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Immunosuppression for Myelodysplastic Syndrome: How Bench to Bedside to Bench Research Led to Success

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Laboratory evidence and clinical evidence suggest that some patients with myelodysplastic syndrome (MDS) have immunologically mediated disease. Autoimmune conditions such as Reynaud syndrome, lupus, rheumatoid arthritis, and polymyalgia are more frequent in MDS patients, and activated cytotoxic T cells and elevated levels of tumor necrosis factor alpha (TNF- α) and interferon γ (IFN- γ)^{1–4} are found in MDS. Cytokine dysregulation and excessive apoptosis are most notable in early MDS,^{3–6} where up-regulation of Fas-receptor and Fas-ligand expression are hallmarks of the disease.^{7–11}

The concept of using immunosuppressive treatment (IST) for bone marrow (BM) failure originates severe aplastic anemia (SAA) patients.¹² Gluckman and colleagues¹³ were the first to show that hematopoietic recovery could occur in SAA following antithymocyte globulin (ATG) treatment alone. As experience and success in IST for SAA accumulated, small numbers of patients with BM failure secondary to hypoplastic MDS were treated successfully. The response of such patients led to the establishment a clinical trial of IST at the National Institutes of Health in 1994 to evaluate the ability of ATG to improve BM failure in all forms of MDS.¹⁴

This study showed improvement in 30% of patients with MDS after ATG. Further efforts to identify the profile characteristics of the IST responder have resulted in better response rates to IST.¹⁵ This article describes the laboratory evidence supporting a role for the immune system in the marrow failure of MDS and clinical trials using IST in these patients.

CYTOKINE DYSREGULATION

TNF is the cytokine most implicated in the pathophysiology of MDS.¹⁶ TNF is overexpressed in cultured cells from patients with MDS.¹⁷ This cytokine is elevated in marrow biopsies¹⁸ and in plasma.¹⁹ TNF-related apoptosis-inducing ligand (TRAIL)⁵ and IFN γ have been reported elevated in peripheral blood lymphocytes from patients with MDS.^{17,19} TNF production correlated loosely with disease subtype,²⁰ being most prominent in patients with low-risk MDS.²¹ Some investigators have suggested that the source of TNF, and perhaps other negative regulators, may be macrophages or other cells located within BM stroma.^{1,22–24} Both TNF and IFN γ up-regulate Fas expression on the CD34 cell and trigger apoptosis.²⁵ TRAIL is a member of the TNF family, which controls apoptosis by binding to agonistic receptors 1 and 2 (TRAIL-R1, TRAIL-R2) and decoy receptors 3 and 4 (TRAIL-

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R3, TRAIL-R4). Although TRAIL is present in negligible amounts in normal marrow, it is constitutively expressed in MDS marrow,⁵ where cells are more sensitive to its cytotoxic and inhibitory effects.⁵ Interestingly, TRAIL produced apoptosis selectively in cytogenetically abnormal cells, identified by fluorescence situ hybridization markers.⁵ These findings were attributed to up-regulation of TRAIL-receptors 1 and 2 on the aneuploid clone and to differential expression (or function) of intracellular inhibitors of apoptosis Fas-associated death domain-like interleukin (IL)-1 -converting enzyme inhibitory protein (FLIP). FLIP is important in controlling apoptosis in normal cells. Isoforms of FLIP, which result from alternative splicing of mRNA, may have either proapoptotic or antiapoptotic properties depending on their length and may regulate apoptosis in normal marrow. In early MDS, when apoptosis is most prominent, the antiapoptotic long isoform of FLIP (FLIP_L) is down-regulated.²⁶

MODEL FOR IMMUNOLOGICALLY MEDIATED MARROW DESTRUCTION

Early MDS is characterized by an increased apoptosis in marrow cells. It is not clear whether programmed cell death is related to alteration in genetic programming or is a result of an immune attack. Evidence that the ineffective hematopoiesis is immunologically mediated comes from experiments showing suppression of hematopoiesis by lymphocytes derived from patients with active disease, but not following successful treatment with ATG.²⁷ Normally, quiescent lymphocyte populations contain a diverse distribution of thousands of individual clones within 23 T cell receptor (TCR) V families. During an immune response, expansion of T cell clones specific for the corresponding antigen occurs. This expansion may be detected by flow cytometry using antibodies directed against the TCR V subfamilies, or more specifically by spectra-typing or CDR3 length analysis. Like patients with multiple sclerosis,²⁸ those receiving vaccinations, and patients with infections,^{29,30} expanded V subfamilies where they appear to be cytotoxic to hematopoietic cells present in some cases of MDS.^{29,30}

