Our perception of preleukaemia as a clinical syndrome has changed during the last 5-10 years. Block et al. (1953) observed that it was sometimes difficult to determine the exact time at which a particular patient with refractory anaemia might be classified as leukaemic and that in any case the diagnosis of preleukaemia could only be substantiated finally by the terminal appearance of overt clinical leukaemia. More recently, the preleukaemic states have been defined more precisely and the French–American–British (FAB) group have proposed that all these conditions are grouped together as the myelodysplastic syndromes (MDS) (Bennett et al., 1982). It is now generally accepted that this represents clonal abnormalities of haemopoietic stem cells characterised by a variety of phenotypic manifestations and with a high risk of eventual leukaemic transformation (Greenberg, 1983; Jacobs, 1985). The clinical syndromes of refractory anaemia, with or without sideroblasts, and with high or low numbers of blast cells in the bone marrow, have been fully described in recent reviews (Francis & Hoffbrand, 1985; Jacobs, 1985).

Increasing clinical awareness has resulted in the diagnosis of preleukaemic states at an earlier stage in their development but we still have no clear conception of how the abnormality arises or progresses to its eventual overtly malignant termination. In attempting to describe a model for this process we need to take account of the available clinical data in this condition, current hypotheses regarding the mechanism of malignant transformation and the probable identity of the target cell in the haemopoietic system.

Clinical data

Most patients with MDS are anaemic and a wide range of morphological, immunological and functional red cell abnormalities have been described (Jacobs, 1985). There may be a variety of metabolic abnormalities (Valentine et al., 1973; Cetto et al., 1982), the reappearance of haemoglobin F (Richard et al., 1979), acquired haemoglobin H (Boehme et al., 1978) and changes in membrane antigens (Levine et al., 1984). These changes are associated with gross dyserythropoietic appearances in the bone marrow with both nuclear and cytoplasmic abnormalities. There may be erythroid hyperplasia, and in such cases sideroblastic granules indicating mitochondrial iron deposition can usually be found in a varying proportion of erythroblasts.

The peripheral blood granulocyte count is usually normal or low and there may be a raised monocyte count. Granulocytes commonly show reduced nuclear segmentation and reduced or absent granules. Cytochemical abnormalities include reduced myeloperoxidase (Schofield et al., 1983) and inappropriately increased z-naphthyl acetate esterase activity (Scott et al., 1983). Abnormalities of both myeloid and macrophage lineage-specific surface markers are common (Clark et al., 1985). Neutrophil function is abnormal in about half of all cases (Bogaerts et al., 1983).

Thrombocytopenia is common and the platelets produced may be abnormal in both morphology (Maldonado & Pierre, 1975) and function (Lintula et al., 1981). Micromega-karyocytes with poor granule formation are common in the bone marrow (Bennett et al., 1982). Occasional patients with sideroblastic anaemia have been reported with an abnormally high platelet count (May et al., 1985; Carroll et al., 1986). Amongst 54 sideroblastic patients seen in Cardiff, 5 had a pathologically high platelet count.

Bone marrow morphology is virtually always abnormal in patients with MDS. Although the combination of hypercellularity with a peripheral blood cytopenia is commonly seen, the marrow may be hypoplastic (Fohlemeister et al., 1985b; Frisch & Bartl, 1986). Progression is marked by a gradual increase in the proportion of blast cells. In the FAB terminology, less than 5% blast cells is designated refractory anaemia (RA), 5–20% blast cells refractory anaemia with excess blasts (RAEB) and 20–30% blasts RAEB in transformation. The one year survival following diagnosis in our patients is: primary acquired sideroblastic anaemia (PASA) 95%, RA 64% and RAEB 35%. Most of these patients eventually die from either haemorrhage or sepsis following suppression of normal haemopoiesis and functional inadequacy of the abnormal haemopoietic clone. The classic picture of acute myeloblastic leukaemia only emerges in 17–40% of patients (Foucar et al., 1985; Todd & Pierre, 1986; Coiffier et al., 1983; Greenberg, 1983).

