

## Review article

# Myelodysplastic syndromes: pathogenesis, functional abnormalities, and clinical implications

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**SUMMARY** The myelodysplastic syndromes represent a preleukaemic state in which a clonal abnormality of haemopoietic stem cell is characterised by a variety of phenotypic manifestations with varying degrees of ineffective haemopoiesis. This state probably develops as a sequence of events in which the earliest stages may be difficult to detect by conventional pathological techniques. The process is characterised by genetic changes leading to abnormal control of cell proliferation and differentiation. Expansion of an abnormal clone may be related to independence from normal growth factors, insensitivity to normal inhibitory factors, suppression of normal clonal growth, or changes in the immunological or nutritional condition of the host.

The haematological picture is of peripheral blood cytopenias: a cellular bone marrow, and functional abnormalities of erythroid, myeloid, and megakaryocytic cells. In most cases marrow cells have an abnormal DNA content, often with disturbances of the cell cycle: an abnormal karyotype is common in premalignant clones. Growth abnormalities of erythroid or granulocyte-macrophage progenitors are common in marrow cultures, and lineage specific surface membrane markers indicate aberrations of differentiation. Progression of the disorder may occur through clonal expansion or through clonal evolution with a greater degree of malignancy. Current attempts to influence abnormal growth and differentiation have had only limited success. Clinical recognition of the syndrome depends on an acute awareness of the signs combined with the identification of clonal and functional abnormalities.

Chronic refractory anaemia, often with a poor prognosis, has been recognised for many years. It has been described variously as "refractory anaemia"<sup>1</sup> or "refractory normoblastic anaemia".<sup>2</sup> Bjorkman described "chronic refractory anaemia with sideroblastic bone marrow" and emphasised the peculiar erythroblast morphology.<sup>3</sup> The concept of preleukaemia was first mentioned by Hamilton-Paterson<sup>4</sup> and Block *et al.*,<sup>5</sup> and this term came to be used to describe any syndrome preceding the onset of overt leukaemia. The diagnosis was usually made retrospectively. Dameshek suggested that acquired sideroblastic anaemia represented an early stage of erythroleukaemia.<sup>6</sup> Several slightly differing syndromes were discussed in subsequent studies describing slightly different groups of patients, including those with "smouldering leukaemia",<sup>7</sup>

"oligoblastic leukaemia",<sup>8</sup> the dysmyelopoietic syndrome,<sup>9</sup> and preleukaemia.<sup>10</sup> Different methods of selecting patients and varying criteria hinder comparison of these various conditions. Saarni and Linman,<sup>10</sup> Linman and Bagby,<sup>11,12</sup> and Greenberg and Mara<sup>13</sup> attempted to define the syndrome more precisely and to separate groups at high risk from those at low risk. In 1982 the French-American-British (FAB) group proposed that all these conditions be grouped together and described as the myelodysplastic syndromes (MDS),<sup>14</sup> and it is now generally accepted that this represents a clonal abnormality of haemopoietic stem cells.<sup>15-17</sup> The condition is characterised by a diversity of phenotypic manifestations with varying degrees of ineffective haemopoiesis and a high probability of eventual leukaemic change.

Peripheral blood cytopenias together with a cellular marrow comprise the haematological picture.

Characteristic morphological abnormalities of dyserythropoiesis, abnormal granulopoiesis, and megakaryocytes, are also a feature, and these have been described in detail.<sup>14</sup> Most authors include only patients with a hyperplastic or normally cellular marrow in their descriptions, although with the known patchy distribution of haemopoietic tissue this may be a difficult criterion to establish without a trephine biopsy. Idiopathic acquired sideroblastic anaemia is included in this syndrome, although distinctions have been made between different types of this disorder.<sup>18, 19</sup> Similarly, chronic myelomonocytic leukaemia is usually included, although this is characterised by a proliferative rather than a cytopenic disorder. Minimal haematological disorders such as unexplained macrocytosis<sup>20</sup> or mild cytopenia affecting only one cell line may represent either a limited form or an early stage of the disorder. Although specific chromosomal abnormalities have been described in many patients,<sup>21</sup> these are not always related specifically to particular clinical syndromes and do not necessarily affect the prognosis.

The diversity of clinical and haematological manifestations reflects the basic abnormality that cytogenetic<sup>21</sup> and glucose 6-phosphate dehydrogenase isoenzyme marker analyses<sup>22-24</sup> indicate is a clonal disorder of haemopoietic stem cells. In some cases this has been shown to evolve through successive mutations progressing by stages to acute leukaemia, which may occur at varying rates, and different progenitors may be the target in different patients. The nature of the defect will determine the phenotypic expression of the disorder at any specific time. In other cases progression may be due to the gradual clonal expansion of the abnormal population, with an increasing clinical expression of ineffective haemopoiesis and bone marrow failure.

### Classification

Bennett *et al* originally defined two premalignant syndromes, refractory anaemia with excess blasts and chronic myelomonocytic leukaemia.<sup>25</sup> Further diagnostic groups in the current FAB classification<sup>14</sup> include idiopathic acquired sideroblastic anaemia, refractory anaemia, and refractory anaemia with excess blasts in transformation. Most of the other reported syndromes can be roughly equated with one of these, although the criteria usually differ somewhat from those of the FAB group.<sup>26</sup>

Despite the value of the FAB classification the demarcation between different clinical groups is necessarily arbitrary, and the need for such a classification has been questioned.<sup>27, 28</sup> It is often difficult to allocate a particular patient to a specific

group as the clinical and haematological features may not always correspond exactly to the definitions given. The designation of patients with more than 15% sideroblasts as having "sideroblastic anaemia" has to be seen in the context of a wide range of sideroblast counts in patients with MDS from 1 to 86%.<sup>20, 29, 30</sup> Many patients have different characteristics at different times, suggesting an evolving process rather than different disease entities. The underlying disease mechanisms are related to the way in which normal stem cells undergo a sequence of changes as part of their transformation to a malignant clone, and it might be valid to try and define this evolution. In morphological terms this can be assessed by the progression of pancytopenia and the percentage of marrow blast cells. The functional characteristics of progenitor cells and marrow precursor cells can be measured by the pattern of clonal growth,<sup>20, 31, 32</sup> erythrokinetic studies,<sup>20, 33</sup> or cell cycle abnormalities.<sup>31, 35</sup> The genetic lesion can be investigated by studies of karyotype and measurement of DNA.<sup>34-38</sup>

### Leukaemogenesis

The increasing number of studies into the mechanisms of malignant transformation<sup>39-45</sup> needs to be seen in the context of the massive number of reports on the production of experimental tumours and human carcinogenesis over several decades. The classic description of skin cancer in chimney sweeps two centuries ago by Percival Pott showed the importance of a carcinogenic insult and the long time lag between the insult and the resulting malignancy.<sup>46a</sup> More recent studies of the effect of carcinogenic hydrocarbons on skin suggested a process in two stages, defined as initiation and promotion, which resulted from two difficult stimuli.<sup>46b-48</sup> Later work on this model suggested that the promotion of a tumour may occur in more than one stage and that inhibition of the process was possible.<sup>49</sup> A similar multistage mechanism for the induction of malignancy has been shown for various tumours in many species.<sup>50</sup> Many agents may behave as both initiators and promoters, and in some cases it may not be possible to differentiate between the stages of development. The initiated cell is irreversibly changed with regard to its susceptibility to a tumour promoting agent, although this change may not be detectable by conventional methods of examination. Promotion is a reversible process, but when a threshold exposure is exceeded this results in the occurrence of recognisable malignant change. Such malignancies may be multifocal, but even when a single tumour emerges this may progress with the evolution of multiple clones of aberrant cells with

different genetic and phenotypic characteristics.

