Autoimmune mechanisms in the pathophysiology of myelodysplastic syndromes and their clinical relevance

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Myelodysplastic syndromes (MDS), first clearly defined as an entity by the pioneering studies of Bernard Dreyfus and others, have long been perceived as pre-leukemic hematopoietic stem cell abnormalities which cause bone marrow failure and ultimately transform to acute leukemia.

The bone marrow failure, resulting in the transfusion dependence and neutropenic infection that characterizes the disease, was assumed to be an intrinsic stem cell defect causing defective maturation. However, accumulating evidence has shown that marrow failure in some MDS is associated with autoimmunity, T-cell mediated myelosuppression and cytokine-induced cytopenias. The novel study by Chamuleau *et al.*[1] in this issue suggests that the innate immune system may play a role in MDS pathophysiology. Study of the autoimmune component in MDS pathophysiology has led to better definition of patient subgroups where immune processes are the dominant cause of the cytopenia, and has led to the development of immunosuppressive treatments which have improved the quality of life and extended survival of selected patients with MDS.

Immune mechanisms in the pathophysiology of cytopenia in myelodysplastic syndromes and the response to immunosuppression

Evidence for autoimmune abnormalities in MDS comes from clinical and experimental sources.[2-6] Hamblin *et al.*[7] first drew attention to an association between MDS and the autoimmune diseases. MDS is sometimes seen in conjunction with Reynaud's syndrome, rheumatoid arthritis and polymyalgia rheumatica. Furthermore, MDS is associated with aplastic anemia, a disease with an established autoimmune pedigree: patients with AA can develop MDS and overlap syndromes of severe aplastic anemia with features of MDS also exist and frequently give rise to diagnostic confusion. Tichelli *et al.* were the first to describe inhibition of erythroid colony growth by autologous T cells in some patients with MDS.[8] Based on the success of antilymphocyte globulin to treat AA and anecdotal accounts of cytopenic patients with clear features of MDS responding to immunosuppressive treatment (IST), we treated a series of transfusion-dependent MDS patients with ATG and achieved sustained red cell transfusion independence in 20%.[9] In several patients studied, we demonstrated that ATG abrogated the T-cell mediated suppression of granulocyte colony growth in responding patients. Subsequently, other investigators confirmed that ATG can lead to improved marrow function and loss of transfusion dependence, accompanied by a normalization of a skewed T-cell repertoire.[6] These findings strongly supported T-cell mediated marrow suppression as the cause of the cytopenia in about 20-30% of MDS patients. Figure 1 illustrates the working hypothesis regarding the relationship between the immune system and cytopenias in MDS. The highest response to immunosuppression occurred in the less common group of younger MDS patients (under the age of 60) with refractory anemia (RA)[10].

Although several factors such as the PNH abnormality, RA, and degree of cytopenia have been identified in various series as predictive factors for response, only age and the presence of HLA DR15 emerged as an independent prognostic factor in multivariate analysis in a large NIH study of 120 patients.[10] Such young MDS responders to ATG included a preponderance of female patients with RA and an over-representation of trisomy 8.

These observations raise many questions concerning the mechanism underlying the immune dysfunction of

