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Myelodysplastic Syndromes

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Table of Contents

Myelodysplastic Syndromes: Introduction ..... *Donald C. Doll and Alan F. List* 1

Classification and Morphologic Features of the Myelodysplastic Syndromes....*Jean E. Goasguen and John M. Bennett* 4

Biology and Pathogenesis of the Myelodysplastic Syndromes.....*Alan F. List and Allan Jacobs* 14

Chromosome Abnormalities in Myelodysplastic Syndromes..... *Peter C. Nowell* 25

In Vitro Marrow Culture Studies in the Myelodysplastic Syndromes.....*Peter L. Greenberg* 34

Leukemias and Myelodysplastic Syndromes Secondary to Drug, Radiation, and Environmental Exposure..... *Ellis G. Levine and Clara D. Bloomfield* 47

Chemotherapy and Bone Marrow Transplantation for Myelodysplastic Syndromes *Bruce D. Cheson* 85

Differentiation-Inducing Agents in the Treatment of Myelodysplastic Syndromes *Masahiro Kizaki and H. Phillip Koeffler* 95

Treatment of Myelodysplastic Syndromes With Hemopoietic Growth Factors *Peter L. Greenberg* 106

Biology and Pathogenesis of the Myelodysplastic Syndromes

Alan F. List and Allan Jacobs

OUR KNOWLEDGE of the pathogenesis of the preleukemic states has evolved considerably since the French-American-British (FAB) Group characterization of the myelodysplastic syndromes into distinct morphological subgroups. It is now clear that the hematologic manifestations that call attention to these disorders represent a late event in the transformation of hematopoietic stem cells, in which a single genetically altered clone dominates blood cell production. Prchal et al,[2] using glucose-6-phosphate dehydrogenase (G6PD) polymorphism in heterozygous females and *cyto*genetic markers, first demonstrated that these disorders may arise in a primitive hematopoietic stem cell common to both myeloid and lymphoid progenitors. Subsequent studies of DNA polymorphisms at X-linked loci[3,4] and cytogenetic analyses of bone marrow progenitors[5,6] have confirmed that these disorders result from clonal expansion of a multipotent or pluripotent hematopoietic progenitor.

Current estimates of the incidence of the myelodysplastic syndromes (MDS) remain imprecise. Because of the relatively recent adoption of the FAB terminology, the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute has not captured specific data regarding the prevalence of these disorders. A Leukemia Research Foundation epidemiologic study in 1984 estimated the incidence of MDS to be approximately 75% that of acute myeloid leukemia (AML).[7] Because MDS is primarily a disease of the elderly, institutions caring for older populations have reported a much higher incidence rate. At the Royal Victoria Hospital in Bournemouth, UK, Hamblin and Oscier[7] reported an incidence six times that for acute myeloid leukemia. Among persons over 60 years of age, the incidence was as high as 0.75 per 1,000 per year. Among a group of patients over 55 years undergoing hematologic screening, the incidence of previously undiagnosed cases of MDS was 1.5 for every 1,000 individuals screened.

In the majority of patients, there is no family history or identifiable genetic predisposition to the development of hematologic malignancy. Although MDS is rare in persons less than 50 years of age, the incidence of familial cases is highest among persons in this age group. Among 550 adults with de novo MDS evaluated by Fenaux et al[8] over a 10-year period, patients under 50 years of age accounted for less than 7% of cases. Familial cases of myelodysplasia, however, represented 14% of patients in the younger cohort, a frequency 35 times that in the older age group. The distribution of FAB types and karyotypic abnormalities differed significantly between the groups. Both refractory anemia with excess of blasts in transformation (RAEB-t) (35% v 9%) and structural or numerical deletions of chromosome 7 (44% v 4%) were more frequent among patients under 50 years of age. This pattern is analogous to that in the pediatric population with myelodysplasia. Though rare in children, two disorders, juvenile chronic myeloid leukemia (JCML) and the monosomy-7 syndrome, account almost entirely for the types of myelodysplasia seen in pediatric patients. There is considerable overlap in the hematologic manifestations of these disorders, which include hepatosplenomegaly, leukocytosis, anemia, thrombocytopenia, excess marrow blasts, and a high frequency of conversion to acute leukemia. Juvenile chronic myeloid leukemia is the pediatric equivalent of chronic myelomonocytic leukemia (CMML), whereas the monosomy-7 syndrome displays a clear familial tendency.[9-15] The congenital chromosome fragility states, such as Fanconi's anemia and other related syndromes, are associated with intrinsic stem cell dysfunction and an increased incidence of MDS and acute leukemia, but account for only a minority of childhood MDS cases.[16-18]