Initiation or promotion may result from a chemical insult, radiation, or infection with an oncogenic virus. Susceptibility may be influenced by the metabolic state of the target cell. In a few instances—for example, retinoblastoma and Wilms's tumour—a specific hereditary factor has been implicated.<sup>51, 52</sup> The role that oncogenes may have in the multistage process has recently been shown.<sup>40, 53</sup> The morphological manifestations of the developing malignant process are seen in the various types of precancer found in many organs,<sup>54</sup> and most pathologists have accepted that these lesions represent a stage in the development of the malignant state. The clinical recognition of precancer depends entirely on the technique used, and experimental studies may prove far more critical in establishing a deviation from the normal than conventional cytology.<sup>54</sup> The experimental evidence that malignancy develops as a multistage process is supported by epidemiological data, and several models have been proposed.<sup>55–57</sup>

The pathogenesis of leukaemia has been especially well studied, although the search for aetiological factors has hardly resulted in a clearly defined picture. In this respect the importance of myelodysplastic syndromes has been considered only recently. Recent data point to a considerable increase in the incidence of leukaemia in both the United Kingdom and Sweden, and clustering has been observed in many countries.<sup>58</sup> It has been suggested that chemical exposure<sup>59–61</sup> or radiation<sup>61, 62</sup> may have an important role. The incidence of myelodysplastic syndrome and the factors entailed in its pathogenesis are unknown, although preliminary data (RA Cartwright, personal communication) suggest an incidence similar to that of acute myeloblastic leukaemia. The preleukaemic nature of this syndrome makes it reasonable to assume that the same genetic or epigenetic factors implicated in leukaemogenesis may also be relevant, although the leukaemia eventually arising in patients with myelodysplastic syndrome differs in its characteristics and behaviour from leukaemia arising independently.

The aberrations of growth and differentiation that distinguish the leukaemic from the normal haemopoietic cell are becoming well characterised. In vitro culture of haemopoietic progenitor cells, together with the purification of growth factors,<sup>63–65</sup> have added greatly to our knowledge of control mechanisms. Cytogenetic techniques have helped to uncover chromosomal abnormalities in both leukaemic and preleukaemic states,<sup>37, 66</sup> and the use of monoclonal antibodies has given a more precise mapping of haemopoietic lineage.<sup>67</sup> The abnormal proliferation and failure of normal differentiation

seen in acute leukaemia are also seen, although to a lesser extent, in myelodysplastic syndrome. Whether this is the result of normal progenitor cells failing to differentiate fully and thus maintaining a phenotype “frozen” in a state of incomplete development<sup>68</sup> or whether it results from a fundamental “misprogramming” of the leukaemic stem cell with the resultant confusion of lineage characteristics described by McCulloch<sup>69</sup> is not entirely clear. Similar uncertainties arise regarding the mechanism whereby proliferation and differentiation are linked, and there is some evidence that specific differentiation factors<sup>70</sup> and differentiation genes<sup>71, 72</sup> may be affected by the process of malignant transformation. The full development of the leukaemic phenotype probably entails several stages that need not necessarily occur in any specific order. Activation of a gene may result in the immortalisation of a target stem cell by changing cell cycle control. Autogenous production of growth factor or an increase in receptors may make cell cycle control less dependent on exogenous growth factors, and the activation of a transforming gene may make such a cell entirely autonomous.<sup>73, 74</sup> These events do not necessarily occur simultaneously. The interrelation between oncogenes, growth factors, and cell cycle control is now emerging.

#### GROWTH CONTROL MECHANISMS

The control of cellular proliferation probably entails the interaction of numerous different factors, many as yet unidentified. Nevertheless, study of the known growth factors and the intracellular events they influence has shown some of the stages at which control can occur. Fig. 1 gives some examples of oncogene products that seem to be related to specific steps in the sequence of events controlling synthesis of DNA. There are certainly many other

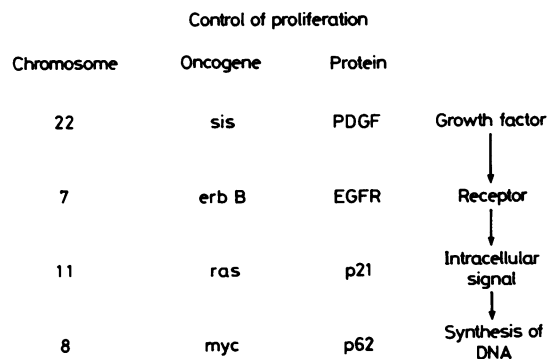


Fig. 1 Association of some known oncogenes with stimulation and control of cell proliferation. Changes in gene expression may be expected to alter growth characteristics.

PDGF = platelet derived growth factor;

EGFR = epidermal growth factor receptor.

such proteins that remain unknown. Current information suggests that the *c-sis* gene probably codes for a subunit of platelet derived growth factor<sup>75,76</sup> and *c-erb B* for the epidermal growth factor receptor.<sup>77</sup> The role of these gene products in the control of cell proliferation has been studied mainly in fibroblasts, and here it has been shown that platelet derived growth factor primes the cell for synthesis of DNA while epidermal growth factor, together with other growth factors, are needed for progress through the cell cycle. Stimulation of quiescent NIH 3T3 fibroblasts with platelet derived growth factor results in a rapid accumulation of *c-myc* transcript that precedes synthesis of DNA.<sup>78,79</sup> In cells that have been transformed by benzopyrene *c-myc* transcript is present throughout the cell cycle, and if it is assumed that the *c-myc* product is important to the cell cycle control mechanism then these cells are immortalised through having a facilitated transit from G0 to G1.<sup>80</sup> In normal human myeloid cells *c-myc* seems to be expressed after exposure to colony stimulating activity and is associated mainly with promyelocytes rather than more mature granulocyte precursors.<sup>65</sup> Although expression of *c-myc* seems to be associated with the transition from G0 to G1, it does not vary in expression through the cell cycle from G1 to G2/M.<sup>81</sup>