Abnormal proliferation of bone marrow cells

Wickramasinghe (1975) showed that an increased number of polychromatic erythroblasts from patients with PASA were in G1 and a reduced number in S phase, compared to normal, a picture similar to that in vitamin B12 deficient marrow. Many cells are arrested in midcycle or in G2 and this state is associated with defective protein synthesis and cell death which is expressed in functional terms as ineffective erythropoiesis. Mitrou and Fisher (1979) made similar observations in six patients with non-sideroblastic refractory anaemia. 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 (Hast & Reizenstein, 1977; Seigneurin & Holland, 1981; Karsdorf et al., 1983). Those patients with the lowest labelling index, indicating the greatest impairment of DNA synthesis, have the poorest prognosis and the highest probability of leukaemic change.

Flow cytometric measurements of DNA in whole marrow confirms that those myelodysplastic patients with the highest proportion of cells in S and G2 phases of the cell cycle have the best prognosis and those with an increased proportion of cells in G2/M have the greatest risk of leukaemic change (Montecucco et al., 1983; Peters et al., 1986).

The evaluation of erythroid production using a mathematical model for the interpretation of ferrokinetic data showed that ineffective erythropoiesis was the major
factor causing anaemia in patients with PASA (Barosi et al., 1978). 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 (Cazzola et al., 1982) and the data resolved into three clusters. The poorest survival was found in the cluster with the lowest erythroid production, and the best survival in the cluster with erythroid hyperplasia and a high level of ineffective erythropoiesis. More recent studies showed ineffective erythropoiesis to be an early manifestation of MDS (May et al., 1985) and erythroid hyperplasia seen in initial marrow aspirates to be associated with the longest survival (Jacobs & Clark, 1987).

Cyto genetic abnormalities

Karyotypic abnormalities in haemopoietic cells are common, being found in about 50% of cases by most workers (Sokal et al., 1980; Jacobs et al., 1986). A higher incidence has been found in some series (Yunis et al., 1986), possibly due to differences in patient selection and in laboratory techniques. Many of the non-random chromosomal aberrations are similar to those found in some cases of acute myeloblastic leukaemia (AML), though certain abnormalities typical of specific subgroups of AML are rarely seen in MDS. These include the 8:21 translocation found in acute myeloblastic leukaemia of FAB type M2, the 15:17 translocation often found with acute promyelocytic leukaemia (FAB type M3), and the 19:22 translocation of chronic granulocytic leukaemia.

The most common abnormalities are monosomy 5 or 7q-, monosomy 7 or 7q- and trisomy 8. Many others have been described. Patients with a normal karyotype usually have a longer survival (Todd & Pierre, 1986), those with an initial abnormality at the time of diagnosis tend to have a less stable karyotype, evolve into a ‘more malignant’ clinical group and have a poorer survival. Only a small minority of patients with erythroid hyperplasia and sideroblastic precursors have an abnormal karyotype, but those showing abnormalities usually have a rapid clinical evolution (Yunis et al., 1986). The presence of monosomy 7 or 7 deletions, usually of the long arm, is associated with a rapidly progressive course and a poor survival (Yunis et al., 1986). Patients with marrow morphology showing myeloid hyperplasia and an increased number of blast cells usually have a high incidence of karyotypic abnormalities, often with multiple complex derangements. They have a very poor prognosis. Multiple independent clones are commonly found in the preleukaemic state.

Although some patients with primary MDS have a history of exposure to chemical agents this does not appear to relate to a specific clinical picture or chromosome defect. Patients treated with chemotherapy or radiotherapy have a high incidence of secondary MDS and AML with a uniformly poor prognosis. This particularly malignant form of preleukaemia is associated with a clonal abnormality of chromosomes 5 and 7 in most cases. The critical regions appear to be 5q23-32 and 7q32-33 or 7q34-35 (Le Beau et al., 1986). Chromosomes 1, 4, 12, 14 and 18 appear to be involved significantly more often in therapy-induced MDS and AML than in the same conditions occurring de novo (Le Beau et al., 1986).