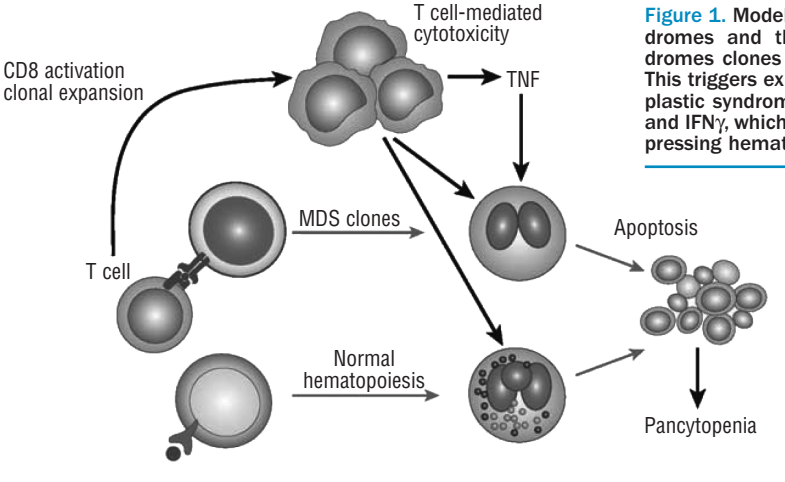


Figure 1. Model of immune interactions with myelodysplastic syndromes and the response to treatment. Myelodysplastic syndromes clones express a neoantigen or overexpress an antigen. This triggers expansion of T cell clones cytotoxic for the myelodysplastic syndromes cell. Activated T cells secrete cytokines, TNFα and IFNγ, which promote apoptosis of normal progenitor cells suppressing hematopoiesis.

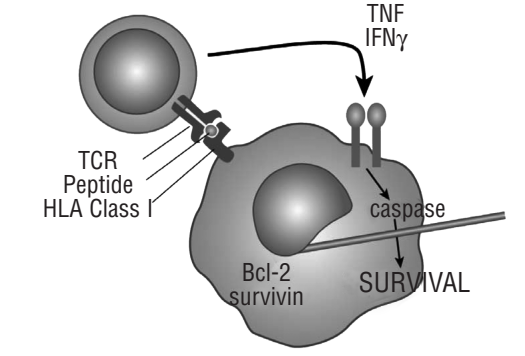
MDS. What are the antigens on MDS cells recognized by auto-reactive T cells? What is the role of cytokines such as TNF-α in the bystander effect on residual normal hematopoiesis? Does the immune-mediated myelosuppression serve as a form of immune surveillance to retard the evolution to leukemia? Much insight into these questions has been provided by studies of the cytopenia associated with trisomy 8 MDS. The use of fluorescent *in situ* hybridization (FISH) to identify the trisomy 8 marker allows the investigator to separately identify T-cell interactions with normal and MDS cells and define their specificity *in vitro*. Using FISH techniques we showed that cytotoxic T cells specifically targeted trisomy 8 bearing cells.[11] Because the Wilm's tumor antigen, WT1, is over-expressed on CD34 cells in many patients with MDS, it is an obvious candidate antigen for an MDS specific T cell response.[12,13] We found that patients, particularly those with trisomy 8 (who have a high probability of response to IST), overexpress WT1. Furthermore, T cells from these patients show repetitive skewing of restricted Vβ 3.2 CD8 T cell and several other Vβ subsets, which diminishes after successful IST.[11] CD8 cells isolated from the Vβ 3.2 fraction contain the preponderance of the myelosuppressive activity of autologous T cells and specifically block proliferation of trisomy 8 hematopoietic progenitors.

Furthermore, the Vβ $CD8^+$ lymphocytes, which are expanded in patients over-expressing WT1 (with or without trisomy 8), contain a high frequency of $WT1_{126}$ peptide-HLA tetramer positive $CD8^+$ T cells which overexpress TNF-α (Sloand *et al., unpublished data, 2008*). These cells are capable of suppressing growth of trisomy 8 cells. These findings strongly indicate that WT1 is at least one of the antigens driving the myelosuppressive T-cell response in MDS.

Thus far, the detailed picture of MDS pathophysiology emerging at the molecular level seems straightforward. However, clinical data is puzzling. Patients with trisomy 8 who recover their blood counts after immunosuppressive treatment revert to normal marrow morphology yet do not lose the trisomy 8 population in the marrow; indeed the percentage of trisomy 8 cells measured by karyotyping or by FISH can actually increase. Despite this increase in karyotypically abnormal cells these patients are stable for extensive periods of time[11] Thus it appears that the removal of T-cell control permits hematologic recovery with a further expansion of the MDS clone. In untreated patients, the persistence of the trisomy 8 clone 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.[14] This stalled apoptotic process results in "living dead" cells with dysplastic morphology. After IST, the loss of MDS specific cytotoxic T cells permits the return to normal morphology and expansion of the now unrestrained trisomy 8 clone. (Figure 2). This scenario would suggest that IST should be used with great caution for fear of removing immune surveillance, which although inconveniently causing cytopenia, might be the only barrier to leukemic progression. Fortunately, from a long-term follow-up of IST treated patients data already exist to refute this concern. We found no increase in progression to leukemia in either responders or non-responders after as much as ten years of follow-up.[10] Responders treated with IST actually had a decreased rate of leukemic progression[10] In addition, apart from patients with trisomy 8, serial FISH studies demonstrated a significant decrease in most karyotypic abnormalities following IST.[11]