Other hematologic disorders associated with

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disturbed stem cell development have been increasingly recognized to predispose to the development of hematologic malignancy, and in particular, the myelodysplastic syndromes. The best characterized are paroxymal nocturnal hemoglobinuria (PNH) and aplastic anemia. The high rate of autologous bone marrow recovery with the use of antilymphocyte globulin (ALG) preparations has altered the natural history of aplastic anemia considerably. Unlike patients undergoing allogeneic bone marrow transplantation, hematologic recovery is generally incomplete with immunosuppressive therapy, implying persistence of an intrinsic stem cell defect. Of 38 patients with severe aplastic anemia surviving more than 2 years after treatment with ALG, Tichelli et al[19] reported emergence of cytogenetically confirmed refractory anemia in five patients (13%), a median interval of 48 months after diagnosis. DePlanque et al[20] similarly noted development of MDS in 8 of 60 (13%) 2-year survivors after ALG, and in 4 patients this preceded development of acute myeloid leukemia. The probability that MDS would develop as a late complication increased with duration of follow-up reaching a frequency of 26% at 7 years' observation.

GENOTOXIC AGENTS

The nonrandom pattern of cytogenetic deletions affecting chromosomes 5 and 7 that characterize the myelodysplastic syndromes correspond to those identified in patients with therapy-related leukemia[21-23] and leukemias arising in patients occupationally exposed to chemical mutagens.[24-26] Indeed occupational or therapeutic exposure to a variety of potentially genotoxic compounds known to induce stem cell injury is associated with an increased incidence of both MDS and AML. The risk of developing either of these hematologic malignancies in workers exposed to benzene has been shown to rise proportionately with cumulative exposure,[27-30] whereas low levels of ionizing radiation appear more leukemogenic than high-dose, therapeutic irradiation.[31,32]

The leukemogenic risks associated with exposure to anticancer therapy are by far the best characterized. Data accumulated from population-based studies of patients with Hodgkin's disease, non-Hodgkin's lymphoma, ovarian cancer, gastrointestinal malignancy, and polycythemia vera have demonstrated a complex relationship between exposure to the offending agent and risk of developing a metachronous myeloid malignancy.[32-39] An excess risk of treatment-related MDS or AML becomes apparent immediately following completion of cytotoxic therapy and reaches its peak nearly 5 years after initiation of treatment, with an excess risk persisting up to 10 years. The cumulative risk is greatest for patients treated with chemotherapy, and alkylating agents in particular, but is proportionate to the dose and duration of alkylator therapy.[32,33,36,38] Treatment with radiotherapy alone is associated with a modest but measurable risk of hemopoietic neoplasia, but does not compound the risk attributed to chemotherapy alone in Hodgkin's patients receiving combined modality therapy.[32,40,41] As in de novo MDS, age remains a consistent variable affecting susceptibility to development of treatment-related myeloid neoplasia.[33,38,40,42,43] The risk is greatest for patients over 40 years of age with relapsed disease, and for patients receiving prolonged treatment with chemotherapy. Leukemias that develop following cytotoxic therapy are invariably preceded by a myelodysplastic phase and are generally accompanied by cytologic evidence of trilineage dysplasia.[45] Males predominate in both de novo and secondary forms of MDS. The reasons for this apparent sexual disparity are not clear; however, exposure differences and the known hemopoietic stimulatory effects of androgens may account for the seeming sexual disparity in MDS prevalence.

Though exposure to genotoxic agents clearly increases the risk of MDS, a history of such exposure can be elicited from only a minority of patients. Among 68 patients with MDS evaluated at the Arizona Lancer Center between 1988 and 1990, 5 (13%) had received prior treatment with chemotherapy, and 5 patients haa a history of radiation or benzene exposure. Whether exposure to unrecognized environmental toxins contributes to the development of MDS in the remaining patients is not clear. In a prospective case-control study performed at the University Hospital of Wales, a higher frequency and intensity of exposure to petrochemicals and other potential chemical mutagens was

observed in persons with de novo MDS ($P < 0.01$).[46] A second study by Goldberg et al[47] did not show a higher frequency of genotoxin exposure. However, patients with occupational exposure to benzene were excluded, and there was an unexpectedly high exposure rate in the control group. Among patients with acute myeloid leukemia, Crane and Keating[48] noted a greater frequency of exposure to genotoxins in patients with an antecedent preleukemic phase. Tobacco smoke, which contains a number of potential leukemogens, including nitrosamines, benzene, and radioactive compounds (polonium-210), has not been specifically addressed as a potential pathogenetic factor in myelodysplasia. Case-control studies have demonstrated a positive association between cigarette smoking and the development of acute myeloid leukemia in adults, suggesting a similar relationship may also exist for MDS.[49-53]

