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REVIEW

A hypothesis for the pathogenesis of myelodysplastic syndromes: implications for new therapies

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To guide development of new clinical strategies, a review of recent investigations in the pathobiology of MDS was performed. Articles were identified through a Medline search. Studies, including reviews, are cited in the references. A multistep pathogenesis is proposed. (1) Targeted injury or mutation within hemopoietic stem cells may be followed by an immunologic response adversely affecting progenitor survival. (2) Accelerated proliferation and premature death of marrow cells is amplified by apoptogenic cytokines (TNF-α, Fas ligand). (3) Establishment of an abnormal clone associated with telomere shortening. (4) Disease progression associated with loss of tumor suppressor activity. Opportunities for therapeutic interventions are possible at each step. Comparisons between the proposed pathogenesis of MDS and severe aplastic anemia (SAA) are also presented. *Leukemia* (2000) **14,** 2–8.
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Introduction

Three decades of investigations into the pathophysiology of the myelodysplastic syndromes (MDS) have confirmed the heterogenicity of MDS and highlighted the complexity in disease biology.[1] Recent advances in technology have yielded provocative observations. The objective of this review is to integrate clinical and laboratory findings into a working hypothesis for the development of idiopathic MDS, differentiate idiopathic MDS from SAA, and suggest new therapeutic strategies.

Interpretation of data from MDS studies remains problematic. Without a reliable disease marker, there can be questions regarding the accuracy of an MDS diagnosis.[2] Additional problems arise when patients with disparate biologies are compared. For example, patients with idiopathic MDS and therapy-related MDS are sometimes included in the same data analyses. The same is true for FAB morphologic type and cytogenetics. A potential source of ambiguity in laboratory studies derives from the mixture of normal and malignant progenitor cells which are known to coexist.[3] The low number of polyclonal progenitor cells in most cases suggests that such studies are valid. However, patients in chemotherapy-induced remission may re-establish polyclonal hemopoiesis.[4,5]

Any hypothesis for the pathogenesis of MDS must support some long-standing clinical observations. Why are cytopenias present with hypercellular marrows? Why does MDS evolve more slowly than AML? Why is idiopathic MDS predominantly a disease of the elderly? Why does MDS sometimes respond to therapies for SAA? Proposals for the pathogenesis of MDS have been suggested previously.[6–9] A specific multistep sequence for the development of adult-onset idiopathic MDS based on cell culture, cytokine, molecular and clinical research is presented (Figure 1).

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Early events in evolution of MDS

Three large (>150 index cases) epidemiologic studies suggest that radiation, smoking and occupational exposure to pesticides, organic chemicals and heavy metals are risk factors for the development of MDS.[10–12] Prevention of MDS will require

