Immunologic Aspects of Hypoplastic Myelodysplastic Syndrome

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The pathophysiology of myelodysplastic syndromes (MDS) is multiple, complex, and poorly understood. In some cases of MDS, especially those in which the bone marrow is hypocellular, there is increasing experimental and clinical indication that an immune-mediated damage to hematopoietic precursors and changes in the hematopoiesis-supporting microenvironment contribute to disease development. Increased serum levels of type-1 cytokines, tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ), and oligoclonal expansion of cytotoxic T cells are observed in human MDS. In some cases, the immunologic attack to the marrow appears to be triggered by MDS-specific antigens, damaging the microenvironment and inducing cell apoptosis especially of normal progenitors. In murine models, dysregulation of osteoprogenitors leads to disrupted hematopoiesis of healthy hematopoietic progenitor and stem cells, eventually resulting in MDS and leukemia. In hypocellular MDS, marrow failure appears to be not only the result of ineffective erythropoiesis of abnormal clones, but also due to inhibition of normal progenitors. Immunosuppressive therapy with cyclosporine, antithymocyte globulin, or alemtuzumab may alleviate cytopenias and in some instances induce cytogenetic remission. However, not all patients respond to immunosuppression, and the identification of relevant biomarkers for an immune mechanism is necessary to identify those patients who may benefit from this treatment modality.
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Myelodysplastic syndromes (MDS) are a very heterogeneous group that represents a spectrum of diseases characterized by ineffective erythropoiesis and marrow failure limited by acute and chronic leukemias and myeloproliferative disorders on one end of the spectrum, in which hypercellular marrow is typical, to aplastic anemia at the other end. Since thresholds are arbitrary and do not necessarily reflect disease biology, it is often difficult to establish the differences between hypoplastic MDS (hMDS) and aplastic anemia, or refractory anemia with excess blasts-2 (RAEB-2) MDS and acute leukemia. A few MDS cases have distinct etiology and pathophysiology, such as 5q⁻ syndrome, in which a specific chromosomal lesion leads to abnormal gene expression (*RPS14*) and disease phenotype[1]; it is likely due to

the distinct genetic basis that 5q⁻ syndrome uniquely responds to specific therapy.[2] This etiologic feature is not present in most types of MDS, which are categorized based on secondary events in disease pathophysiology due to our inability to understand their etiology.

hMDS refers to a morphologic feature in which the marrow cellularity is low for age (<30% cellularity if age <60 years or <20% cellularity if age >60 years), representing approximately 10% to 15% of all MDS cases, but which does not necessarily take into account disease mechanism.[3] However, hMDS does not represent a defined MDS category according to the World Health Organization (WHO) classification, but rather denotes the morphologic status of other MDS categories. Nevertheless, patients with hMDS tend to be younger; have more profound neutropenia and thrombocytopenia and a lower percentage of blasts; and are less likely to display abnormal karyotype in comparison to patients with normocellular or hypercellular MDS.[4] The prognostic significance of hMDS is still debatable, according to the WHO classification; patients with hMDS had a more favorable overall survival compared to normo/hypercellular MDS in some studies[5,6] but not in others.[7]

HYPOPLASTIC MDS AND APLASTIC ANEMIA

Due to its similarities and often difficult differential diagnosis,[8] hMDS is inferred to share pathophysiologic

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mechanisms with acquired aplastic anemia, and more likely to be immune-mediated.[9] However, evidence for an immune process is not restricted to this hMDS. The differential diagnosis between aplastic anemia and hMDS is based mainly on the presence of dysgranulopoiesis, dysmegakaryocytopoiesis, increased percentage of blasts, and abnormal karyotype, all of which favor the diagnosis of hMDS. In addition, an abnormal antigen expression pattern in marrow CD34$^+$ cells,[10] indicating an aberrant clone, and elevated hemoglobin F-containing erythroblast production[11] suggest the diagnosis of hMDS. However, findings compatible with an immune process (relative lymphocytosis in the marrow and oligoclonal T-cell expansion, increased cytokine levels) do not contribute to the differential diagnosis, as these elements are present in both diseases. The overlap of immunologic aspects suggests that hMDS and aplastic anemia share an immune-mediated mechanism.

