly the role of the cytokine are lacking.

Granulocyte macrophage-colony stimulating factor

There has now been extensive experience using GM-CSF in many clinical settings in a variety of haematological disorders and infectious diseases, including marrow failure, bone marrow transplantation and following cancer chemotherapy. It is now clearly accepted that GM-CSF in the setting of myeloablative therapy and autologous bone marrow transplantation will reduce the duration of leucopenia,[53] though only a few studies have reported accelerated platelet recovery.[54,55]

GM-CSF has, however, been used to potentiate the collection of peripheral blood progenitor cells (PBPCs) following chemotherapy prior to collection, and these cells have been shown to be capable of restoring haemopoiesis, especially granulocyte and platelet recoveries, after subsequent high-dose therapy.[56] This has led to an improvement in hospital stays with a reduction in transfusion support.

Granulocyte-colony stimulating factor

G-CSF shows no effect *in vitro* on megakaryocytopoiesis and no augmentation of platelet recov-

ery has been observed following its use clinically,[57] although as with GM-CSF it will mobilise peripheral blood stem cells whose transplantation markedly accelerates platelet recovery following high-dose chemotherapy.[58]

GM-CSF / IL-3 fusion protein (PIXY 321)

PIXY 321 is a recombinant hybrid molecule of IL-3 and GM-CSF designed to take advantage of the synergistic action of these two growth factors. It has been shown *in vitro* to promote megakaryocytopoiesis and may enhance platelet recovery after chemotherapy.[59] In the non-human primate model duration of both neutropenia and thrombocytopenia were reduced and in early phase I/II trials following cancer chemotherapy initial beneficial effects have been observed.[60]

Interleukin 6

IL-6 is a multi-functional cytokine affecting the immune response, modulating the acute phase reaction, in addition to its effects on haemopoiesis. Peripheral blood platelet counts have been shown to increase in animal models and in addition megakaryocyte size and ploidy increase following IL-6, indicating its activities as a maturation factor.[61] Phase I studies in patients with advanced malignancies demonstrated a significant platelet response following subcutaneous IL-6 and currently phase I and II trials are underway combining IL-6 with chemotherapy in a variety of malignancies.[62–64] Minor side effects including fever, chills, malaise, nausea and vomiting, headaches and myalgia have been observed in many of these studies. IL-6 has also been shown to enhance the platelet count when administered with sequential IL-3, and clinical trials are underway to identify the optimal combinations and dose schedules of these cytokines.[65] A combination of predominantly proliferation-inducing cytokines such as IL-3 with a later acting cytokine such as IL-6 potentially offers an ideal synergistic combination to enhance recovery post chemotherapy and following bone marrow transplantation.

Stem cell factor

SCF is the ligand for the C-KIT oncogene receptor which is thought to stimulate the earliest haemopoietic progenitors and may also play a role in lymphocyte proliferation. However, studies in culture have shown that it has negligible effects on proliferation of megakaryocyte progenitors. Studies in primates have shown that it augments megakaryocyte numbers without any appreciable effect on the platelet count. As with GM- and G-CSF it will stimulate appearance in the circulation of progenitor cells capable of enhancing platelet recovery following high-dose chemotherapy or lethal irradiation. However, its use is associated with significant side effects and its clinical value is unknown, although studies are currently underway to assess its clinical utility.[66]

Thrombopoietin

The proto-oncogene c-Mpl encodes a cell surface receptor that is a member of the haemopoietic growth factor receptor super family. The murine myeloproliferative leukaemia virus transduces the gene for a cellular protein MPL in its envelope gene region. The gene for the MPL protein was cloned and found to have considerable homology with the gene encoding haemopoietic growth factor receptors and studies found that strong c-Mpl message could be detected only in purified CD34+ cells, highly enriched megakaryocytes and platelets. In 1994 the murine and human c-Mpl ligand was cloned and *in vitro* and *in vivo* studies indicated that it stimulated megakaryocytopoiesis and thrombopoiesis and had all the properties expected of thrombopoietin. It causes a 4-fold rise in platelet counts in normal mice [45,67] and increases megakaryocyte numbers and ploidy *in vivo* and *in vitro*. Thus the purified protein shows activity capable of causing proliferation and increased ploidy of megakaryocyte precursors.[68] Its potential impact on platelet support and thrombopoiesis is immense and preclinical studies are awaited.

Other cytokines with thrombopoietic activity

Numerous other cytokines have been described that affect thrombopoiesis. These range from established products such as EPO, through other cytokines such as IL-11 that are undergoing evaluation, to factors such as LIF that currently remain laboratory based. IL-11 and LIF may promote megakaryocytic maturation when used alone, or in conjunction with proliferation-promoting factors such as IL-3. IL-11 will also promote progenitor proliferation.

Phase I studies of IL-11 in advanced breast cancer have demonstrated some effect on platelet recovery but are associated with an early acute-phase reaction that may lead to an initial thrombocytopenia.[69]

IL-1 is a cytokine with multiple biological effects and is one of the major pro-inflammatory cytokines. It has been shown to augment the platelet count in animals and humans and will accelerate platelet recovery in patients following chemotherapy-induced thrombocytopenia.[70] However, quite severe toxic side effects have been noted and there must be significant concern that these will preclude its general use.[70,71]

EPO has also been shown to be capable of promoting megakaryocyte colony growth and differentiation but *in vivo* animal studies suggest that this response is only transient[72] and there is no evidence from its use in patients with chronic renal failure that there is any consistent stimulation of platelet production.[73] It is therefore unlikely to be a significant contributor, at least as a single agent.