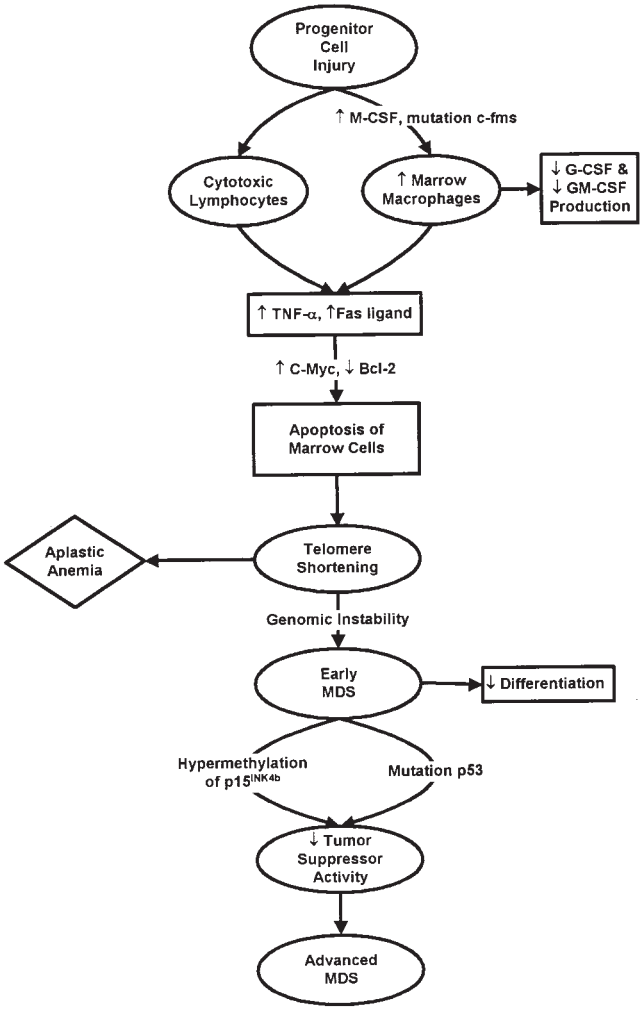

Figure 1 A proposed multistep sequence for the development of idiopathic myelodysplastic syndrome. M-CSF, macrophage colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimul-

further delineation of disease-associated toxins and possible polymorphisms in toxin metabolism that may predispose to a higher risk of MDS.[13] Clearly, additional epidemiologic studies are needed.

One proposal is that progenitor cells damaged by toxin exposure or spontaneous mutation evoke an immunologic response that further compromises progenitor cell growth and maturation. What is the evidence for the existence of an aberrant immunologic response when over 25 years of studies indicate a diminished immune state in MDS?[14] Incubation of marrow cells with cyclosporine or removal of T cells enhance colony formation in some patients.[15,16] Studies reported by Molldrem and colleagues[16] at the NIH indicate that suppression of CFU-GM may be mediated by $CD8^+$ cells directed against MHC class I restricted antigens. The anti-CFU-GM response does not appear to be mediated by MDS-derived immune cells since, most, but not all, studies indicate that T cells are not clonal in MDS.[17–22] Clinical observations also support the notion of immune suppression of progenitor cell growth in MDS. Treatment with anti-thymocyte globulin or cyclosporine can improve cytopenias in select MDS patients.[23–26] In contrast, attempts to augment an immunologic response with roquinimex or IL-2 have met with very limited suceess indicating that the diminished immune response may be the result rather than the cause of MDS.[27,28]

Non-clonal lymphopoiesis provides indirect evidence for a lack of stem cell involvement in MDS. Using precursors sorted by flow cytometry and subsequent FISH to define clonal hemopoiesis, primitive progenitors ($CD34^+$, $Thy1^+$) lacked the cytogenetic marker whereas more committed progenitors ($CD34^+$, $CD33^+$) display a clonal chromosome abnormality.[29] Conceivably, these non-clonal primitive stem cells could be utilized as a stem cell graft for autologous transplantation.

The growth and differentiation of the progeny of clonal progenitors is further compromised by an accelerated rate of apoptotic cell death. In cell culture, cytokines such as TNF-α and IFN-γ can suppress the growth of hemopoietic progenitors and induce Fas expression on CD34 cells.[30–32] What is the evidence for a functional role of apoptogenic cytokines in MDS? Elevated serum levels of TNF-α in patients with MDS is well documented (see Table 1).[33–35] Increased TNF-α production by blood mononuclear cells in one study was restricted to patients with RA and RARS, but not RAEB or RAEBt.[36] Furthermore, overexpression of TNF-α mRNA from marrow was detected in most cases of MDS, but not in normal controls or AML patients.[37] One probable source of TNF-α overproduction is marrow macrophages which are increased in MDS.[38,39] The increased density of marrow macrophages may occur in response to elevated serum levels of M-CSF.[40] Point mutations in c-fms, which encodes the M-CSF receptor, may also promote macrophage development in some cases.[41] The physiological significance of TNF-α in MDS is supported by several lines of investigation: (1) enhanced *in vitro* formation of CFU-GM by antibody neutralization of TNF-α in MDS but no effect on AML CFU;[42] (2) inverse correlation between serum TNF-α concentration and hemoglobin in one study;[34] (3) inverse correlation between clinical response to erythropoietin and TNF-α levels;[35] (4) inverse correlation between a platelet response to IL-3 therapy and TNF-α serum levels;[13] (5) positive correlation between TNF-α producing cells in the marrow and apoptosis;[44] and (6) correlation between plasma TNF-α concentration with nucleotide oxidation in marrow MDS $CD34^+$ cells.[45]