Paroxysmal nocturnal hemoglobinuria (PNH) clones, which are deficient for glycosyl-phosphatidylinositol (GPI)-anchored proteins, also are observed in up to 20% of MDS cases.[12,13] The emergence of a PNH clone derived from mutant hematopoietic stem cells is frequently observed in acquired aplastic anemia and is interpreted as an "escape" from the immune attack directed against hematopoietic progenitors. For reasons poorly understood, GPI-anchored protein-deficient cells appears to be spared by the immune process mediated by T cells in marrow failure and the occurrence of a PNH clone is interpreted as a sign of an immune pathophysiology.[14] In MDS, the presence of a detectable PNH clone is more common in low risk cases (refractory anemia and refractory cytopenias with multilineage dysplasia.[15] Patients with refractory anemia MDS with a PNH clone, suggesting an immune mechanism, have a distinct feature: the morphologic dysplastic abnormalities are less dramatic, the presence of abnormal karyotype is less common, and progression to acute leukemia is infrequent.[13] However, the presence of a PNH clone is not restricted to hMDS.

Aplastic anemia and hMDS also may share genetic defects. Mutations in genes encoding the telomerase complex, responsible for maintaining the length of telomeres, result in excessive telomere shortening in hematopoietic progenitors, are etiologic in dyskeratosis congenita (an inherited bone marrow failure syndrome), and also are found in some cases of apparently acquired aplastic anemia.[16] Dyskeratosis congenita has a high probability for progression to MDS and acute leukemia.[17] Aplastic anemia in patients with telomerase mutations also often evolves to hMDS and acute myeloid leukemia.[18] Acquired aplastic anemia patients with shorter telomeres (lowest quartile for telomere length) are those with higher risk of disease evolving to MDS, especially those with monosomy 7.[19]

Mutations in *TERC* (encoding the RNA component of telomerase) or *TERT* (encoding the telomerase reverse transcriptase enzyme) also are found in a minority of patients with MDS.[20,21] Hypoplastic MDS also may be the primary clinical manifestation of telomerase deficiency, especially in children.[22,23]

IMMUNOLOGIC CHANGES IN hMDS

Similar to acquired aplastic anemia, several abnormalities indicative of an active immune process mediated by a Th1-cell response are observed in MDS, including hMDS.

Abnormal Cytokine Profile

Cytokines are usually abnormally expressed in MDS. TNF-α is consistently high in bone marrow samples of patients with MDS, regardless of morphologic subtype.[24,25] However, TNF-α is most prominent in low-risk MDS.[26] Serum levels of TNF-α also are increased in MDS.[27] In addition, the TNF–related apoptosis-inducing ligand (TRAIL), a member of the TNF family, also is overexpressed in MDS.[28] TRAIL, usually not present in healthy marrows, induces apoptosis by activating agonistic receptors 1 and 2 and decoy receptors 3 and 4.[9] In MDS, TRAIL preferentially targets abnormal clonal cells with aberrant chromosomes, inducing apoptosis.[28] The expression of TRAIL agonistic receptors is significantly higher in MDS marrow cells as compared to healthy marrows. In addition, MDS marrow cells display lower levels of FLIP (Flice-like inhibitory protein), a cytoplasmic inhibitor of apoptosis, which may explain the higher sensitivity to TRAIL in cytogenetically abnormal clones. In fact, the FLIP long isoform (FLIP$_L$) is underexpressed in marrow CD34$^+$ cells of patients with MDS. INF-γ also is overexpressed by marrow mononuclear cells in MDS.[25,27] Both TNF-α and IFN-γ activate the expression of induced nitric oxide synthase (iNOS) by MDS cells, which can potentially mediate the dysregulation of hematopoiesis in MDS.[29] In hematopoietic cells, iNOS appears to engage Fas-mediated apoptosis[30] and this may be an additional immune-mediated mechanism contributing to ineffective hematopoiesis in MDS.