Related work in fibroblasts has shown that *c-fos* is expressed transiently even earlier in the cell cycle than *c-myc*.<sup>82-84</sup> After stimulation of quiescent macrophages, however, maximum concentration of *c-fos* product occurs later than that of *c-myc*, suggesting that it may have a different role in different cell types.<sup>85</sup> Evidence suggests that the *c-fos* protein may play a specific part in the differentiation of murine<sup>71,84</sup> or human<sup>85</sup> myeloid leukaemia cell lines *in vitro*.<sup>85</sup> When differentiation of mouse erythroleukaemia cells<sup>86</sup> of HL-60 cells<sup>72,87</sup> is induced *in vitro* expression of *c-myc* and cell proliferation are suppressed. Conversely, removal of the differentiating agent allows re-expression of *c-myc*. Suppression of *c-myc* during cell differentiation is accompanied by increased expression of *c-fos* when TPA is used as a differentiating agent but not when 1,25-dihydroxycholecalciferol or retinoic acid is used.<sup>85</sup>

The full, and probably complicated, picture of control of growth and the acquisition of a malignant phenotype will not emerge for some time, but there are clear indications that the multistage process of transformation may result from a variety of stimuli. Deuel and Huang suggested that any genes that code for proteins mediating the cellular response to growth factors may be potential oncogenes.<sup>44</sup> Any abnormality in the expression of these genes, whether as a result of deletion, duplication, abnormal activation, suppression, or mutation, can result

in a change in the control of proliferation, which may contribute to the malignant process. The characteristics of the malignant cell include both immortalisation and autonomy from exogenous growth factors, both of which could result from genetic lesions. Sporn and Todaro introduced the concept of transforming growth factors; these are produced by the cell and stimulate its growth by binding to its own receptors.<sup>88</sup> This autocrine hypothesis has been extended to include possible inhibitory factors, secretion of which may be absent in the transformed cell,<sup>45</sup> possibly as a result of a gene deletion. An increase in exogenous growth factor or other stromal influences may also play a part in the development of malignancy.<sup>73</sup> Boettiger *et al* produced an *in vitro* model of haemopoietic cell immortalisation that could be considered to be analogous to a preleukaemic state.<sup>89</sup> In long term marrow cultures infected with an RNA virus containing the *src* oncogene there is a dramatic change in the balance of early progenitors to mature myeloid cells with an increase in the former and a greater capacity for self renewal at the expense of differentiation. Although this system permits the continuous generation of stem cell clones *in vitro*,<sup>90</sup> it does not result in malignant transformation.

Several systems for cell culture have now been described in which single insults by themselves do not produce malignant transformation but a succession of two such events may.<sup>40</sup> An example of this is the inability of the *ras* oncogene to transform mouse embryo fibroblasts unless these have first become immortalised. If, however, transfection of both the *ras* gene and a *myc* gene are carried out together malignant transformation results.<sup>91</sup> Initial immortalisation of the target cell may be induced by treatment with mutagenic chemicals or radiation.<sup>53</sup> Acquisition of the malignant phenotype does not signify that the genetic evolution of the stem cell has ended. The process of tumour progression seen in many cancers is similar to the clonal evolution of leukaemia and occurs with changes in cellular and clinical characteristics.

#### EXPERIMENTAL MYELODYSPLASIA

The pathogenesis of myelodysplastic states and their relation to leukaemia may be seen during experimental induction of leukaemia in animals. An acute blast cell leukaemia can be rapidly induced in rats by five successive doses of dimethylbenzanthracene.<sup>92</sup> If only a single dose is given the initial marrow hyperplasia recovers, and the subsequent hyperplasia is usually myeloid but may be erythroid in type.<sup>93</sup> Dyserythropoiesis and neutrophil and monocyte abnormalities occur, and death eventually results from anaemia or septicaemia. A T cell leukaemia

can be induced in mice by the administration of butylnitrosourea, and in this case too a preleukaemic phase occurs.<sup>94</sup> Several such models exist, and in some cases such as benzene tumours may be induced outside the haemopoietic system.<sup>95</sup>

Other workers have used leukaemia induced by viruses to study the developing disease. Erythroleukaemia induced by the Friend virus in mice is characterised by two stages. Firstly, erythroid hyperplasia and polychaemia occur but no leukaemic cell line can be isolated. At this point there is an increase in circulating burst promoting activity, and an increase in the erythroid progenitors BFU-E and CFU-E, showing increased sensitivity to burst promoting activity and erythropoietin, respectively, and probably an increase in stem cells.<sup>96</sup> The second stage is overt malignancy. This picture was interpreted as an initial production of burst promoting activity induced by virus with a consequent stimulation and expansion of BFU-E and the stem cell population. This facilitates their infection with a virus and a consequent increase in sensitivity to circulating burst promoting activity and leukaemic change. In vitro the Friend virus can transform erythroid<sup>97</sup> or myeloid<sup>98</sup> progenitors. Heard *et al*<sup>99</sup> infected long term mouse marrow cultures and defined three stages in the development of myeloblastic leukaemia.<sup>99</sup> Firstly, an abnormal response of progenitors to granulocyte-macrophage colony stimulating factor (GM-CSF), promoting proliferation rather than differentiation; secondly, autonomous growth of progenitors in the absence of adherent cells, possibly associated with autocrine production of GM-CSF; and, finally, the ability of cells from the cultures to form tumours when inoculated into suitable recipient mice. This type of model permits further study of the nature of the cellular changes at each stage. Although most of this work concentrates on the transformation process in the potentially leukaemic target cell, the effect of mutagenic insults on the immune system with the consequent failure of defence against an emerging malignant clone is of unknown importance. The correlation of resistance to the leukaemogenic effects of irradiation in mice with their resistance to natural killer cell suppression induced by radiation<sup>100</sup> suggests that this factor may be important, but exactly how important has not yet been determined.

#### LEUKAEMIA AND PRELEUKAEMIA INDUCED BY TREATMENT

The carcinogenic potential of radiation and many of the drugs used in human cytotoxic treatment are well recognised,<sup>101 102</sup> and permanent bone marrow damage is not an uncommon complication in the treatment of malignancy. Myelodysplastic syndrome may occur after such treatment, and this in turn may

evolve into frank leukaemia.<sup>102-105</sup> There are analogies between this situation and the chemical induction of preleukaemia in animals. Although short term exposure to a cytotoxic drug reduces the size of the pluripotential haemopoietic progenitor compartment, recovery may occur at varying rates and hypoplasia may be prolonged. Much will depend on whether further exposure to the drug occurs before the stem cells have fully recovered from the initial insult. Return of the stem cells to normal numbers does not necessarily mean that they will behave normally. After treatment with busulphan the CFU-S in mice has a reduced capacity for self replication<sup>106</sup> and a decreased ability to repopulate the marrow of an aplastic recipient.<sup>107</sup> Morley *et al* showed that mice given short term treatment with busulphan apparently recover haematologically only to die some time later from marrow hypoplasia.<sup>108-110</sup> During the period of apparent normality progenitor cells are reduced in number. Late haemopoietic damage after treatment with cytotoxic drugs always seems to be related to residual stem cell abnormalities or a reduction in their number. Whether such abnormalities are enough in themselves to cause progression to a myelodysplastic or preleukaemic state is uncertain. Possibly, the damaged stem cells are less able to deal with subsequent toxic insults, respond abnormally to later physiological stress, or have already proceeded part of the way to malignant transformation and are awaiting the final transforming event.