The mechanism for immunologically mediated marrow damage in MDS has been defined most clearly in patients presenting with trisomy 8 as a single karyotypic abnormality. The authors chose trisomy 8 to investigate, because such patients are likely to respond to IST.¹⁵ Additionally, the dysplastic clone can be identified readily by FISH. Initially, the authors showed that early apoptotic changes appeared to be restricted to the trisomy 8 clone, because flow cytometric-sorted Fas-positive, annexin-positive cells were enriched in, or entirely composed of trisomy 8 cells.³¹ T cells from expanded V families selectively killed trisomy 8 cells in vitro, indicating that T cells could target trisomy 8 cells specifically. Consistent with this finding was the observation that depletion of cytotoxic T cells in BM cell improved growth of trisomy 8 colonies. Despite such specificity, it remained a possibility that normal BM cells could be killed as innocent bystanders by TNF and IFN- γ elaborated by activated T cells.²⁵ Trisomy 8 cells survive despite showing signs of early apoptosis; DNA degradation did not develop, and cell death did not occur.³² The resistance of trisomy 8 cells to apoptotic cell death might be attributed to up-regulation of c-myc present on chromosome 8, and survivin,³² which may be responsible for resistance of trisomy 8 cells to T cell attack. Indeed, when these proteins are knocked down or inhibited, trisomy 8 cells generally die in vitro.

It may be hypothesized that CD8+ T cells recognize a neoantigen or an overexpressed self-antigen presented by major histocompatibility locus (MHC) class I molecules on trisomy 8 cells. The authors searched for candidate antigens by examining microarray data on CD34 cells obtained from MDS patients with trisomy 8 and other cytogenetic abnormalities.³³ Wilms tumor protein 1 (WT-1) was markedly overexpressed in trisomy 8 cells. WT-1 is a zinc finger transcription factor located on chromosome 11p13 and was implicated in the

pathogenesis of Wilm tumor. WT1 is expressed at low levels in normal tissues, including kidney, ovary, testis, and spleen, but not in normal BM or peripheral blood (PB). It is overexpressed in various hematological malignancies including leukemia, MDS and paroxysmal nocturnal hemoglobinuria (PNH).³³⁻⁴⁰ Cytotoxic T cells recognize a number of epitopes from the WT1 protein.

In MDS, WT1 transcript levels increase with disease progression and correlate with the International Prognostic Scoring System (IPSS) stage.³⁶ In the authors' studies, WT1 mRNA and protein were increased in many IPSS-intermediate 1 (int-1) MDS patients, but were highest in patients with trisomy 8. CD8+ T cells from patients who ultimately became IST responders generated TNF- α and IL-2 in response to WT1 peptide stimulation.⁴¹ CD8 cytotoxic T cells, recognizing WT1₁₂₆ peptide, also could be detected by tetramer analysis in HLA-A*0201-positive patients. These findings do not exclude the possibility that other antigens on MDS cells are recognized by the immune system, and that their identification will require further testing of peptide libraries. In this regard, PR1, a peptide derived from myeloid-restricted azurophil granule proteins, can provoke T cell responses in patients with MDS.⁴² In summary, therefore, the authors' studies showed that MDS cells could overexpress tumor-specific antigens that are responsible for inducing autologous T cell cytotoxic T cell responses against the MDS lineage. In the case of trisomy 8, however, the MDS target cells are relatively resistant to such T cell-mediated apoptosis. Fig. 1 outlines the authors' working model of T cell interactions with trisomy 8 cells that may be extrapolated to other MDS patient responsive to IST.

Patients with trisomy 8 have a high probability of responding to IST. Curiously, the authors found that selective patients may have normal bone marrow cytology despite the persistence of this karyotypic abnormality with trisomy 8 who recover their blood counts after IST to normal marrow morphology yet do not lose the trisomy 8 populations in the marrow. Indeed the percentage of trisomy 8 positive cells by FISH in blood and marrow can actually increase. Despite the increase in karyotypically abnormal cells, these patients remain stable for years and have shown a very low risk of progressing to acute leukemia.⁴³ These observations reveal unique and unexpected consequences of the interaction of the immune system with MDS. First, in untreated patients, the survival of the trisomy 8 clone despite in the presence of the T cell response suggests that the MDS cells are adapted to survive T cell attack. Experimental data support this idea; trisomy 8 cells show signs of apoptotic induction with increased caspase and BCL-2 but appear to avoid destruction from T cell-mediated apoptosis by up-regulating survivin. This stalled apoptotic process results in "living dead" cells with dysplastic morphology. Second, the recovery of normal morphology in the trisomy 8 cells after IST strongly suggests that morphologic features of dysplasia at least in trisomy 8 disease may represent cellular responses induced by T cell attack. Lastly, and surprisingly, in the absence of T cell attack the expanded trisomy 8 clone can establish normal hematopoiesis, which remains stable for many years.