DNA content of bone marrow cells

It is often difficult to evaluate either the frequency or the extent of cytogenetic aberrations in patients with preleukaemia because chromosome spreads suitable for analysis may be few in number and poor in quality (Nowell, 1982). When ploidy is measured in marrow cells by DNA content with high resolution flow cytometry (Barlogie et al., 1983), aneuploidy is found in about half the patients (Clark et al., 1986), those with a low percentage of blasts tending to be hyperdiploid. A few patients with increased blast cell numbers show two separate G1/G2 peaks. Sideroblastic anaemia appears to be associated with hyperdiploidy, while hypodiploidy was found most commonly in patients with high numbers of marrow blasts (Clark et al., 1986). Patients with hypodiploid marrow cells had a significantly shorter survival time than other patients and hypodiploidy appeared to be a better indicator of prognosis than the marrow blast count. These data suggest that there is a relationship between the loss of chromosomal material and progression towards a leukaemic phenotype.

Haemopoietic progenitors

Impaired in vitro growth of CFU-GM colonies from marrow cells has been widely observed (Milner et al., 1977; Greensberg & Mars, 1979; Verma et al., 1979) and in some cases growth patterns have been related to prognosis (Verma et al., 1979; Spitzer et al., 1979). Erythroid colony growth is usually decreased in preleukaemic marrows (Milner et al., 1977; Amato & Kahn, 1983; May et al., 1985) but in PASA patients a combination of impaired or absent erythroid growth with normal or high CFU-GM growth is common (Ruuta et al., 1984; May et al., 1985).

Clinical progression

The evolution of one clinical type of MDS into another with a poorer prognosis and the eventual emergence of AML in a substantial number of cases is commonly observed. Fohlemeier et al. (1985b) have suggested, on the basis of sequential observations in individual patients, that in many cases an initial marrow hypoplasia is followed by a phase of erythroid hyperplasia and ineffective erythropoiesis. This, in turn, is succeeded by a phase of myeloid hyperplasia with the later emergence of circumscribed nests of blast cells, suggesting new subclones arising within the pre-existing dysplastic clone. This scheme is compatible with the association of sideroblastic change, erythroid hyperplasia and hyperdiploidy with early stage disease and a relatively good survival. It must be rare for a patient to proceed from a ‘myeloid’ to an ‘erythroid’ phase.

Possible models

The multistate nature of malignant transformation is well recognised (Knudson, 1973; Land et al., 1983; Klein & Klein, 1985), though even in a specific tumour type the nature and sequence of these steps may vary and the malignant phenotype may be reached by different routes. The gradual evolution of a haemopoietic stem cell through the sequential stages from an initial genetic insult, the development of preleukaemia and progression to leukaemia is clearly in accordance with this general model (Jacobs, 1985). The initially damaged stem cell may well be undetectable by conventional methods of examination and many such cells, or even minor clonal populations, may fail to survive either due to a metabolic disadvantage or through destruction by the host immune defences. A further genetic change may be necessary before growth control mechanisms are altered sufficiently to give the new clone a proliferative advantage over normal haemopoietic cells. Such an advantage and the consequent clonal expansion may be related to increased sensitivity to growth factors, autocrine stimulation or inhibition of normal haemopoiesis. Clinical, morphological and cytogenetic data suggest that the progress of preleukaemia is marked by greatly varying rates of clonal expansion, or by clonal evolution with new populations having greater malignant potential (Tricot et al., 1985; Tomonaga et al., 1984). Except in specific instances, such as post-chemotherapy marrow damage, the nature of the external attack is undefined, though more than one agent
can be involved. Similarly we have no clear information regarding the possibility of inherited genetic lesions predisposing to malignant change (de Vinuesa et al., 1985; Krontiris et al., 1986).