Of all the human malignancies related to previous treatment for cancer, acute myeloblastic leukaemia is the most common. Kapadia *et al* found nearly 6% of all cases could be attributed to this cause.<sup>111</sup> The median interval between initial treatment and the occurrence of secondary leukaemia is about five years.<sup>112 113</sup> Incidence depends on the type of drug regimen, dosage, combination with radiotherapy, and the duration of follow up, but secondary leukaemia may occur in up to 7% of patients with myeloma,<sup>114</sup> 10% of those with Hodgkin's disease,<sup>103</sup> 1.5% of those with ovarian cancer,<sup>115</sup> and 0.3% of those with breast cancer.<sup>116</sup> Leukaemia is commonly preceded by a myelodysplastic phase, often with pancytopenia, and the bone marrow may show the morphological features of refractory anaemia with excess blasts with dyserythropoiesis and abnormal megakaryocytes.<sup>103 117</sup> Karyotypic abnormalities and impaired growth of granulocyte-macrophage progenitors (CFU-GM) in vitro are also common.<sup>100 101</sup>

#### EVOLUTION OF THE MYELOYDYSPLASTIC SYNDROMES

The stages in the development of the myelodysplastic process (Fig. 2) evolve differently in different

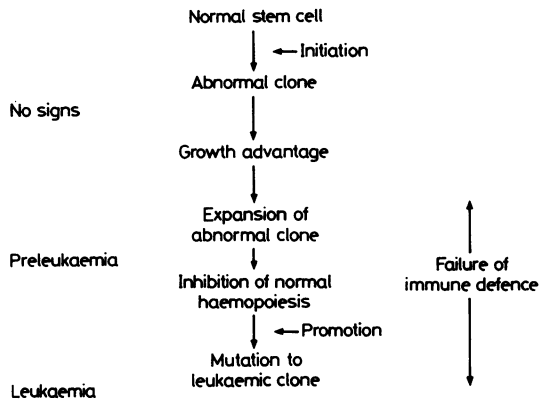


Fig. 2 Hypothetical pathway for evolution of myelodysplasia. Death may occur after expansion of normal clone and suppression of normal haemopoiesis before gross leukaemic changes are seen.

patients. The first mutation giving rise to an abnormal clone may be more likely to occur in an expanded and rapidly proliferating haemopoietic system, but we have no evidence of this in man. Expansion of the abnormal population and emergence of a leukaemic cell line may become more likely when failure of immune surveillance has occurred.

New clones may emerge after initiation but before clinical leukaemia is recognised, and clonal evolution may continue after the first overtly leukaemic cells are recognised. Expansion of an abnormal haemopoietic population may result from the acquisition of "immortality" through a loss of normal control mechanisms for synthesis of DNA and autonomy from normal growth or inhibitory factors. The suppression of normal haemopoiesis may result from inhibition by the abnormal cells. None of these factors has yet been clearly shown in patients with myelodysplastic syndrome. In clinical terms prognosis is related to the haematological state as determined by examination of the blood and marrow, cytogenetic abnormalities, and abnormal progenitor cell behaviour. Many patients succumb to infection or bleeding without any manifestations of frank leukaemia, and it is not clear whether the variety of haematological changes represent different disease entities or stages in the development of malignant change. Evidence in support of clonal evolution is beginning to emerge. In a group of 65 patients in whom serial observations were made<sup>36</sup> six of 17 progressing to acute myeloblastic leukaemia showed karyotypic evolution either before or at the time of leukaemic change. Thirteen patients with no leukaemic progression also showed karyotypic change, but no record was made of any change in their haematological state. Clonal evolution in

myelodysplastic syndrome has been observed in other patients, associated with both leukaemic transformation<sup>9 117 119</sup> and an aplastic phase in a pre-existing refractory anaemia.<sup>9 120</sup> Tricot *et al* suggested that three patterns of evolution can be distinguished: a long period of stability with only occasional karyotypic evolution; an abrupt change in a stable state, often with karyotypic evolution and the emergence of clinical acute myeloblastic leukaemia; and a gradual increase in the percentage of bone marrow blasts with increasing haemopoietic failure but no new cytogenetic changes.<sup>121</sup> Those patients with unstable clones and a high risk of sudden emergence of acute myeloblastic leukaemia were characterised by a high incidence of karyotypic abnormalities when first seen.

### Functional abnormalities

#### BLOOD AND MARROW CELLS

Most patients with myelodysplastic syndrome are anaemic and have a normal or low reticulocyte count. Red cells are usually normocytic or macrocytic, although microcytosis may occur,<sup>122</sup> and abnormal shapes, hypochromasia, and stippled cells and nucleated red cells may be seen. These changes are associated with gross dyserythropoietic appearances in the bone marrow with both nuclear and cytoplasmic abnormalities. There may be erythroid hyperplasia, and sideroblastic granules can usually be found in a varying proportion of erythroblasts, reflecting either ferritin aggregates or iron loaded mitochondria. There does not seem to be a clear demarcation between sideroblastic and non-sideroblastic cases.<sup>20 30</sup> The mature red cells show a wide variety of metabolic abnormalities,<sup>123 124</sup> which may be associated with the reappearance of haemoglobin F,<sup>125 126</sup> and changes in membrane antigens.<sup>126 127</sup>

The peripheral blood granulocyte count is usually normal or low. Those patients with a raised monocyte count are designated as having chronic myelomonocytic leukaemia. Granulocytes may show reduced segmentation (pseudo-Pelger phenomenon) or reduced or absent granulation. A few myelocytes and blast cells may be present, and some cells may be difficult to classify as either myeloid or monocytic. Cytochemical abnormalities in granulocytes include reduced myeloperoxidase<sup>128 129</sup> and the presence of increased monocyte type esterase.<sup>130</sup> These cells also show defective phagocytosis, bactericidal activity, adhesion, and chemotaxis.<sup>128 129</sup> Clark *et al* showed abnormalities of both myeloid and macrophage lineage surface markers.<sup>131</sup>

Thrombocytopenia is common in myelodysplastic

syndrome, and the platelets produced may be abnormal in both morphology<sup>132</sup> and function.<sup>133</sup> Micromegakaryocytes are common in the bone marrow, and these are not only smaller than normal but have poor formation of granules.<sup>14 134</sup>