This model suggests that secretion of TNF-α or other pro-apoptotic cytokines plays a pivotal role in the ineffective hematopoiesis of MDS, but the relationship to disease progression remains ill defined. This implies that strategies which effectively neutralize TNF may inprove hematopoiesis. Pentoxifylline at micromolar concentrations suppresses TNF-α mRNA transcription. Combination therapy with pentoxifylline + ciprofloxacin yielded no hematologic benefit in one study but a triple drug regimen of pentoxifylline + ciprofloxacin + dexamethasone produced hemopoietic responses in 35% (18/51) of patients and 28% (5/18) of responders demonstrated a cytogenetic response.[46,47] An alternative approach is to neutralize circulating TNF-α by administration of soluble TNF receptors. *In vitro*, incubation of MDS marrow with TNFR:Fc enhanced CFU-GM formation.[42] Strategies which reduce the impact of multiple soluble mediators of progenitor cell apoptosis or increase the threshold for apoptosis induction may be more effective than single agent therapy. For instance, interruption of progenitor cell apoptosis could be attempted with soluble TNF receptor (to inhibit the initiation of apoptosis) plus amifostine (to raise the threshold for apoptosis). Another possibility for combined therapy is simultaneous inactivation of more than one inducer of apoptosis.

Table 1 Cytokine levels in MDS compared to normal controls

	Colony-stimulating factors			*Pro-apoptotic factors*		
	Serum	*Blood cells*	*Marrow cells*	*Serum*	*Blood cells*	*Marrow cells*
G-CSF	↑[34]	↓[79]	U[51]			
GM-CSF	↑[76], U[34]		↓[80,82], U[51], N[39]			
M-CSF	↑[40]					
IL-3	↑[34], U[76]					
IL-6	↑[34]	↑[36*]	↑[51], N[80]			
SCF	↓[75,83]					
BPA			↓[84]			
FLT3 ligand	↑[85*]					
TNF-α				↑[33–35], ↑[42*]	↑[36*]	↑[37,39], N[80,82], U[51]
Fas ligand						↑[42], ↑[59‡]
IL-1β				↑[35]	↑[36*]	↑[39,51]
TGF-β						↑[39], N[51]
IFN-γ						↑[37], U[49]

One potential approach includes simultaneous blockade of the activity of Fas ligand and TNF-α.[48]

IFN-γ or IL-1β are apoptogenic cytokines that could contribute to ineffective hematopoiesis in MDS. In two series, IFN-γ gene overexpression was detected in only 5/12 and 0/11 MDS cases.[37,49] Increased production of IL-1β by blood and marrow cells has been reported.[36,50,51] Increased production of IL-1β by cultured marrow mononuclear cells was detected in 13/32 MDS patients.[50] Patients with RA tended to have the highest IL-1β production.[36,50] IL-1β levels have been correlated with the extent of apoptosis, but not proliferation.[50] Deficient production of the IL-1β receptor antagonist by MDS stromal cells may give rise to unopposed apoptotic activity from IL-1β.[52] These studies suggest that IFN-γ and IL-1β do not contribute to apoptosis in the majority of patients with MDS.

Several investigators have reported increased marrow cell apoptosis in MDS and have implicated the potential role of the Fas/Fas ligand system.[53–58] Increased Fas expression was detected on marrow CD34 cells from MDS patients.[42,55,56] Lack of correlation between Fas expression and apoptosis suggests that multiple mediators of cell death are operational.[55] Gupta and coinvestigators[59] have examined Fas ligand expression in marrows of MDS patients. The mean percentage of Fas ligand expressing cell was higher in MDS (17%) than from normal controls (6%). In contrast to normals where Fas ligand was detected mostly in lymphocytes, Fas ligand was expressed in erythroblasts, myeloblasts, megakaryocytes, maturing myeloid cells and dysplastic cells. In another study, Fas ligand expression in MDS patients was localized to marrow macrophages.[60] Further investigations revealed more Fas ligand positive marrow cells in RA/RARS (9%) than in RAEB/RAEBt (20%).[59] However, this appears to be inconsistent with the finding that the extent of marrow cell apoptosis inversely correlates with clinical stage of MDS. A higher degree of apoptosis was seen in early FAB classes for both $CD34^+$ cells or marrow aspirates in most studies.[53–56] Furthermore, as MDS clinically progresses, apoptotic signals decrease (Fas antigen, c-Myc oncoprotein) whereas anti-apoptotic signals increase (bcl-2 oncoprotein).[54,55,61] Fas-associated phosphatase-1 (fap-1) is a negative regulator of fas. Recent studies have shown that fap-1 expression is reduced in MDS marrow cells compared to marrow cells from either normals or AML than has progressed from MDS.[62] Several investigators have suggested that the clinical sequelae of apoptosis is cytopenia. Since MDS is usually detected by cytopenia and apoptotic activity is most pronounced in the early phases of MDS, it is possible that apoptosis precedes the clinical recognition of MDS.