Finally, cytokine expression profile also may have prognostic significance. In a recent study, interleukin-4 (IL-4) and C-C motif chemokine 3 (CCL3) serum levels were consistently underexpressed in MDS and independently associated with survival.[31]

T-Cell–Mediated Attack

In acquired aplastic anemia, there is compelling laboratory evidence that marrow failure is the result of an antigen-driven lymphocyte destruction of the hemopoietic tissue.[32,33] In these patients, there is an expansion of cytotoxic T cells expressing defined T-cell-receptor (TCR) Vβ chain, indicating the oligoclonality of the

T-cell repertoire.[32] Similar Vβ-chain usage is observed in MDS; skewed T-cell populations are observed in treatment-naïve MDS patients, which are reduced or disappear after response to immunosuppressive therapy.[34-37] Conversely, in patients who fail to respond to immunosuppression, the dominant T-cell clone persists after treatment.

Although the antigens triggering the immune response in MDS are not known, some molecules are potential candidates. Trisomy 8 patients often respond to immunosuppression, indicating a strong immunologic mechanism underlying marrow failure in this disease.[9] Trisomy 8 cells express high levels of WT1, a zinc finger transcription factor implicated in the pathophysiology of Wilms tumor. CD8^{+} T cells of patients with trisomy 8 are able to recognize WT1 peptides and engage INF-γ expression in vitro, suggesting that this antigen may contribute to elicit an immune response.[9] Whether WT1 antigenicity may be used therapeutically is still unknown.

Further evidence supporting an autoimmune component in MDS is genetic. Several autoimmune diseases are associated with specific human leukocyte antigens (HLAs) and among marrow failure syndromes, acquired aplastic anemia has been linked to HLA-DR15, a serologic split of HLA-DR2.[33,38] HLA-DR15 antigen also is overrepresented in patients with refractory anemia MDS when compared to healthy controls[39]; HLA-DR15 allele frequency also is significantly higher in patients with MDS bearing a PNH clone.[13] Taken together, these observations further suggest that some MDS cases are immune-mediated.

MICROENVIRONMENT CHANGES IN MDS

Recent observations have changed the way we understand leukemogenesis and how the marrow microenvironment (niche) may contribute to malignant clonal evolution.[40] Conditional deletion of *Dicer1*, an RNAse III endonuclease essential for microRNA biogenesis and RNA processing, in murine osteoprogenitors led to various degrees of cytopenias and dysplastic changes in myeloid and megakaryocytic lineages in spite of hematopoietic cells having intact *Dicer1*. Interestingly, transplantation of hematopoietic cells from conditionally knock-out *Dicer1* animals, which showed dysplastic changes but normal *Dicer1* gene, into wild-type mice resulted in recovery of normal hematopoiesis after engraftment; conversely, wild-type marrows transplanted into *Dicer1* knock-out mice showed dysplastic abnormalities after engraftment, both indicating that myelodysplasia was induced by the marrow microenvironment. *Dicer1*-knockout mice also evolved to tissue infiltration with blasts (myeloid sarcomas), splenic infiltration, and circulation monocytoid cells, resembling human acute myelomonocytic or mono-

blastic leukemia. However, in this mouse model, the marrows did not become hypocellular.