THE PLACE OF IMMUNOSUPPRESSION IN THE TREATMENT OF MDS

Although BM transplantation is the only curative therapy in MDS, it is important to point out that about half of all deaths are related to cytopenias rather than leukemic progression. Any improvement in hematopoiesis thus may translate into improved survival for this disease.^{30,44} Following the considerable success of IST in SAA patients, this treatment was applied with some success first to hypoplastic MDS and then to normo- or hypercellular MDS. About one third of patients had become transfusion independent and enjoyed normal counts for years, but more commonly counts improved but did not revert to normal.³⁰

CYCLOSPORINE-BASED TREATMENTS

Many early studies were performed using cyclosporine in low-risk MDS. Jonasova⁴⁵ who treated 17 cytopenic patients with refractory anemia (RA) and variable marrow cellularity with cyclosporine (CsA) reported prolonged response in 82% of patients. All responders achieved transfusion independence. A multicenter study in Japan⁴⁶ reported responses to CsA in 60% of 50 low-risk patients. Although CSA generally was well tolerated, renal failure occurred in a minority, necessitating stopping the drug. Others have reported no substantial responses to CsA⁴⁷ and unacceptable toxicity. Together these findings indicate a potential role for CsA in the treatment of MDS with careful followup of renal function. It is important to note that responses occurred both in patients with hypocellular and hyper- and normo-cellular marrows.

ATG-BASED REGIMENS

Horse ATG and more recently rabbit ATG (rATG) have been used to treat both aplastic anemia and MDS. Horse ATG rapidly depletes peripheral and lymphoid T cells, but its effect is transient. Rabbit ATG induces more profound and more prolonged immunosuppression. In vitro data suggest that cocultivation of rATG with normal human peripheral blood mononuclear cells (PBMCs) resulted in marked expansion of functional T regulatory cells by converting CD4+CD25- T cells to CD4+CD25+ T cells.⁴⁸ Horse ATG did not expand but rather decreased T regulatory cells.

Formal trials of ATG in MDS first were instituted in 1995.⁴⁹ Since then, many other investigators have reported improvement in cytopenias in MDS patients treated with ATG-containing regimens.^{50–52} One-hundred-twenty-nine MDS patients were treated with ATG-containing regimens in a trial at the National Institutes of Health (NIH) from 1995 through 2003. Thirty-nine (30%) patients receiving a single course of immunosuppression responded with significant improvement in cytopenias and transfusion independence.³⁰ Thirty-one percent (12 of 39) of the responses were complete, resulting in transfusion independence and near-normal blood counts. Factors affecting responses included younger age, low IPSS score, and the presence of HLA DR15. Although responses were equal in patients regardless of cellularity, complete responses were more common in patients with hypocellular MDS. Similar to SAA, the addition of CsA improved the response rate ($P = .048$) and decreased the relapse rate. No controlled trial, however, has yet been performed that compares ATG versus ATG and cyclosporine. Age was a continuous variable, so that the younger the patient, the better was the response to treatment. Median survival of responders was superior to that of nonresponders. Comparison of survival of int-1 MDS less than or equal to 60 years of age treated with IST was superior to that of the historical sample of similar patients treated with supportive care.³⁰ Multifactorial analysis showed that cellularity did not affect survival or response to therapy. In contrast, Lim and colleagues⁵³ in the United Kingdom studied a group of 96 patients with MDS, 40 (42%) of whom achieved a hematological response. This group reported that patients with hypocellular BM were most likely to respond and that age did not influence response. The average age of patients in that trial was only 56 years, however, and despite the relation of response to cellularity, 26% of patients with normo- or hypercellular marrows showed improved blood counts.

Trials with younger patients tend to show improved response rates and decreased morbidity associated with the ATG.⁵⁴ Geary and colleagues⁵⁰ from Manchester, United Kingdom, reported the use of ATG with CsA or oxymetholone in 13 cases of hypoplastic MDS with cytogenetic abnormalities (average age 40). All patients ultimately responded to immunosuppression (with or without androgens), but three eventually relapsed into aplasia. In contrast, Steensma and colleagues⁵⁵ from the Mayo Clinic studied a group of older