The lymphoid system is affected in myelodysplastic syndrome, and Prchal *et al* showed that the abnormal cells may be derived from the same clone as the pathological erythroid and myeloid cells.<sup>22</sup> There is often a peripheral blood lymphopenia with a reduced number of T helper cells.<sup>135</sup> T cells may also show increased radiosensitivity, reduced response to mitogens, and poor colony formation in vitro.<sup>136</sup> B cells may be deficient in EBV receptors.<sup>137</sup> Reduced natural killer activity is associated both with reduced numbers of natural killer cells<sup>138</sup> and, possibly, with inadequate production of  $\alpha$  interferon and a failure of natural killer cells to respond to interferon.<sup>139</sup>

#### *Proliferation of bone marrow cells*

The "megaloblastoid" appearance of the erythroid precursors in refractory anaemia with or without sideroblasts indicates an underlying abnormality of synthesis of DNA. Wickramasinghe reported on bone marrow cells from normal subjects and patients with idiopathic acquired sideroblastic anaemia after estimation of cellular DNA content and synthesis of DNA by incubation with tritiated thymidine and Feulgen staining.<sup>140</sup> Polychromatic erythroblasts from patients with idiopathic acquired sideroblastic anaemia showed an increased number of G<sub>2</sub> cells and a reduced number in S phase, a picture similar to that in vitamin B<sub>12</sub> deficient marrow. Mitrou and Fisher made similar observations in six patients with refractory anaemia, those cells with the most pronounced "megaloblastoid" change showing the greatest deviation from normal.<sup>141</sup> A parallel study of proliferating myeloid cells showed similar abnormalities in the group as a whole, but within the same marrow specimen it was possible for severe myeloid abnormalities to be accompanied by normal erythropoiesis. A low labelling index for nucleated red cells and myeloid cells in patients with myelodysplastic syndrome has been confirmed by several workers.<sup>142-144</sup> Those patients with the lowest labelling index, indicating the greatest impairment of synthesis of DNA, have the poorest prognosis and the highest probability of leukaemic change.

The application of flow cytometry to measurements of DNA in whole marrow populations confirms that those patients with myelodysplastic syndrome with the highest proportion of cells in S and G<sub>2</sub> phases of the cell cycle have the best prognosis and those with an increased proportion of cells

in G<sub>1</sub> have the greatest risk of leukaemic change.<sup>34</sup> Patients with refractory anaemia have a greater proportion of cells in G<sub>2</sub>/M than normal, while in patients with refractory anaemia with excess blasts there seems to be an increase in G<sub>1</sub> cells similar to that seen in acute myeloblastic leukaemia.<sup>35</sup>

The evaluation of erythroid production by ferrokintic techniques has developed from early studies, showing that in refractory anaemia total erythroblast numbers, erythrocyte production, and lifespan are all reduced.<sup>145</sup> In 10 patients with sideroblastic anaemia Singh *et al* found a progression from mild impairment of synthesis of haemoglobin through a phase of ineffective erythropoiesis to eventual complete failure of red cell production.<sup>146</sup> Barosi *et al*, using a mathematical model for the interpretation of their data, showed convincingly that ineffective erythropoiesis was the major factor resulting in anaemia in patients with idiopathic acquired sideroblastic anaemia.<sup>147</sup> In an extension of this work quantitative data on total marrow iron turnover, ineffective erythropoiesis, and red cell lifespan in 43 patients with myelodysplastic syndrome were studied by cluster analysis.<sup>33</sup> The data resolved into three clusters, one consisting almost entirely of patients with refractory anaemia with excess blasts, one of patients with sideroblastic anaemia, and the third a mixture of patients with refractory anaemia and sideroblastic anaemia. Survival curves of these three groups were significantly different, the poorest survival being in the first group, with the lowest marrow iron turnover, and the best survival in the second group with erythroid hyperplasia and a high level of ineffective erythropoiesis. More recent studies by May *et al* showed major defects in erythropoiesis in all types of myelodysplastic syndrome with ineffective erythropoiesis being an early manifestation.<sup>20</sup> There was no correlation between erythroid output measured by ferrokinetics and reticulocyte count in the peripheral blood, percentage erythroblasts in the marrow, or the number of erythroid progenitors measured by clonal assay in vitro; nor was increased ineffective erythropoiesis related to morphological evidence of dyserythropoiesis.

#### HAEMOPOIETIC PROGENITORS

In vitro culture of haemopoietic progenitors has proved useful in diagnosing myelodysplastic syndrome and in predicting prognosis. Although variations in technique and the assessment of results make a strict comparison of different data difficult, a general consensus view has emerged. Milner *et al* showed that in refractory anaemia, refractory anaemia with excess blasts, chronic myelomonocytic leukaemia, and CFU-GM cultures from bone mar-

row displayed a reduction of colony and cluster growth.<sup>31</sup> This has been confirmed by many workers.<sup>13 32 148-150</sup> The MD Anderson group showed that the growth patterns of CFU-GM in vitro could be classified into five categories, two of which they described as non-leukaemic, and suggested that these could be related to prognosis.<sup>32 149</sup> In five of 19 patients with refractory anaemia they found a pattern of leukaemic growth characterised by reduced colony growth and increased numbers of microclusters.<sup>32</sup> This was observed in 48 of 65 patients with "oligoleukaemia—that is, with up to 50% marrow myeloblasts.<sup>144</sup> In both cases progression of the clinical disease was associated with a progression of in vitro growth to a pattern more representative of leukaemia. Similar abnormalities of growth were seen using a diffusion chamber technique in place of agar culture.<sup>151</sup> In those cases in which repeated marrow cultures have been carried out the abnormalities increased as the disease advanced.<sup>150</sup> Ruutu *et al* found normal CFU-GM growth in vitro in half of the 44 patients of myelodysplastic syndrome they studied, and these included all the patients with idiopathic acquired sideroblastic anaemia.<sup>152</sup> May *et al* noted that six of 40 patients with myelodysplastic syndrome had increased colony growth, four of whom had sideroblastic anaemia.<sup>20</sup> Normal growth in sideroblastic anaemia has also been noted by others.<sup>153 154</sup> Leukaemic cultures have an increased proportion of low density progenitors, and this is also seen in myelodysplastic syndrome.<sup>13</sup>

The abnormalities of in vitro growth are compatible with the gradual replacement of normal haemopoietic cells by an abnormal clone. This may occur through an intrinsic stem cell defect giving the new clone a growth advantage, either through insensitivity to normal feedback regulation or by inhibiting normal haemopoiesis. There may, of course, be an abnormality of stromal or regulating cells with abnormal suppression<sup>155</sup> or failure to produce growth factors. Greenberg and Mara found that patients with myelodysplastic syndrome had normal marrow and urinary concentrations of colony stimulating activity,<sup>13</sup> although Francis *et al* showed that many patients had increased amounts of endogenous marrow colony stimulating activity.<sup>156</sup> High colony stimulating activity was associated with a high risk of early leukaemic transformation.