The genetic lesions giving rise to the preleukaemic process may be of two types. Firstly, each step may be related to a specific abnormality resulting in gene activation, amplification or deletion. At least three relevant groups of genes have been described, oncogenes (transforming genes), suppressor genes (anti-oncogenes) (Green & Wyke, 1985) and modulator genes (Klein & Klein, 1985). The recent burst of information linking oncogene proteins to specific functional roles in the regulation of cell proliferation (Burgess, 1985) and their place in multistep carcinogenesis has made it attractive to suppose that defects in the function of these genes play an important, if not an essential, role in the development of malignancy (Land et al., 1983; Deuel & Huang, 1984; Klein & Klein, 1985; Bradshaw, 1986). The evidence in relation to human disease is, however, circumstantial and some doubts have been expressed as to whether oncogene abnormalities are sufficient or even necessary to cause cancer (Duesberg, 1985; Editorial, 1986). Secondly the evolution of leukaemia is associated with increasing chromosomal instability characterised grossly by aneuploidy, translocations, marker chromosomes and, in some cases, a progressive loss of chromosomal material. Knuttila et al. (1984) suggest that in MDS chromosome instability is associated with hypodiploid clones and aberrations in chromosome 7, and Clark et al. (1986) have shown that hypodiploidy is associated with poor survival. Increasing genomic instability appears to be an important feature in the progression of preleukaemia and may well provide a mechanism for the emergence of multiple abnormal clones, usually with chromosome loss. Most of these will not survive but some will have increased malignant potential.

Functional abnormalities in MDS

Both leukaemia and myelodysplasia are characterised by gradual expansion of an undifferentiated self perpetuating stem cell population with poorly differentiated progeny. Knowledge of the mechanisms controlling the balance between self-renewal and maturation is imperfect and it is still not clear whether differentiation is precisely programmed at a genetic level or whether it is coupled to proliferative activity by humoral interactions (Sachs, 1982; Nicola & Metcalf, 1985).

In clinical terms the earliest manifestations of MDS commonly include evidence of ineffective erythropoiesis associated with cell cycle abnormalities and premortem cell death. An analogous, though not so well characterised, process probably occurs in myeloid precursors. At this stage an accompanying marrow hyperplasia compensates for cell loss and maintains the supply of end cells to the peripheral blood, though these may be functionally defective. This suggests that the normal feedback mechanisms regulating the flow of mature blood cells from the marrow are still maintained. At a later stage the production of mature cells gradually fails, either due to a growth factor/receptor abnormality or to the gradual expansion of the undifferentiated cell population, seen clinically as an increase in marrow blast cells.

It is tempting to try and implicate abnormalities of those genes, such as src, thought to be related to cell cycle control in the early phase of MDS and to speculate on the possible role of src in stem cell self renewal (Boettiger & Dester, 1986) or fos in differentiation (Muller, 1986), but at present we have no evidence for the involvement of these oncogenes in the preleukaemic process. It is simplistic to suggest that only a few specific genes are involved in the heterogeneous picture of myelodysplasia but the accumulating evidence that chromosomess 5, 7 and 8 are the most common sites for karyotype abnormalities does draw attention to the possible role of oncogenes at these locations. In addition to fms (5q34), coding for the M-CSF receptor (Sherr et al., 1985), myc (8q24), mos (8q22) and met (7q21-q31), the gene for GM-CSF is located at 5q21-q32 (Huebner et al., 1985), the A-chain of PGDF is coded on chromosome 7 (Bethsholtz et al., 1986) and M-CSF on chromosome 5 close to GM-CSF (Rowley, personal communication). However activated N-ras has also been identified in human myeloid leukaemia (Bos et al., 1985; Hirai et al., 1985) and abnormal expression of other oncogenes has been observed (Blick et al., 1984).

What is the target cell?

