Occasional articles

Pathogenesis of idiopathic myelofibrosis: Role of growth factors

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Introduction
Idiopathic myelofibrosis, or agnogenic myeloid metaplasia, is a chronic myeloproliferative disorder that is characterised by marrow fibrosis and extramedullary haematopoiesis. The origin of the disease remains an enigma and a source of confusion in management, not least because until recently no meaningful progress had been made regarding its pathophysiology. Research during the past decade in three interrelated areas may offer some hope for the future. First, it is now accepted that the fibroblast proliferation is a reactive, or secondary process and is not, as previously thought, a component of the underlying clonal haematopoiesis. Second, the nature and composition of both normal and myelofibrotic bone marrow stroma has largely been elucidated, and lastly, the pivotal role of inappropriate growth factor release from megakaryocytes as mediators of fibrogenesis has been recognised. This article aims to review the current understanding of the disease's pathogenesis, with particular emphasis on the part played by growth factors.

Clonality of haematopoiesis
It was originally believed that bone marrow fibroblasts were derived from pluripotent haematopoietic stem cells and that the proliferative defect present in myelofibrosis also affected the fibroblasts. Jacobson et al., however, using G6PD isoenzyme analysis in a heterozygous female patient, showed for the first time that the fibroblasts were polyclonal, in contrast to the erythrocytes, platelets, and granulocytes. Subsequent studies, using cytogenetic analysis and fibroblast proliferation kinetics, have confirmed this observation. Recently, two independent strategies have added further support for haematopoietic clonality, namely probing for X-linked restriction fragment length polymorphisms by taking advantage of the non-random inactivation of the X chromosome, and N-ras gene mutation analysis. Interestingly, the latter study has provided evidence for a role for both T and B lymphocytes, a fact that could explain the observed immunological abnormalities. Further investigations are needed to determine if lymphocytic involvement is a consistent feature or whether lineage heterogeneity can occur as reported in other myeloproliferative disorders.

The concept of a clonal expansion of single multipotent stem cells has been strongly supported by the finding of increased numbers of multilineage (CFU-GEMM) and committed (CFU-GM, BFU-E, and CFU-MK) circulating progenitor cells. The molecular events that confer such a growth advantage remain unknown, although cytogenetic evidence points to abnormalities at specific chromosomal locations. One of the most common findings is deletion of 13q with consistent loss of 13q14, the site of the retinoblastoma gene. The pathogenetic importance, however, of the loss or inactivation of this tumour suppressor gene, as well as the reported N-ras oncogene mutations, requires further study on a larger number of patients. A proposed model of the disease's pathogenesis is outlined in fig 1.

Composition of myelofibrotic stroma
The constituents of normal and myelofibrotic extracellular matrices have a greater diversity and complexity than was apparent from routine histological examination. Immunological analysis, using specific antibodies, has shown a variety of collagen types, including I, III, IV and V. Myelofibrotic stroma is characterised by an increase in total collagen, as measured by hydroxyproline content, with, in particular, deposition of the interstitial collagens type I and III. Type III collagen was assumed to form fibrous structures only after specific peptide extensions had been removed from the ends of the procollagen or “precursor” molecule. It is now apparent that a significant proportion of collagen type III still retains its amino-terminal peptide, even when assembled into fibrillar structures. In myelofibrotic stroma, therefore, procollagen type III deposition is also increased, especially in advanced disease, where it has a distribution similar to that of reticulin. The increase in interstitial collagen is also reflected in the circulation, where serum concentrations of the carboxy-terminal peptide of procollagen type I (personal observation) and the amino-terminal peptide of procollagen type III are raised, especially in patients with active disease.

Type IV collagen and laminin are major components of all basement membranes together with the glycoproteins nidogen, entactin, and heparan sulphate proteoglycan. In normal bone marrow staining for collagen type IV and laminin is limited to discontinuous
Role of growth factors

Several observations led to the belief that the megakaryocytic lineage may be pathogenetically important in the development of myelofibrotic stroma; (i) megakaryocytic hyperplasia, often with atypical or dysplastic forms (fig 3A), is a constant and prominent characteristic of idiopathic myelofibrosis; (ii) fibroblast proliferation and collagen deposition is often maximal in areas of megakaryocyte necrosis (fig 3B); (iii) morphological abnormalities of megakaryocytes are more prominent in myelofibrosis than other myeloproliferative conditions; and (iv) myelofibrosis is a well recognised feature of both acute megakaryoblastic leukaemia and the rare "grey platelet syndrome". More recently, marrow fibrosis has been associated with myelodysplasia, another condition characterised by dysplastic megakaryocytopenia.

PLATELET DERIVED GROWTH FACTOR (PDGF)

Castro-Malaspina and colleagues provided the first tangible evidence in support of the above hypothesis, by showing that megakaryocytes contain a growth factor, PDGF, capable of inducing the proliferation of fibroblasts. Human PDGF is a 30 000 dalton heterodimeric protein that is stored in platelet a granules and released on activation. It is the major mitogenic factor in serum and is able to stimulate the growth and cell division of fibroblasts as well as other cells. Several groups have reported reduced platelet PDGF activity, as measured by 3H-thymidine uptake, in myeloproliferative disorders, a feature thought to reflect an abnormal leakage or release of PDGF from megakaryocytes in the bone marrow. Similar results have been obtained using a specific radioimmunooassay, while interestingly, Baglin et al reported that the low concentrations may be reversed following chemotherapy.