SENESCENCE AND CLONAL SELECTION

The clear age dependence for development of both de novo and secondary MDS implies factors inherent to hematopoietic senescence as well as spontaneous and/or chemical-induced genetic insults represent important pathogenetic features. Evidence from murine as well as human studies has shown an age-related decline in stem cell reserve and proliferative capacity.[54-58] These changes are accompanied by decreased recovery of erythroid progenitors,[55,59-61] due in part to defective local priming by the micro-environment.[61-63] The relation between these age-related changes in hematopoiesis and development of MDS remain speculative, however such changes may favor selective outgrowth of individual clones with a proliferative advantage.

Perhaps the best model in which to assess these changes and the relation to malignant transformation is in populations with a defined risk of MDS, as in the setting of exposure to recognized leukemogens. It is clear from animal models and clinical observations that the latent interval between exposure to an offending genotoxin agent and clinical detection of MDS may extend over several years. Animal studies have shown that stem cell self-renewal capacity is compromised proportionate to the degree of toxic insult.[64-66] This loss in stem cell reserve is permanent and appears limited to agents such as ionizing radiation and alkylating agents, and is not observed with repeated exposure to cycle-specific agents such as the antimetabolites. In large animals with compromised stem cell reserve following high doses of a bifunctional alkylator, hematopoiesis is maintained by a succession of individual stem cell clones which persists for the lifetime of the animal.[64-66] A similar pattern of clonal succession is demonstrable in bone marrow allograft recipients by analysis of restriction-fragment-length polymorphism.[67]

Clonal hematopoiesis per se does not necessarily imply a predisposition to malignancy and requires cautious interpretation. Nevertheless, in persons with compromised hematopoietic reserve, sustained clonal hematopoiesis may be a requisite event for initiation of neoplasia. In patients with aplastic anemia, for example, who have a recognized excess risk of MDS, patterns of X-chromosome inactivation in peripheral blood and marrow have shown clonal hematopoiesis in up to 50% of patients.[68,69] Investigators at the University Hospital of Wales have examined clonality of hematopoiesis among hematologically normal individuals in complete remission following conventional chemotherapy for malignant lymphoma for a minimum of 1 year.[70,71] Interestingly, monoclonal hematopoiesis was demonstrable in up to 57% of female subjects using an X-linked probe. Conversely, 3 of 18 (17%) normals, each of whom were over 70 years of age, had evidence of clonal restriction, suggesting an association between age and clonal hematopoiesis. These patients will be followed prospectively to assess their risk for secondary myeloid neoplasia. Nonetheless, these data provide preliminary evidence that clonal restriction, whether due to age or toxin exposure, represents an early event in susceptibility to MDS.

MOLECULAR EVENTS

Identification of nonrandom karyotypic abnormalities in the myelodysplastic syndromes confirmed the clonality of these disorders and their relation to specific clinical and biological features.[1] Mounting evidence indicates that acquisition of chromosome aberrations represents a relatively late event in the pathogenesis of

MDS. Indeed, clonal cytogenetic abnormalities are lacking in up to 50% of patients at initial diagnosis and may be acquired during the course of hematologic progression. This has led to the proposal that at least two steps are involved in the pathogenesis of MDS: an early step that leads to expansion of a genetically unstable stem cell clone, followed by acquisition of a chromosome abnormality, which may confer some selective growth advantage.[73,74]

Studies of experimental neoplasia indicate that early events may result from genetic mutations that alter cell growth." Mutations affecting the regulatory domains of *ras* proto-oncogenes in particular have been associated with mutagen-induced neoplasia in animal systems and have been extensively evaluated in MDS. *Ras* gene products are guanine-nucleotide-binding proteins that couple membrane receptors to phospholipase-C.[76] The growth-promoting effect of these oncoproteins derives in part from their ability to augment protein kinase-C activation.[77,78] Selective hybridization studies using synthetic oligo-deoxynucleotide probes specific for activating mutations of each of the three *ras* gene alleles have demonstrated mutations predominantly affecting N-*ras* in 30% to 40% of patients with MDS.[79-81] Mutant *ras* alleles are detected in all FAB subtypes but are found with greatest frequency in CMML. Among 75 patients evaluated by Jacobs and associates (Table 1), 21 of 31 patients with CMML (68%) had mutant *ras* alleles compared to a prevalence of 36% among 44 patients with other FAB types. The frequency of leukemic conversion was significantly higher in patients harboring mutant *ras* alleles (**14** of 39 [36%] v 2 of 36 [5.5%]).