Erythroid colony growth is usually decreased in most marrow<sup>20 31 153 157</sup> and peripheral blood cultures,<sup>158</sup> although Koeffler *et al* found increased growth in a few cases, together with normal sensitivity of CFU-E to erythropoietin.<sup>159</sup> CFU-GEMM also seemed to be reduced in patients with myelodysplastic syndrome.<sup>160</sup> A technique for culturing blast cell colonies in patients with acute

leukaemia has been described, and similar colonies have been grown from the peripheral blood of these patients.<sup>160</sup> The clinical importance of these findings is yet to be explored.

#### CYTOGENETIC ABNORMALITIES

Karyotype aberrations are common, and abnormal clones are found in about 50% of cases.<sup>21 36-38 161</sup> In a study of bone marrow cells in 35 patients with myelodysplastic syndrome, using flow cytometry after DNA staining, aneuploidy was confirmed in over half the patients, and in two cases distinct populations of different DNA content were seen.<sup>35</sup> Many of the non-random chromosomal aberrations are similar to those found in some cases of acute myeloblastic leukaemia.

The common abnormalities in myelodysplastic syndrome are monosomy 7 or 7q-, trisomy 8, monosomy 5 or 5q-, and loss of the Y chromosome. We can speculate on the relevance of lost genes on these chromosomes to the pathogenesis of the disorder (Table). Chromosome 5 has two identified oncogenes, c-fos and c-fms, on its long arm, and the genes for leucocyte interferon and dihydrofolate reductase are also found here.<sup>162</sup> Chromosome 7 contains the oncogene erb B, the genes for neutrophil membrane glycoprotein 130, and the  $\beta$  chain of the T cell antigen receptor.<sup>162</sup> Trisomy 1q, 9 and 21, 20q-, and iso 17q have also been reported.<sup>21</sup> Certain chromosomal aberrations found in acute myeloblastic leukaemia are rarely found in myelodysplastic syndrome, suggesting that patients with these abnormalities have not developed their disease from a pre-existing myelodysplastic syndrome. These include the 8:21 translocation often found with acute myeloblastic leukaemia of FAB type M2, the 15:17 translocation often found with acute promyelocytic leukaemia (FAB type M3), and the 19:22 of chronic granulocytic leukaemia.

The presence of chromosomal abnormalities seems to be associated with leukaemic change and a more rapid progression of the disease.<sup>21 26</sup> Sagar presented the hypothesis that an initiating preneoplastic

Table *Interrelationship between chromosomal abnormalities, clinical features, and gene deletions in the myelodysplastic syndrome*

5q-(15→30)	Monosomy 7	Trisomy 8
<i>Clinical defects:</i>		
Macrocytosis	Pancytopenia	
Anaemia	Reduced leucotaxis	
Thrombocytosis	Infection	
Abnormal megakaryocytes		
<i>Affected genes:</i>		
Leucocyte interferon	Neutrophil GP130	
c-fos	erb B	c-myc
Dihydrofolate reductase	q11-22 T antigen R ( $\beta$ )	
c-fms	q34	



genetic event may predispose to an increasing incidence of transpositions, which then occur in an accelerated manner, and that the subsequent chromosomal chaos is more important in the evolution of malignancy than the precise mutations present.<sup>163</sup> This is consistent with the observations of Tricot *et al.*, who suggested that those myelodysplastic patients who have cytogenetic abnormalities when first seen are likely to have unstable clones with an increased risk of further genetic changes and the appearance of highly malignant growth characteristics.<sup>121</sup> In those patients with no initial chromosomal abnormalities but an independent growth pattern the subsequent appearance of an abnormal karyotype is rare but is usually associated with the onset of acute myeloblastic leukaemia. Patients developing myelodysplastic syndrome after treatment with cytotoxic drugs and irradiation more commonly show multiple chromosomal abnormalities, and such patients have a higher incidence of subsequent leukaemic change. Anderson and Bagby suggested that in patients with a single chromosomal abnormality the incidence of leukaemic change may not be especially high and that in patients with no history of exposure to alkylating agents cytogenetic abnormalities may not indicate an appreciably higher risk of leukaemic change.<sup>164</sup> It is not entirely clear whether those patients with some residual cells with a normal karyotype have a better prognosis than those with entirely abnormal cells.<sup>21</sup> Rowley *et al.* noted that in 23 of 26 patients with myelodysplastic syndrome or acute myeloblastic leukaemia after previous treatment for malignant disease there was loss of part or the whole of chromosome 5 or 7, or both.<sup>118</sup> Van Den Bergh *et al.*<sup>165</sup> and Mitelman *et al.*<sup>166, 167</sup> found the same abnormalities in patients with a history of occupational exposure to myelotoxic chemicals who developed acute myeloblastic leukaemia *de novo*, and this has prompted the suggestion that non-random loss of chromosomes 5 and 7 may indicate previous exposure to an environmental mutagen.

Clinical progression of myelodysplastic syndrome may be associated with the development of a new clone with a greater growth advantage than the original abnormal cells that gave rise to the syndrome. The emergence of new clonal karyotypic abnormalities in patients with myelodysplastic syndrome has been observed in relation to clinical leukaemic change<sup>9, 118, 121</sup> or non-leukaemic change in clinical signs<sup>120</sup> or without any obvious change in clinical condition.<sup>118</sup> In the rare case in which myelodysplastic syndrome terminates in the syndrome of chronic granulocytic leukaemia this does not seem to be associated with the Ph<sub>1</sub> chromosome,<sup>168</sup> although one case of sideroblastic anaemia with one third of

the marrow mitoses possessing the Philadelphia chromosome has been recorded.<sup>169</sup> In other cases progression may simply be the clinical manifestation of a gradually expanding abnormal clone or a change in the balance between proliferation and differentiation within the clone giving rise to a predominance of more primitive cells. It is not known whether changes in the immune system or the production of growth factors influence clinical evolution or whether such changes that have been observed are secondary phenomena.<sup>156, 170</sup>

Although the presence of clonal karyotype abnormalities in patients with myelodysplastic syndrome is common and seems to be of prognostic importance, it should be noted that the disappearance of abnormal clones has been reported,<sup>171</sup> and not all patients with a persistent abnormality proceed to a rapid leukaemic transformation.

**5q- Syndrome** The now classic syndrome of refractory anaemia associated with a deletion in the long arm of chromosome 5 has been described by many workers.<sup>26, 38, 172-174</sup> Patients usually have macrocytic anaemia and erythroid hyperplasia in the bone marrow. Thrombocytosis and poorly lobed megakaryocytes may occur, but granulocyte-macrophage production is usually normal. Tinagat *et al.* found five such cases among 37 patients being investigated for refractory or aplastic anaemia and suggested that the condition may be underdiagnosed.<sup>175</sup> The suggestion that it is not a pre-leukaemic condition<sup>176</sup> does not seem to have been borne out, although it seems to follow a chronic course with a low incidence of leukaemic change.<sup>26</sup> The syndrome is more common in women than men.