This suggests that although the immune response to MDS causes cytopenias, such immune surveillance may be unrelated to what appears to be an intrinsic tendency of MDS stems cell to progress to leukemia. Another possibility is that the immune system fosters development of genomic instability. Provocative evidence in other inflammatory states suggests that aneuploidy may result from oxidative stress and nitric oxide (NO)-induced cell cycle arrest. Conditions such as Barrett's esophagitis, hepatitis, graft versus host disease of the skin, and ulcerative colitis result in aneuploid and tetraploid lesions which can precede malignant development.[15-18] This mechanism could account for the high frequency of clonal progression in patients with aplastic anemia and for the decrease in leukemic progression among responders to IST.[10]

Given the existence of a natural T-cell response to WT1 in MDS, the use of WT1 vaccines to treat MDS appears difficult to justify. The induction of a T-cell response to WT1 by vaccination could provoke undesired marrow suppression. Currently there are not enough data on the use of WT1 vaccine in MDS to con-



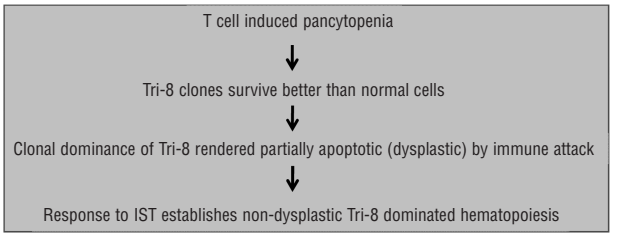


Figure 2. Working hypothesis for induction of an immune response to MDS and the selection of trisomy 8 clones. Trisomy 8 cells overexpress normal antigens such as WT1 which results in induction of an immune response. Cytotoxic T cells, which recognize the antigen, expand. However, these cells are ineffective in eliminating the trisomy 8 clone. Apoptosis is triggered in the trisomy 8 cell but is blocked upstream of caspase 8 activation because trisomy 8 cells overexpress survivin.

firm or refute these concerns. We chose to select for WT1 vaccine treatment only those patients with progressing MDS with excess of blasts, because prevention or delay of overt leukemia could outweigh the disadvantage of inducing cytopenia.

Finally it remains a possibility that the entire T-cell response to MDS is a side-show, and we should direct our attention more to myelosuppression by NK cells shown by Chamuleau *et al.*[1] to be strongly and specifically cytotoxic to MDS cells and perhaps in some patients (e.g. non-responders to ATG) responsible for myelosuppression and immune surveillance. In conclusion, the relationship between the immune system, marrow suppression and MDS remains confusing. Comprehensive studies in a large group of patients as performed by Chamuleau *et al.* are critical steps forward in trying to establish a global view of competing mechanisms contributing to the two major outcome determinants of MDS – marrow failure and leukemic progression.

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Cord blood transplantation: state of the art

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Hematopoietic stem cell transplantation (HSCT) can be curative in a large variety of selected malignant and non-malignant diseases. Cord blood is an unlimited source of hematopoietic stem cells for allogeneic hematopoietic stem cell transplant. Umbilical cord blood transplantation (UCBT) has extended the availability of allogeneic HSCT to patients who would not otherwise be eligible for this curative approach. Since the first human cord blood transplant performed twenty years ago,[1] cord blood banks (CBB) have been established for related or unrelated UCBT with more than 400,000 units available and more than 20,000 umbilical cord blood transplants performed in children and in adults. UCB has many theoretical advantages due to the immaturity of newborn cells. UCB hematopoietic progenitors are enriched in primi-