patients (median age 69), finding no responses and considerable toxicity in eight MDS patients (two RA and six refractory anemia with excess blasts) treated with ATG 40 mg/kg/d for 4 days (Table 1). At MD Anderson Cancer Center, 32 MDS patients were treated with ATG (40 mg/kg/d intravenously for 4 days plus CsA daily orally for 6 months and methylprednisone 1 mg/kg/intravenously before each dose of ATG). Of the 31 evaluable patients, 16% responded; three of these responses were durable remissions (12 to 60+ months). The inclusion of older patients with excess blasts may have accounted for the relatively poorer results compared to other studies (see Table 1). Of interest is the fact that IST appears to improve nonclonal hematopoiesis. One study from Dusseldorf, Germany⁵⁶ examined this phenomenon in 10 female patients with low-risk MDS treated with ALG or ATG. The four responders demonstrated a nonclonal marrow defined by X chromosome inactivation patterns (XCIP), suggesting improved normal progenitor function. It may be hypothesized that this improvement is related to possibly from reduction in bystander damage from TNF and IFN .

The comparative effectiveness of hATG versus rATG has not been studied intensively in either MDS or AA. One small study⁵¹ comparing the efficacy of horse ATG (15 mg/kg/d) with rATG (3.75 mg/kg/d) in a cohort of 35 MDS patients treated for 5 days found no significant differences in responses between the two treatments. In patients with RA, overall response rate was 34% and 42% respectively for horse and rabbit ATG. Larger trials will be required to adequately compare these two treatments.

Patients, who relapse following ATG and receive CsA, generally respond to reinstituting CsA. In the NIH study,³⁰ there were 13 relapses among the 39 responders. Among the 12 patients with complete responses, four relapsed within the first year, but all responded to reinitiation of immunosuppression. Of these, only two required retreatment with ATG. Three of these patients remain in remission without further treatment at a median follow-up of 6.2 years. Median relapse-free survival was greater than 10.5 years. Of the 40 responders in a European study, 30 (75%) had durable hematological response lasting a median 31.5 months (range: 6 to 92 months).⁵³ Of the 10 patients with a transient response to ATG therapy, two of three patients subsequently achieved a hematological response following a second course of ATG.

Despite concerns regarding the potential for escape of a malignant clone, IST has not been associated with an increased progression to leukemia.³⁰ With the notable exception of trisomy 8 (as described previously) samples examined by FISH showed no significant expansion of karyotypically abnormal cells. FISH and cytogenetic analysis of the patient's BM before and following immunosuppression have demonstrated no consistent increase in chromosomal abnormalities indicative of clonal expansion other than in patients with trisomy 8.⁴³ The trisomy 8 patients remained stable and more likely to respond and maintain their response provided CsA was used as maintenance therapy.⁴³ Newer immunosuppressive treatment such as alemtuzumab may prove equally or more effective when compared to ATG in patients with MDS.⁵⁷

ANTI-TNF DRUGS

Of all cytokines, TNF- appears to be the most prominently expressed in MDS. Consequently, many trials have been conducted to evaluate the role of anti-TNF agents as treatment for MDS. Initial trials used thalidomide, which has multiple activities directed against angiogenesis and TNF production. Response rates were modest, but potentially benefit some MDS patients. Thalidomide has significant toxicity in the elderly population. CC5013 or lenalidomide was developed as an oral analog of thalidomide with a better toxicity profile, notably with fewer neurologic adverse effects, and possibly additional

immunomodulatory activities. Its actions however are significantly different than those of thalidomide. Anti-TNF- monoclonal antibodies,^{58–60} soluble TNF- receptors,⁶¹ and chemical inhibitors of TNF^{62–64} have generally been disappointing when used alone.⁶

SUMMARY

Some patients with MDS have an immune-mediated process that compromises hematopoiesis. Improving hematopoiesis in those individuals appears to improve survival. Careful selection of patients to be treated with IST doubtless will improve response rates and eliminate toxicity of those least likely to respond.

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Fig. 1.
Working model for immune dysregulation in MDS.

Table 1

Clinical trials of ATG to treat cytopenia in MDS

Center	N	Median Age	% RA	Response n (%)	Response RA (%)	Median Duration (Months)	Response Hypocellular
NIH ⁶⁵	129	60	87 (67)	39 (30)	36 (40)	>44	18 (44)
London ⁵²	30	54.5	13 (65)	10 (50)	8 (62)	15.5	27 (51)
MD Anderson ^{26,66}	31	59	18 (58)	4 (16)	2 (11)	12–60	NE ^a
Hanover ⁵¹	35	63	24 (68)	12 (34)	10 (42)	>9	2 (50)
Mayo Clinic ⁵⁵	8	71	2 (25)	0	0	-	0
Karolinska ⁵⁴	20	85		6 (30)	5 (29)	7	2 (50)
Total	253			71 (28%)	61 (41)		

^aNE data not given.