The nature of the lesion is not known. Tinagat *et al.* found that the breakpoints on 5q were not constant, although 5q- (15→30) was always deleted.<sup>175</sup> A gene related to interferon has been located in this region, and patients with 5q- or -5 have been shown to have deficient leucocyte interferon production.<sup>177</sup> Various syndromes, however, have been described in patients with this chromosomal anomaly, including one with macrocytosis but no anaemia<sup>20</sup> and others with polycythaemia or idiopathic acquired sideroblastic anaemia.<sup>178</sup> The 5q- anomaly may be found in combination with other chromosomal abnormalities and in such cases leukaemic change is common.<sup>26</sup> It is also found as one of the multiple aberrations in acute myeloblastic leukaemia and lymphoblastic leukaemia.<sup>26, 175</sup>

**Monosomy 7** When monosomy 7 occurs together with myelodysplastic syndrome a hypoproliferative marrow with pancytopenia associated with defective neutrophil chemotaxis and recurrent infection, followed by progressive evolution into acute myeloblastic leukaemia, is the characteristic profile.<sup>175</sup> The

condition may be found in children. Defective chemotaxis in the monosomy 7 syndrome has been well recognised,<sup>179</sup> leading to the suggestion that a gene on this chromosome may have a role in neutrophil function. Although this is supported by more recent studies, it seems that peripheral blood neutrophils derived from residual normal progenitors in such cases also show reduced chemotaxis, indicating a less simple mechanism than a single clonal abnormality.<sup>180</sup> Gahmberg *et al* noted a deficiency of a neutrophil glycoprotein GP130 in the cells of a patient with monosomy 7,<sup>181</sup> although it is not known whether this can be related either to defective chemotaxis or to defective control of granulopoiesis. Leukaemic transformation in Fanconi's anaemia may be associated with the emergence of a clone characterised by -7,<sup>182</sup> suggesting a possible role for this deletion in the process.

### Therapeutic possibilities

Until fairly recently treatment for patients with myelodysplastic syndrome relied largely on giving blood components to remedy cytopenias, antibodies to counter infection, and cytotoxic drugs in patients with more obvious malignant manifestations.<sup>183-184</sup> Chemotherapy has not been conspicuously successful either in the preleukaemic phase or in acute myeloblastic leukaemia arising in such patients. The suggestion that growth and differentiation may be modified both in preleukaemic and possibly in leukaemic patients by either specific biological factors or pharmacological means presents an exciting prospect that has stimulated widespread clinical activity.<sup>185-186</sup>

Differentiation in suspension cultures has been studied largely in leukaemic cell lines with myeloid (HL-60, KG1, ML3), monocytic (U-937), or erythroid (K-562) characteristics. A wide variety of differentiation inducers have been used, including dimethylsulphoxide, tetradecanoylphenol acetate, retinoic acid, 1,25-dihydroxycholecalciferol, interferon, and less well defined products of mononuclear cells.<sup>185-187</sup> Some of these may have physiological importance, others are too toxic or carcinogenic for systemic use in man.

The rationale for using differentiating agents in treating myelodysplastic syndrome depends on a view of the abnormal clone existing in a state of arrested differentiation along a normal maturation pathway.<sup>62</sup> In some leukaemias poorly differentiated cells from different lineages may be derived from a common abnormal stem cell, sometimes called "biphenotypic leukaemia".<sup>188</sup> The alternative hypothesis that the abnormal clone arises from a

misprogrammed stem cell<sup>69</sup> makes differentiation induction less feasible. Lineage infidelity has been described both in acute myeloblastic leukaemia<sup>69,189</sup> and in leukaemic cell lines.<sup>190-191</sup> The presence of inappropriate surface markers on granulocytes and monocytes in myelodysplastic syndrome suggests that abnormal rather than arrested differentiation may also occur in this condition.<sup>131</sup> Whatever the mechanism, there is ample evidence that differentiation can be induced experimentally *in vitro* and, possibly, *in vivo*. Some agents, such as tetradecanoylphenol acetate, retinoic acid, and 1,25-dihydroxycholecalciferol seem to accelerate differentiation with the production of non-dividing mature end cells. Inhibitors of synthesis of DNA such as cytosine arabinoside (ara-C) may slow proliferation while allowing differentiation to continue.

### RETINOIDS

Retinoic acid is known to prevent chemically induced skin cancer in mice, but other epithelial tumours do not always respond so well. Many hundreds of retinoid analogues have been tested for their ability to cause regression of skin papillomas, and interest in their capacity to reverse malignant transformation has grown considerably.<sup>192-194</sup> The effect of retinoic acid on skin tumours appears to be the inhibition of tumour promotion.<sup>195</sup> Retinoids are known to inhibit proliferation of many cell types, and this seems to be linked to their effect on differentiation.<sup>194-196</sup> HL-60, a promyelocytic cell line, and U-937, a histiocytic cell line, respond to retinoic acid by differentiating into morphologically and functionally mature cells similar to granulocytes and monocytes, respectively,<sup>197-199</sup> although they stop proliferating at the same time. The mechanism of action is unknown but may be related to inhibition of ornithine decarboxylase,<sup>200-201</sup> microfilament formation, or the effect on cell membrane components.<sup>193</sup> The importance of cellular retinoic acid binding proteins is not known.<sup>202</sup>

In a few patients with promyelocytic leukaemia differentiation of these cells has been induced by retinoic acid both *in vitro*<sup>203</sup> and *in vivo*.<sup>204</sup> Clonal growth of CFU-GM is usually inhibited by retinoic acid, although some stimulation may occur at low concentrations.<sup>205</sup> Bradley *et al* found inhibition of both proliferation and differentiation under these conditions.<sup>157</sup> Bailey-Wood *et al* found that CFU-GM from some patients with myelodysplastic syndrome may have a greater sensitivity than normal to the inhibitory effect of retinoic acid *in vitro*,<sup>207</sup> suggesting that individual clinical responses may vary.

Individual cases of clinical response to retinoic acid in promyelocytic leukaemia have been

recorded.<sup>204 208</sup> Gold *et al* treated 15 patients with myelodysplastic syndrome with oral retinoic acid and found a haematological response in five, including a reduction in marrow blasts and increases in leucocyte count, platelets, and haemoglobin concentration.<sup>209</sup> Hepatotoxicity, hyperkeratosis, and hyperostosis may be limiting factors in prolonged treatment.