Amifostine is a phosphorylated aminothiol with dual biologic activities including free radical scavenging, by addition of reducing equivalents, and inhibition of TNF-α and other inflammatory cytokine elaboration.[63] In this way, amifostine may protect against TNF-induced apoptosis in MDS.[43] In one trial, amifostine responses were noted in 83% (15/18) patients.[64] In another study with 12 patients, none satisfied the criteria for a partial or complete response.[65] Theoretically, amifostine activity should be more pronounced in early, rather than, late MDS. However, the potential application of this agent in MDS awaits further investigation. Inhibition of apoptosis by agents that decrease the c-Myc/Bcl-2 ratio could also be attempted. There are candidate agents to decrease c-Myc (Vitamin D_3, agents that potentiate intracellular

tigate the cause(s) of cytopenia in established MDS. Numerous studies indicate deficient growth of myeloid, erythroid and megakaryocytic colonies.[69] Similar to AML, blast cell colonies (CFU-L) can be detected in MDS.[70] One cause for cytopenias may be related to diminished capacity for differentiation.[71] Therapeutic trials of differentiating agents have not demonstrated consistent efficacy in ameliorating cytopenia.[72–74]

Interpretation of the results from investigations of colony-stimulating factor levels in MDS patients is difficult to discern. Results appear inconsistent (Table 1) and CSF levels may be altered by clinical events (ie infections). Furthermore, in most studies, serum CSF levels did not correlate with clinical parameters.[34,75,76] Given these limitations, the decreased cellular production of G-CSF, GM-CSF and burst-promoting activity (BPA) plus low serum levels of SCF suggest that low CSF levels may be related to cytopenias and provide a basis for CSF therapy of MDS. Diminished elaboration of CSF may arise from apoptosis and functional abnormalities of stromal cells.[44,77,78] Studies indicate decreased production of GM-CSF and G-CSF by MDS monocytes.[79,80] The use of colony-stimulating factors for the treatment of MDS is beyond the scope of this paper.[81]

MDS disease progression

The next proposed step is reduction of telomere length. Telomeres are located at the ends of eukaryotic chromosomes, and function to stabilize chromosomes. Telomere length is progressively reduced by cell divisions. Accelerated apoptosis and proliferation may lead to the reduction in telomere length observed in MDS.[53,86,87] Reduction in telomere length is probably not directly responsible for disease progression since only 42% of patients with RA have telomere length reduction.[87] Instead, telomere shortening may give rise to genomic instability that leads to cytogenetic evolution and disease progression. This assertion is supported by the finding that telomere shortening is associated with advanced MDS, cytogenetic abnormalities, percentage of marrow blasts, leukemic transformation and poor prognosis.[86,87] Since telomerase activity (a DNA polymerase that can synthesize the telomeric sequence) is normal to low in most patients, telomere length may be a better reflection of the pathophysiology in MDS.[86] Progenitor cells with shortened telomeres may be more susceptible to elimination by telomerase inhibitors. Telomerase inhibitors are currently in development.[88]

Progression to advanced MDS and AML has been linked to inactivation of the tumor suppressor genes, $p15^{INK4b}$ and to a lesser extent, p53, and thereby contribute to clonal expansion (see Figure 1). p53 is a tumor suppressor gene which serves as a major control of the G_1 checkpoint.[89] Studies indicate that mutations of p53 occur in less than 20% of MDS cases.[90–93] At the molecular level, p53 mutations in MDS are usually point or missense mutations of one allele, associated in some cases with 17 p deletion of the alternante allele.[94] Evidence that suggests mutation of p53 is related to progression of MDS includes the limitation to advanced MDS (RAEB, RAEBt), association with complex cytogenetic abnormalities, and risk of secondary leukemia.[89–91] In view of the low frequency of mutations, introduction of wild-type p53 into MDS cells by gene therapy offers limited therapeutic potential.[95] Bishop and coinvestigators[96] investigated whether a rebound in p53 after brief inactivation of p53 RNA by antisense oligonucleotide could inhibit clonal proliferation. In a phase I trial that included 10 high risk MDS patients (three RAEB, seven RAEB-