Table 1. *Ras* Mutations in Patients With Myelodysplastic Syndrome

		Ras Mutations			
	No.	N	K	H	Total
Refractory anemia (RA)	18	3	3	—	6
RA with ringed sideroblasts (RARS)	1 6	3	1	3	7
RAEB/RAEB-t	10	2	—	**1**	**3**
CMML	**31**	11	**6**	**4**	**21**
Total	75	19	10	**8**	**37**

NOTE. From the University of Wales Preleukernia Unit.
Adapted with premission.[83]

The prevalence of mutations affecting regulatory domains of the ***C-FMS*** proto-oncogene has also been investigated. The ***C-FMS*** gene encodes the receptor for macrophage-colony stimulating factor (M-CSF) and possesses ligand-dependent tyrosine kinase activity.[84,85] Mutations in codons 969 and 301 produce unrestricted tyrosine kinase activity[84,85] and increase in vitro transformation efficiency.[86,87] The gene encoding ***FMS*** is located on the long arm of chromosome 5 (5q33.3) in the region commonly deleted in hematologic malignancies.[88] The Cardiff group detected ***FMS*** mutations in 9 (13%) of 67 patients with MDS and 5 (10%) of 48 patients with acute myeloid leukemia.[89] Mutations at codon 969 predominate and are detected primarily in patients with chronic myelomonocytic leukemia (6 of 30 patients; 20%) and myelomonocytic variants of acute leukemia. Tobal et al[90] found a similar distribution in patients with MDS, suggesting that ***FMS*** mutations may play a role in monocytic lineage commitment.

Although ***ras*** mutations may be acquired at any stage of MDS evolution,[80,91] recent studies suggest that ***ras,*** and ***FMS*** mutations in particular, may represent early events in the pathogenesis of secondary myeloid neoplasia. Using assays to detect mutations in X-linked genetic loci, somatic mutations can be identified in circulating blood cells years after exposure to gamma or neutron radiation in atomic bomb survivors.[92-94] A similar pattern of mutational injury is detected in patients following exposure to therapeutic irradiation and/or chemotherapy.[95] In a study described earlier,[96] DNA extracted from peripheral blood specimens of hematologically normal individuals previously treated with chemotherapy for malignant lymphoma displayed *ras* point mutations in 9 of 70 cases (13%) tested. These mutations were not identified in archival lymphoma specimens, indicating their somatic origin. Eleven patients (16%) harbored *FMS* mutations at codon 969, 3 of whom also had *ras* mutations. Analysis of clonality using an X-linked probe in 6 of the female patients with mutant proto-oncogene alleles demonstrated a monoclonal pattern in each patient."' ***N-ras*** mutations were not detected in specimens screened from 18 normal subjects. However, two patients harbored mutations in H-*ras* alleles. Among 111 normal volun-

teers screened for *FMS* mutations, only one harbored a constitutional mutation at codon 969.[89] These findings indicate that *FMS* and *ras* mutations occur commonly after conventional cytotoxic therapy and may be responsible for emergence of clonal hematopoiesis in many but not all patients in this setting. Nevertheless, it is clear from these data that activating mutations of *ras* and/or *FMS* alone are insufficient to confer a malignant phenotype. Whether such mutations increase susceptibility to subsequent transforming events and thereby predispose individuals to development of MDS must await the results of longitudinal studies.