#### VITAMIN D<sub>3</sub>

Proliferation of both murine and human myeloid leukaemia cells<sup>210 211</sup> and normal murine myeloid-macrophage progenitors (CFU-C)<sup>212</sup> are suppressed by 1,25-dihydroxycholecalciferol. The differentiation of HL-60 by 1,25-dihydroxycholecalciferol is associated with binding to a specific cytosol protein, similar to that found in other vitamin D<sub>3</sub> target tissues, followed by transport to the nucleus.<sup>213</sup> The mechanism seems to be similar to that generally associated with steroid hormone effects. Honma *et al* were able to prolong the survival of mice inoculated with murine myeloid leukaemia cells by giving vitamin D<sub>3</sub> analogues.<sup>214</sup>

The exposure of HL-60 to physiological concentrations of 1,25-dihydroxycholecalciferol results in the acquisition of monocyte like features,<sup>215 216</sup> and this is preceded by decreased expression of the c-myc oncogene.<sup>87</sup> Removing the vitamin results in re-expression of the oncogene, showing a relation between 1,25-dihydroxycholecalciferol and expression of c-myc, proliferation, and differentiation. Both normal and leukaemic human myeloid stem cells can be induced to macrophage differentiation by 1,25-dihydroxycholecalciferol.<sup>217</sup>

#### CYTOTOXIC DRUGS

It has been suggested that low concentrations of some cytotoxic drugs may induce differentiation of leukaemic or preleukaemic cells.<sup>218–220</sup> The clinical use of low dose ara-C in myelodysplastic syndrome, however, usually results in considerable toxicity with increased pancytopenia before any beneficial effect is observed.<sup>221</sup> When an abnormal clone can be identified by specific cytogenetic changes treatment with low dose ara-C results in suppression of this clone rather than maturation,<sup>222</sup> suggesting that the effect of even low concentrations of drugs is due to cytotoxicity rather than stimulated differentiation.

Wisch *et al* treated eight patients with myelodysplastic syndrome with seven to 21 day courses of intravenous low dose ara-C and obtained delayed remissions after eight of 13 courses had been taken.<sup>221</sup> Tricot *et al* administered 38 courses to 26 patients and found either a "good" or a "partial" response after 19 of the courses had been taken.<sup>223</sup> The difficulty of evaluating a response combined

with the unpredictable course of the disorder does not allow firm conclusions to be drawn at the moment.

#### BIOLOGICAL DIFFERENTIATION FACTORS

In normal myelopoiesis proliferation and differentiation occur together under the stimulus of several colony stimulating factors. In myeloid leukaemia cell lines, in which proliferation occurs in the absence of differentiation, granulocyte colony stimulating factor (G-CSF) appears to have a specific differentiating effect not shown by other colony stimulating factors. This is sometimes called differentiating inducing factor.<sup>224 225</sup> There may be several differentiating inducing factor proteins, probably with molecular weights 20 000–40 000, and these seem to be produced by macrophages, T lymphocytes, and various cultured cells.<sup>226</sup> Sachs showed that in mice differentiating inducing factor, also known as macrophage granulocytic inducer-2 (MGI-2), is produced by the myeloid cells themselves.<sup>227</sup> Failure to differentiate in leukaemic states might be due either to a failure of response to MGI-2 or to a failure of production. This hypothesis is not universally accepted.<sup>226</sup> Differentiating inducing factor may be induced by the administration of endotoxin,<sup>228</sup> although such treatment also results in the production of tumour necrosis factor and interleukin-1. Possibly, the increasing number of pure growth regulators becoming available may have some therapeutic potential.

#### CURRENT PRACTICE

Therapeutic support for the patient with myelodysplastic syndrome has not changed radically despite recent attempts to modulate the abnormal haemopoiesis. The backbone of treatment remains replacement treatment for anaemia and other cytopenias, together with antibiotics when indicated. Although individual and variable responses occur with short courses of low dose ara-C, no good evidence is available with regard to patient survival. Similarly, there is little evidence to support the routine administration of retinoids.

#### Clinical diagnosis and investigation

The clinical diagnosis of fully developed myelodysplastic syndrome presents no problem, and the features described by the FAB group are well recognised.<sup>14</sup> For practical purposes it may be useful to apply arbitrary threshold values for haemoglobin concentration, neutrophil count, platelet count, etc, to avoid misdiagnosis in patients with minimal, and possibly irrelevant, morphological abnormalities<sup>20</sup>—namely, neutrophils less than 1.5

$\times 10^9/l$ ; circulating myelocytes, erythroblasts, or blasts; monocytes greater than  $1.0 \times 10^9/l$ ; haemoglobin less than 12.0 g/dl; mean cell volume greater than 104 fl. Other diagnostic criteria should be marrow blasts less than 30% with or without ring sideroblasts and non-response to hydroxocobalamin 1000  $\mu\text{g}$ , with folic acid 5 mg for 21 days, and pyridoxine hydrochloride 50 mg for 21 days.

The anaemia, or other cytopenia, that usually draws attention to the disorder should be confirmed as a persistent feature, unassociated with any other systemic disorder such as renal or hepatic failure or an excess alcohol intake. Some elderly patients with cytopenias and normal serum  $\text{B}_{12}$  and folate concentrations respond well to a short course of these vitamins and occasionally to pyridoxine. Such occult deficiencies should be excluded before making the diagnosis. Such patients usually have the expected morphological changes in the blood and marrow associated with dyserythropoiesis and abnormal granulocyte maturation, possibly with more specific anomalies such as ring sideroblasts, micro-megakaryocytes, or an increased number of blast cells. Further investigation may show cytochemical or surface antigen abnormalities in the bone marrow or failure of progenitor cells to grow normally in culture.

The potentially serious nature of this disorder warrants a reasonably high index of suspicion and a precise diagnosis in each patient. Confirmation of the haematological diagnosis depends on the facilities available, but the detection of chromosomal abnormalities or aneuploidy in bone marrow preparations gives direct evidence of a genetic abnormality. Defective colony growth in culture confirms a functional change in haemopoiesis that is not found in other haematological states.<sup>148</sup> All these abnormalities seem to relate to prognosis, but whether they enable a more precise prediction of outcome to be made than an assessment of peripheral blood count alone<sup>229</sup> remains to be seen. In those patients with myelodysplastic syndrome with minimal haematological changes such as morphological changes but no cytopenia, the more specialised investigations may be the only way to confirm the diagnosis.

### Unanswered questions

Myelodysplastic syndrome poses a fascinating and important clinical and biological problem. It illustrates the way in which minor aberrations of growth and differentiation can gradually evolve into a leukaemic state and provides an opportunity to study leukaemogenesis in man. There may well be an opportunity to apply our newly found knowledge

of genetics and growth control mechanisms to the study, and hopefully, to modify a clinical condition that has not so far yielded to any form of treatment. While the fully developed syndrome shows abnormalities in the haemopoietic stem cell and its progeny, we remain unclear about the parts played by growth factors, bone marrow stromal cells, and immune mechanisms. We do not know whether the observed abnormalities are linked to abnormal gene function, and we have no knowledge regarding the underlying factors initiating damage to stem cells. We are probably unable to detect the earliest subtle changes occurring in damaged stem cells, and this prevents us from defining those at risk from further evolution of the disease. We also do not know whether any of the abnormalities can be reversed by either biological or pharmacological agents, or whether it is possible to prevent clonal evolution in an already damaged cell population. We look forward to the answers to at least some of these questions.

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