Gene expression analysis indicated consistent downregulation of the *Sbds* gene. Biallelic mutations in human *SBDS* is etiologic in Shwachman-Diamond syndrome, an inherited bone marrow failure syndrome characterized by aplastic anemia, pancreatic exocrine insufficiency, and proclivity to develop MDS involving chromosome 7.[41]

CLINICAL RESPONSE TO IMMUNOSUPPRESSION

That some patients with MDS respond to immunosuppressive therapy is compelling evidence for an immune-mediated mechanism. As for most patients with low-risk MDS, mortality is associated with complications from marrow failure and not evolution to acute leukemia, therapies aiming to alleviate cytopenias are warranted. Immunosuppressive regimens are effective to induce hematopoietic response in acquired aplastic anemia, ameliorating peripheral blood cytopenias, and recovering marrow cellularity.[33] Several investigators around the world have tried immunosuppressive therapy for MDS using different agents: cyclosporine,[42] methylpredisolone and cyclosporine,[43] antithymocyte globulin and/or cyclosporine,[7,44-48] or alemtuzumab[9] (Table 1).

In a Czech study 17 patients with MDS were treated with cyclosporine alone.[42] Most patients had refractory anemia MDS and the majority were hMDS. The authors observed a significant persistent response with transfusion independence in 14 of 17 patients; in a minority of patients, cyclosporine was discontinued due to toxic side effects. Conversely, in a small British study (six patients), none of the patients responded to cyclosporine alone.[49] Cyclosporine was added to methylprednisolone in a Japanese study of 18 patients with MDS[43]; only six patients received cyclosporine and most patients had refractory anemia MDS. One third of patients responded to therapy.

The combination of anti-thymocyte globulin and cyclosporine is known to be more effective than any of the agents alone in acquired aplastic anemia.[50] Several trials have investigated this immunosuppressive therapy in MDS.[7,45,48] The response rate varied from 16% to 45%, but patients with hMDS did not show a statistically significantly better response to immunosuppression than normo/hypercellular MDS.[7] In a larger trial at the National Institutes of Health (NIH), patients received either anti-thymocyte globulin as a single agent (n = 74), cyclosporine alone (n = 13), or a combination of anti-thymocyte globulin and cyclosporine (n = 42). Combination therapy appeared to reach superior response rates in comparison to single agents (Table 1).

More recently, the monoclonal antibody against CD52 alemtuzumab was used as single agent in a

Table 1. Immunosuppressive Therapies for MDS

Center	N	Treatment	Median Age (yr)	% RA	% Response
Prague[42]	17	CsA	57	94	82
St. George's[44]	30	ATG	55	62	50
M.D. Anderson[45]	32	ATG + CsA	59	58	16
Germany[46]	35	ATG	63	68	34
Gifu, Japan[43]	18	MP + CsA	67	56	33
Karolinska[48]	20	ATG + CsA	62	85	30
NIH[7]	129		60	40	30
	74	ATG	—	—	24
	42	ATG + CsA	—	—	45
	13	CsA	—	—	8
NIH(51)	21	Alemtuzumab	—	—	81

Abbreviations: RA, refractory anemia MDS; CsA, cyclosporine A; ATG, anti-thymocyte globulin.

pilot study of 21 selected patients with MDS.[51] Patients were selected based on their likelihood to respond to anti-thymocyte globulin (young age, low International Prognostic Scoring System [IPSS] score, and the presence of HLA-DR15). The authors observed a significant response rate among patients with intermediate-1 MDS (15 of 16 patients) and also among patients with intermediate-2 MDS (two of five patients). Interestingly, five of seven patients with abnormal karyotype had complete cytogenetic remission, including one patient with monosomy 7.

CONCLUSION

Evidence for an immune-mediated process in some cases of MDS, including hMDS, has been accumulated in the last decades. This knowledge has clinical implications in the selection of patients who might benefit from immunosuppressive regimens to alleviate cytopenias or even induce cytogenetic responses, and novel, less toxic agents may be very beneficial to selected patients. An immune-mediated mechanism is not restricted to hMDS, and the bone marrow cellularity in MDS may be the consequence of a delicate dynamic balance among the marrow microenvironment, proliferation capacity of the abnormal clone, intensity of the immune attack towards the marrow, and inhibition of normal hematopoiesis. Although hMDS is not classified as a defined category according to the WHO classification, MDS patients with a hypocellular marrow tend to have a less dramatic clinical course and a lower probability to evolve to leukemia.

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