BIOLOGIC FEATURES

The hematologic abnormalities that characterize MDS reflect an uncoupling of proliferation and differentiation in clonal hematopoietic progenitors. Ineffective hematopoiesis predominates, accompanied by marrow hypercellularity, increased intramedullary cell death, and peripheral cytopenias.[97-102] The latter are compounded by a shortened survival of mature blood elements.[98,103] Progenitor cell assays have shown reduced or absent growth of multipotent progenitors (CFU-GEMM), irrespective of FAB type.[105-107] Recovery of lineage-committed progenitors varies, but generally corresponds to peripheral blood abnormalities. The abnormalities in progenitor growth arise principally from an intrinsic stem cell defect. Although long-term bone marrow cultures have yielded phenotypic abnormalities in the adherent cell layer, no consistent functional defect in MDS stroma is demonstrable.[107] The propensity for abnormalities in progenitor cell growth to progress and extend to other myeloid lineages over time likely relates to the inherent instability of the stem cell clone and accumulation of genetic

In patients with pure sideroblastic anemia (RARS), however, dyserythropoiesis and isolated abnormalities of erythroid progenitor growth predominate.[111-113] This may explain the low incidence of leukemic conversion in this FAB type, although clonal restriction of all myeloid lineages and, to a variable extent, lymphoid progenitors remains demonstrable.[3,4] Ferrokinetic investigations have shown the greatest degree of ineffective erythropoiesis and erythroid hyperplasia in patients with RARS.[97,98] This profound disturbance in erythropoiesis may relate in part to abnormalities of mitochondrial iron metabolism. Multiple mitochondrial enzyme defects are demonstrable in RARS that give rise to intramitochondrial deposition of insoluble, nonferritin iron.[112,114] This is associated with impairment in cell cycle transition limiting entry of cells into S phase, defective protein synthesis, and accelerated cell death. Aberrant intercellular junctions demonstrable on erythroblasts from patients with dyserythropoietic anemias may further compromise red cell release and contribute to intramedullary hemolysis.[115,116]

Although impaired progenitor cell growth is a consistent feature of MDS, CMML remains an important exception. Both JCML and CMML are distinguished by excessively increased growth of CFU-GM in the absence of supplementation with an exogenous source of colony-simulating activity.[102,118,119] Analysis of CMML-conditioned media shows substantially increased levels of interleukin-6 (IL-6) and granulocyte/macrophage-colony stimulating factor (GM-CSF).[120] Antibody neutralization of cytokine activity produces near complete inhibition of spontaneous CFU formation.[120,121] These observations have been proposed to support autocrine regulation by GM-CSF and explain the spontaneous proliferation of CMML progenitors in vitro. However, adherent cell depletion prior to culture of mononuclear cells from patients with JCML abrogates recovery of spontaneous colony-forming units.[117] The latter finding supports an enhanced sensitivity of CMML myeloid progenitors to cytokine stimulation and suggests that GM-CSF is an endogenous paracrine regulator of myeloid progenitor proliferation in these disorders.

The cellular abnormalities that underlie ineffective hematopoiesis in MDS and how they relate to molecular events have not been well-defined. It is clear from in vitro culture data that impaired response to hematopoietic growth factors is a consistent feature accompanying reduced recovery of hematopoietic progenitors.[106,123-125] Observations that colony growth can be restored with supersaturating concentrations of recombinant growth factors that do not augment growth of normal progenitors indicates a disturbance in

receptor-signal transduction and/or receptor quantity or function.[125] One mechanism may relate to activation of growth regulatory genes such as *ras,* which alter cellular responsiveness to hemopoietic cytokines.[77,126] Nonetheless, impairment in terminal differentiation and premature death of hematopoietic elements may relate to decreased cellular responsiveness to regulatory stimuli. Recent investigations indicate that growth factors promote the proliferation and differentiation of blood cell precursors by suppressing programmed cell death or apoptosis,[127-129] thereby sustaining developmental programs for terminal differentiation. Deprivation of hematopoietic cytokines triggers a complex sequence of events that ultimately results in DNA fragmentation and cell death. Conceivably, apoptosis may provide a physiologic rationale for the high rate of intramedullary cell death and the accompanying cytologic abnormalities identified in MDS. Interestingly, pharmacologic differentiators such as retinoic acid appear to act principally through suppression of apoptosis.[130] Delineation of the role of this physiologic process in the biology of MDS warrants investigation and may provide insight for therapeutic manipulations.

