

THE END OF A LONG SEARCH: AT LAST THROMBOPOIETIN

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HAEMATOLOGICA editorials normally comment original papers published in the journal. The present editorial is an exception, justified by the discovery of a new hematopoietic growth factor: thrombopoietin. The Editorial Board believes that we must point out to our readers this major scientific advance.

More than 15 hemopoietic growth factors have now been biochemically characterized, genetically cloned and tested for *in vitro* and/or *in vivo* biologic activity.¹ Of these, erythropoietin and G-CSF have already become powerful tools in the hands of clinicians, and their clinical applications have already been documented in books.^{2,3}

Recombinant human erythropoietin corrects anemia, eliminates the need for transfusion, improves quality of life and is cost effective in anemic patients with renal failure.^{2,4,5} Autologous blood donation can be significantly potentiated by administering erythropoietin to subjects with Hct values lower than 40%, in order to avoid recourse to allogeneic blood.² Recombinant human erythropoietin is also effective in several other anemias, although only a part of these patients is responsive.²

Recombinant human G-CSF has had a major impact in ameliorating one of the primary side effects of cancer chemotherapy: granulocytopenia.³ It has also proved to be useful in the treatment of chronic neutropenia, in accelerating neutrophil engraftment following bone marrow transplantation and in mobilizing peripheral stem cells.³

Although some of the known hematopoietic growth factors and cytokines had been found to stimulate megakaryocytopoiesis and platelet production, none of them appeared to behave as a true thrombopoietin.⁶ Nevertheless, a factor called thrombopoietin was recognized in the plasma of thrombocytopenic patients at least 30 years ago.⁶ Four papers in a recent issue of

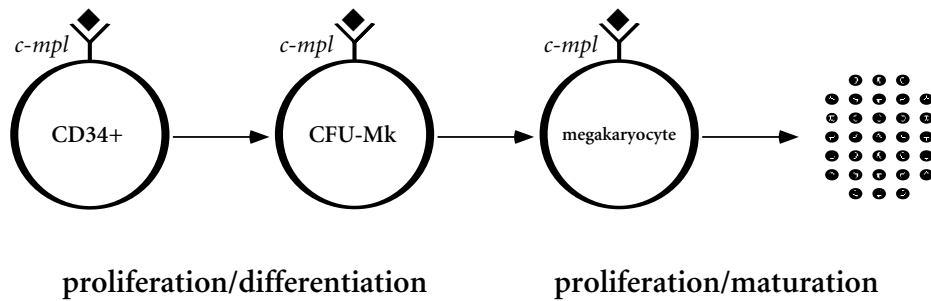
*Nature*⁷⁻¹⁰ and an article in a recent issue of *Cell*¹¹ mark the end of this long search.

The cellular developmental process that leads to platelet production involves megakaryocytopoiesis and thrombopoiesis. At least two humoral factors have long been thought to regulate these processes: megakaryocyte-colony stimulating factor (meg-CSF), which induces the proliferation and differentiation of megakaryocyte progenitors (CFU-Mk), and thrombopoietin, which is a megakaryocyte maturation factor.⁶

The *c-mpl* proto-oncogene is a newly described – and, until recently, orphan – member of the hematopoietic receptor superfamily.¹² This human gene was cloned using probes derived from the highly leukemogenic murine myeloproliferative leukemia virus (mplv). It is likely that one oncogenic version of *c-mpl* had been naturally transduced in the genome of mplv. Wendling and coworkers identified this oncogene as *v-mpl* and found that it was a potent dysregulator of hematopoiesis.¹³ Until recently, however, they could not find any clue to the function of the normal proto-oncogene *c-mpl*.