These findings, however, could result from either impaired megakaryocytic synthesis or from platelet activation and release of a granule contents. The reports of decreased intraplatelet plasminogen activator and platelet factor 4, accompanied by increased plasma and urinary concentrations, would favour a platelet or megakaryocyte release mechanism. Recently,

Figure 1 Proposed model for the development of idiopathic myelofibrosis.

sinusoidal basement membranes. In contrast, myelofibrotic stroma contains continuous sheets of both proteins (fig 2A), resulting from pronounced neovascularisation and endothelial cell proliferation. These findings are mirrored in the circulation where increased serum concentrations of laminin related peptides and collagen type IV have been reported; facts that may be of clinical value in monitoring disease activity. Fibronectin, a non-collagenous glycoprotein, is widely distributed throughout the body, being found in loose connective tissue and basement membranes. In normal marrow it is localised to megakaryocytes and the walls of blood vessels, whereas in myelofibrosis (fig 2B) there is a pronounced stromal deposition. Vitronectin, another adhesive glycoprotein like fibronectin, is also increased, although to a lesser extent.

Figure 2 Immunological studies of myelofibrotic stroma. (A) Extensive neovascularisation of myelofibrotic bone marrow as demonstrated by antibodies against laminin (immunoperoxidase). Identical results were obtained using anticollagen type IV. (B) Pronounced fibronectin deposition in advanced myelofibrosis (APAAP).
increased plasma and urinary PDGF protein concentrations have also been reported in myeloproliferative diseases. Two independent groups have documented high platelet PDGF concentrations in a small number of myelofibrotic patients. Katoh et al also reported increased mRNA expression, for both PDGF A and B chains, in megakaryocytes from myeloproliferative disorders. It is therefore possible, at least in some cases, that increased PDGF synthesis may be pathogenetically important. Most studies, however, have found that myeloproliferative platelets contain reduced z granule proteins and support the hypothesis of an abnormal leakage or release of PDGF in bone marrow, with consequent fibroblast proliferation.

The pathogenesis of myelofibrosis in acute megakaryoblastic leukaemia may be related to the release of PDGF from the blast cells. Several observations support this view; the c-sis gene, which encodes the chain of PDGF, may be expressed by megakaryoblastic leukaemia cells; increased plasma PDGF concentrations have been reported in acute micromegakaryocytic leukaemia (fig 4), and breakpoints around the site of the PDGF- gene, 22q13, are strongly associated with megakaryoblastic leukaemia in infants. The release of PDGF, while undoubtedly inducing fibroblast growth, cannot account totally for the complexity of myelofibrotic stroma. PDGF does not, for example, have angiogenic properties, nor does it induce the transcription of genes coding for laminin, fibronectin, or the collagens. Additional growth factors must be involved, the most important of which is likely to be transforming growth factor-β.

**Figure 3** Trephine biopsy specimens showing (A) hyperplastic haematopoietic tissue with increased megakaryocytes; some exhibiting dysplastic features. (B) Silver impregnation staining showing increased reticulin fibres in an area of megakaryocyte hyperplasia.

**Figure 4** Acute micromegakaryocytic transformation in a patient with long standing myelofibrosis (peripheral blood). Transformation was associated with a sharp increase in serum PDGF, TGF-, soluble laminin and procollagen type III concentrations.

**TRANSFORMING GROWTH FACTOR-β (TGF-β)**

TGF-β is a highly conserved 25 kilodalton polypeptide, ubiquitously expressed by mammalian cells and recognised by specific receptors on virtually every cell type analysed. Like PDGF it is synthesised by megakaryocytes, stored in high concentration in platelet z granules, and released by degranulation at sites of injury. The importance of TGF-β in relation to the pathogenesis of myelofibrotic stroma, lies in its function as a regulator of extracellular matrix synthesis. It increases the expression of genes that code for fibronectin, collagens type I, III, and IV, as well as chondroitin/dermatan sulphate proteoglycans. It also has powerful angiogenic properties, with new capillary formation occurring within 48 hours of injection. TGF-β also decreases the synthesis of various collagenase-like enzymes that degrade extracellular matrices, while at the same time stimulating the synthesis of protease inhibitors, such as plasminogen activator inhibitor-1. The net effect, therefore, of these complex interactions is the accumulation of extracellular matrix.

The reports of active TGF-β synthesis by megakaryoblasts and the finding of increased plasma concentrations in acute micromegakaryocytic leukaemia (fig 4) that correlated with increased bone marrow stromal turnover, provide further evidence for its pathogenetic role. Similar findings have been documented in hepatic fibrosis where the amount of TGF-β mRNA correlates with both collagen type I mRNA and serum procollagen terminal peptide III.

**The future**

The past 10 years have seen a substantial increase in our understanding of the mechanisms involved in myelofibrosis. Many questions, however, remain to be answered. What, for example, are the roles and interactions of platelet factor 4, epidermal growth factor, and endothelial cell growth factor, components co-released with PDGF and TGF-β? Are the reported autocrine or paracrine mechanisms during bone marrow fibroblast growth important in disease progression? What is the importance of raised M-CSF concentrations and their correlation with myeloid metaplasia and bone marrow expansion?

The prognosis of idiopathic myelofibrosis,
when compared with other myeloproliferative disorders, remains poor and has not changed significantly during the past 20 years. It is to be hoped, therefore, that the recent advances in this once neglected disease will eventually lead to effective therapeutic measures.

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