Physiologic features intrinsic to hematopoietic stem cells may explain the relative insensitivity of MDS and their leukemia counterparts to conventional induction chemotherapy active in de novo AML. Multidrug resistance (MDR) is a cellular phenotype expressed in drug-resistant tumor cell lines and represents an inherent cellular phenotype in some epithelial tissues and corresponding carcinomas. The mdr 1 gene product, P-glycoprotein, serves as a transmembrane drug efflux pump that is induced by exposure to certain chemical carcinogens and a broad range of naturally occurring antineoplastic compounds.[131,132] Because MDS has been linked to xenobiotic exposure, we examined the prevalence of P-glycoprotein expression by immunocytochemistry in bone marrow specimens from patients with MDS or acute leukemia.[133] P-glycoprotein was detected in 8 of 32 (25%) specimens of de novo MDS and 4 of 19 (21%) cases of de novo AML. Reactivity was restricted to blast forms and leukemic monocytes, but was otherwise absent from terminally differentiated blood cells. In contrast, acute leukemias preceded by a myelodysplastic phase (9 of 12; 75%) and treatment-related hematologic disorders (7 of 9; 77%) exhibited a comparatively high frequency of P-glycoprotein expression. Although the gene encoding P-glycoprotein (mdrl) is localized to the long arm of chromosome 7,[130] we found no relation between specific karyotypic abnormalities and P-glycoprotein expression. Rather, we found a significant association between P-glycoprotein expression and monocytic and stem cell blast phenotypes. P-glycoprotein was detected in up to 90% of cases expressing the progenitor cell antigen CD34, compared to 21% in cases lacking CD34-reactivity ($P = 0.001$). The latter cohort was principally restricted to patients with CMML or acute myelomonocyticleukemia. Chaudhary and Roninson[134] have subsequently shown that P-glycoprotein is expressed by normal hematopoietic stem cells. These findings indicate that multidrug resistance is an inherent feature of hematopoietic stem cells that may be conserved in neoplastic counterparts. Indeed, CD34 expression in AML has been linked to inferior rates of complete remission and shorter remission duration in patients receiving conventional anthracycline-containing induction regimens.[135,136] The high prevalence of P-glycoprotein expression in high-risk acute leukemia, its association with lineage and stage of differentiation, and the recently recognized prognostic importance of MDR in de novo acute leukemia[137] suggest that MDR may be an important determinant of chemotherapy resistance in these disorders potentially amenable to therapeutic manipulation. P-glycoprotein–mediated drug extrusion is inhibited in vitro by a number of agents such as verapamil, calmodulin inhibitors, and cyclosporin A. Results of a phase 1/11 trial at the Arizona Cancer Center using cyclosporine as a chemosensitizer in patients with high-risk AML have shown that this agent can be added to conventional induction therapy without significant added toxicity and possibly improved rates of complete remission.[138]

CONCLUSION

While our knowledge of the pathogenesis of MDS continues to evolve, several conclusions can be made from studies to date. The major body of evidence indicates that a concatenation

of factors including host susceptibility, age, sex, cumulative exposure to leukemogens, and other unrecognized factors influence the risk for development of **MDS** (Fig **1).** Preliminary observations indicate that emergence of clonal hematopoiesis as a result of diminished stem cell reserve and/or somatic mutations involving growth regulatory genes can be detected years after genotoxin exposure. Ongoing longitudinal studies will determine whether such changes have an initiating role in the pathogenesis of **MDS.** Nonetheless, activation of individual cellular oncogenes alone appears insufficient to confer a malignant phenotype, implying additional events are necessary mediators of neoplastic change. Indeed, gross karyotypic aberrations may represent **a** relatively late event in a series of adaptive changes that reflect genetic instability of the affected clone. Abnormalities at the cellular level have only begun to be explored. Alterations in growth factor receptor number and/or affinity cannot be excluded, and require

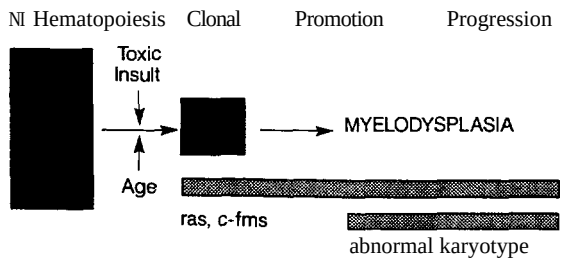

Fig 1. Putative model of the pathogenesis of the myelodysplastic syndromes.

investigation. To this end, development of an animal model for **MDS** should be a focus of future investigations. The myelodysplastic syndromes are an important group of hemopoietic disorders that will assuredly increase in incidence with the continued aging of our population. Understanding the pathobiology of these disorders will no doubt yield critical information concerning fundamental controls of hematopoiesis and provide new avenues for therapeutic manipulation.

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