Evidence of the underlying clonality of haemopoiesis in MDS comes from an abundance of karyotype data and a few well studied cases with G6PD heterozygosity (Jacobs, 1985). There is, however, no clear evidence regarding the nature of the clonogenic cell. The patient studied by Prchal et al., (1978) showed the same G6PD isoenzyme in myeloid and erythroid cells, platelets, macrophages, T and B cells, suggesting a disordered pluripotential stem cell. In the patient of Abkowitz et al. (1984) T cells were polyclonal (B cells were not studied), suggesting either that the clonogenic cell had restricted lineage potential or that a substantial number of long-lived normal T cells persisted in the circulation. Raskind et al. (1984) investigated a patient with both enzyme and karyotypic evidence of clonality and showed that although B cell lines produced by Epstein-Barr virus transformation contained only the single G6PD isoenzyme found in erythroid and myeloid cells, none of them contained the clonal karyotype abnormality found in the marrow. These workers hypothesised that an initial lesion may be found in the pluripotent stem cells and a secondary lesion mediating chromosome abnormalities may occur in one of its progeny.

Similar evidence from the myeloid leukaemias suggests that the transformed cell may have restricted lineage potential. Fialkow (1985) found that in 5 elderly women with acute myeloblastic leukaemia (AML) the leukemic stem cell was pluripotential for myeloid, erythroid, megakaryocytic and probably B-cell lines. However, in 10 younger AML patients the abnormal stem cell appeared to be restricted to the myeloid lineage. Lineage restriction to the myelocytic pathway has also been observed (Metcalf, 1984). There are two possible explanations for lineage restriction in the abnormal clone. Either the target for the leukenaemonic insult is already a partially committed cell or the multipotential stem cell is damaged in a way that determines its subsequent maturation pathway. Three steps can be defined in the evolution of some cases of preleukaemia, an initial genetic change producing an abnormal clone with a growth advantage, a second change associated with the appearance of an abnormal karyotype, possibly in a stem cell with a more restricted differentiation potential, and thirdly the emergence of a leukemic clone. If we accept that in some cases MDS may evolve clinically from an initially erythroid phenotype, through to a myeloid and then a more primitive phenotype, we need to consider whether erythroid progenitors are specifically damaged at an early stage of the disorder, with subsequent damage occurring at other points in the haemopoietic stem cell system or whether the entire process can be explained by a specific sequence of stem cell lesions determining the observed disease progression.

It is interesting that in the hierarchy of haemopoietic cells proposed by Johnson (1981) in which stem cell maturation involves progressive, sequential loss of differentiation potential, the final stage in the process results in a progenitor cell only capable of producing erythroid colonies, the penultimate stage being bipotential for erythroid and megakaryocytic colonies. The clinical sequence described by Fohmleister et al. (1985b) almost parallels this maturation sequence and suggests that an early lesion might affect an
crythroid committed progenitor cell. Juvonen et al. (1986) have suggested that both erythroid and megakaryocyte colony formation are abnormal at an early stage in the clinical development of MDS, often when CFU-GM forma
tion syndrome is the only abnormality. Johnson (1981) suggests the possibility of a lesion in a bipotential progenitor cell. If this were so then, in the light of Johnson’s (1981) model, one might speculate that the probability of any particular stem cell maturation stage being a susceptible target for leukae
genic insults may be related to intrinsic physiological differences in the develop-
mental hierarchy such as population size, mitotic activity or self renewal capacity. We still have no way of determining the number of insults or the number of targets.

It would be difficult to provide a precise model to describe the human preleukaemic process, and it is likely that not all cases follow the same pathway. However, our present knowledge of the clinical syndromes is entirely consistent with current hypotheses of the multistage development of malignancy and oncogene function. In addition, and perhaps more importantly, clinical preleukaemia provides us with a definitive model of human leukaemogenesis that can be explored in detail. We now need to characterise the earliest abnormalities, their progression and the interrelations between genetic lesions and functional changes in pre-
leukaemic haemopoiesis.

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