As noted earlier, Fas expression decreases as the blast percentage increases.[55] The inhibitory effect of TGFβ on leukemic colony formation in early MDS is diminished after transformation to AML.[97] Transcription of p15^{INK4b}, a cyclin-dependent kinase inhibitor represents one mechanism by which TGFβ exerts its inhibitory effect. Hypermethylation of the p 15^{INK4b} gene occurs in 38–50% of MDS patients and may contribute to loss of proliferative regulation.[98,99] Evidence implicating a causal role for inactivation of p 15^{INK4b} with disease progression is provided by the increased frequency of hypermethylation observed in advanced MDS and secondary AML compared to early MDS. Agents which promote hypomethylation of DNA may impact the evolution of the leukemic clone by derepression of p 15 transcription.[100] 5-Aza-2′-deoxycytidine and 5-azacytidine promote DNA hypomethylation by inhibition of DNA methyltransferase.[101] However, most of the activity of 5-azacytidine, a drug demonstrated to have clinical efficacy, is through mechanisms other than DNA demethylation.[101,102] Incubation of cell lines harboring methylation silenced p 15 with 5-Aza-2′-deoxycytidine leads to re-expression of p 15.[103] In one study, a 72 h infusion of 5-Aza-2′-deoxycytidine to 29 high risk MDS patients yielded a 29% complete response rate and 24% partial responses.[104] Prolonged myelosuppression was the sole cause for a 17% drug-related mortality. Using a lower dose of 5-Aza-2′-deoxycytidine in 61 patients produced similar clinical effects without marked myelosuppression.[105] Additional studies with 5-Aza-2′-deoxycytidine should be performed.

Clinical relevance of model

How does this model account for MDS being a disease of the elderly? Accumulated environmental exposures provide a cumulative probability of mutational events that increases with time. Age-related telomere shortening fosters heightened susceptibility to genomic instability that can lead to emergence of clonal disease. A similar explanation can account for the differences between MDS and SAA. The younger age of SAA patients may reflect exposure to a progenitor cell toxin or a genetic event in a patient not susceptible to genomic instability from age-related telomere loss. This proposal is supported by the finding of shorter telomere lengths in MDS than SAA.[106] Another difference between MDS and SAA may be the milieu of hemopoietic inhibitory cytokines that drive apoptosis. In SAA, IFN-γ gene overexpression was detectable in most patients.[49,107] As described earlier, IFN-γ mRNA was usually not detected in MDS patients, but TNF-α was overexpressed in 11/14 cases.[37,49] These data suggest a tendency for IFN-γ-mediated apoptosis in SAA and TNF-α-driven apoptosis in MDS. Some features of MDS and SAA are shown in Table 2. We propose that MDS and SAA may have a similar pathophysiology but the disease expression is dependent on patient age.

There are a few other points suggested by the model. Marrow failure has a multifactorial etiology including decreased marrow production of colony-stimulating factors, increased elaboration of hemopoietic inhibitors and accelerated apoptosis of progenitor cells. As proposed previously, cytopenia with a hypercellular marrow can be attributed to simultaneous progenitor cell apoptosis, increased proliferation with a differentiation block.[44,53,71]

The sequential development of MDS does not explain all observations. This proposal provides a basis for designing new studies for what remains an enigmatic disease.

Table 2 Pathophysiologic factors in MDS and SAA

	MDS[a]	*SAA*
↑ Hemopoietic inhibitors	Yes	Yes[36,49,108]
↓Progenitor cells	Yes	Yes[109]
Apoptosis of marrow cells	Yes	Yes[110,111]
Telomere shortening	Yes[b]	Yes[106]
Clonal hemopoiesis	Yes	Rare[112]
Production of G-CSF and GM-CSF	Often ↓	Normal or ↑[113]

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