In 1993, using an antisense strategy the French authors provided evidence that this gene was involved in megakaryocytopoiesis.¹⁴ First, they examined *c-mpl* expression in human purified hematopoietic cell populations: *c-mpl* transcripts were detected in purified CD34-positive cells, megakaryocytes, and platelets. Second, they studied the effect of oligodeoxynucleotides antisense to *c-mpl* on differentiation and maturation of hematopoietic cells. *In vitro* exposure of CD34-positive cells to antisense oligomers significantly reduced *c-mpl* mRNA synthesis and markedly inhibited CFU-Mk growth, whereas BFU-E and CFU-GM growth was unaffected. These results raised the possibility that *c-mpl* encoded the receptor for a cytokine specifically

◆ thrombopoietin (c-mpl ligand)



regulating thrombocytopoiesis.

These findings suggested that a putative ligand for *c-mpl* might be a megakaryocyte lineage-specific growth factor similar to meg-CSF and thrombopoietin, and stimulated research aimed at cloning a ligand for *c-mpl*. Whereas previous studies on *v-mpl* and *c-mpl* had been performed in academic institutions or research laboratories exclusively, cloning the new factor involved collaboration with biotechnology companies⁷⁻⁹ or was performed by the company itself.¹¹

Different strategies were employed. de Sauvage et al.⁷ first showed that plasma obtained from irradiated pigs stimulated human megakaryocytopoiesis and contained a *c-mpl* ligand, called ML. They purified ML from aplastic porcine plasma and used the amino-acid sequence information to isolate a human ML complementary DNA. Human ML shares homology with erythropoietin. ML gene was expressed in mammalian cells, and human recombinant ML was found to stimulate both megakaryocytopoiesis and thrombopoiesis *in vitro* and *in vivo*.

Lok et al.⁸ cloned a murine complementary DNA encoding a *c-mpl* ligand. The encoded polypeptide has a predicted molecular mass of 35,000 and a two-domain structure with an amino-terminal domain homologous with erythropoietin. Intraperitoneal injections of mice with recombinant protein increase circulating platelet levels by greater than fourfold after 7 days. Through procedures involving *c-mpl* receptor affinity chromatography, Bartley et al.¹¹ identified in aplastic canine plasma a novel fac-

tor called *megakaryocyte growth and development factor* (MGDF). They cloned its cDNAs from canine, murine, and human sources. Human, dog, and mouse cDNAs for MGDF are highly conserved and encode open reading frames for proteins of 353, 352, and 356 amino acids, respectively.

Kaushansky et al.⁹ analyzed the effects of the recombinant murine *c-mpl* ligand on murine hematopoiesis. They first found that the recombinant factor was relatively lineage specific. It worked both alone and synergistically with early acting factors to support megakaryocyte colony formation. In addition, it acted at a late stage of development to increase megakaryocyte size, polyploidization and expression of differentiation markers such as glycoprotein Ib. *In vivo* administration to mice resulted in marked expansion of marrow and splenic megakaryocytes and their progenitors, and increased platelet production. Kaushansky et al.⁹ concluded that the new factor has the expected characteristics of the major regulator of megakaryocytopoiesis and thrombopoiesis and proposed that it be termed thrombopoietin. Findings by Wendling et al.¹⁰ indirectly confirm this conclusion. Bartley et al.¹¹ showed that both canine and recombinant human MGDF support the development of megakaryocytes from human CD34-positive progenitor cell populations in liquid culture, and promote the survival of a factor-dependent murine cell line (32D) engineered to express *mpl*. In addition, these biological activities were blocked by the soluble extracellular domain of *mpl*.

In summary, thrombopoietin appears to be a lineage-specific factor capable of regulating megakaryocytopoiesis at multiple cellular levels: proliferation and differentiation of megakaryocyte progenitors and maturation of megakaryocytes (Figure). These features (lineage-specificity and multiple cellular levels) resemble those of erythropoietin with respect to erythropoiesis.^{15,16} The availability of recombinant thrombopoietin now makes it possible to study the physiopathology of platelet production. Obviously, clinicians are eagerly looking forward to using this powerful new agent.

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