Peripheral blood progenitor cell rescue

With the increasing intensity of chemotherapy, thrombocytopenia has become an increasingly profound problem requiring enhanced platelet transfusion support. As has been described there have been numerous attempts, using a variety of cytokines, to overcome this problem. Another approach is to infuse mobilised peripheral blood stem cells or marrow cells after chemotherapy. Several studies now using stem cells, mobilised by growth factors used either in combination or as single agents, with or without chemotherapy as an alternative source of haemopoietic support, have shown a more rapid recovery of circulating neutrophils with consistently faster platelet recovery compared with bone marrow transplantation alone or post chemotherapy. Recent studies in non-myeloid malignancies have shown that if sufficient CD34+ cells are transplanted there is a highly predictive increase in platelet recovery, achieving a platelet count of greater than 20×10^9/l with a median time of 10 days,[74] and a more recent phase III randomised study confirmed this more rapid platelet recovery with shortened hospitalisation, reduced antibiotic use and an overall reduction in the costs of bone marrow transplantation.[75]

A more recent innovative approach has been the use of growth-factor mobilised PBPCs as a source of autologous support in patients receiving multiple cycles of high-dose chemotherapy. PBPCs collected during an initial cycle of chemotherapy following GM-CSF permitted the administration of four repetitive cycles of high-dose chemotherapy, offering the possibility of a reduction in treatment cycles with overall dose intensification. This sort of approach will allow a wider range of patients to benefit from more rapid platelet recovery with a reduced morbidity and mortality and with potential benefits in tumour response.[76]

Summary

Platelet transfusions have increased dramatically over the last decade with enhanced chemotherapy schedules coupled with a wider range of diseases treated plus patients undergoing chemotherapy.

At times platelet usage appears indiscriminate and although transfusion protocols are available, usage is increasing and is governed by many factors that show varying degrees of logic. These include the platelet count, the state of the patient and the physician's fear of haemorrhage. This repeated and often excessive platelet usage, although frequently life-saving, results in the inevitable transmission of many blood-borne infections, as well as leading to allo-immunisation. The logistics of blood product collection and fractionation and the financial burden are also not insignificant and must not be forgotten.

In view of the difficulties in maintaining safe and adequate platelet transfusion support, the use of cytokines has been seized on as a potential salvation in these circumstances. The cloning, production and clinical use of many cytokines over the last few years has shown the potential value of this approach. Numerous cytokines are available that may potentially influence thrombopoiesis; although it is in the other cell lines that real clinical benefit has been seen. The use of EPO has virtually eliminated the need for red cell transfusions in anaemic states associated with reduced epo production, and the development of the growth factors GM-CSF and G-CSF has been shown to enhance neutrophil recovery and in many though not all studies has had an impact on febrile episodes, with reduced morbidity and mortality. The interest in thrombopoietic factors, however, lies in the numerous areas in which they may be of value (Figure 2).

The impact however on platelet recovery has been far less and the use of agents in these fields has lagged behind that in the others. Of the factors currently available, IL-3 has undoubted activity on

(1) Therapy of congenital cytopenias
  (a) Fanconi's anaemia
  (b) Thrombocytopenia
    — *Amegakaryocytic*
    — *Dysmaturation*
(2) Aplastic anaemia
(3) Myelodysplastic syndrome
(4) Reduction in cytopenia post-conventional chemotherapy
(5) Recovery from high-dose chemotherapy / radiotherapy
(6) For peripheral blood progenitor cell mobilisation
(7) AIDS
(8) ? Autoimmune thrombocytopenia

Figure 2. Potential areas of use for cytokines with megakaryocyte stimulating activity.

platelet recovery, although its use post bone marrow transplantation and following intensive chemotherapy is still under debate. Of the other agents available, IL-6 is potentially of value and undoubtedly has an effect on megakaryocyte maturation and platelet production. However, even before these agents are widely accepted in practice, it is becoming clear that their best use may be more in combination than as single agents; and this has been demonstrated quite clearly, *in vivo* and *in vitro*, using the sequential administration of IL-3 followed by GM-CSF and with the combination of IL-6 and GM-CSF.

This supports the hypothesis that the simultaneous or sequential use of proliferation and maturation cytokines might show synergistic effects *in vivo* compared with either of the individual agents alone. Clinical studies are currently underway to look at these concepts.

It is important to consider the potential adverse clinical effects of these cytokines. Several of them, such as SCF and IL-1, cause significant problems that may well preclude their general clinical use, and it must also be borne in mind that some of these cytokines may have other as yet undetected effects on the underlying malignancy or on other tissues in the body.

The effect of peripheral blood progenitor cell support has also had an impact on the need to find a thrombopoietic agent, and careful studies will be needed to balance the cost–benefit analysis of chemotherapy alone, dose intensification, additional use of cytokines and the use of peripheral blood progenitor cells. A clear statement of the relative overall value of each of these procedures in cancer chemotherapy and their bearing on patient survival and cure rates will need to be made. Therefore, it will be necessary, while the studies are being undertaken, to find relevant cytokines for the amelioration of thrombocytopenia, to undertake a critical analysis of their actual role in the treatment of patients with malignancies.

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