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## EDITORIAL

# Defects of immune surveillance offer new insights into the pathophysiology and therapy of myelodysplastic syndromes

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Myelodysplastic syndromes (MDS), whose pathophysiology remains poorly known, are myeloid disorders that may evolve toward acute leukemic transformation, and for which allogeneic bone marrow transplantation, largely through the 'graft versus leukemia' (GvL) effect, remains the only curative treatment. A peculiar and yet unexplained relationship between MDS and immune disorders including hypo- or hyper-$\gamma$-globulinemia, peripheral lymphopenia, abnormal B- or T-cell functions, monoclonal gammopathy, autoantibodies or diseases like relapsing polychondritis, vasculitis and seronegative arthritis has been noticed.[1,2] The immune system also seems to contribute, in some cases, to the progressive cytopenias observed in MDS.[3] On the basis of those observations, a role of the immune system in the pathophysiology and progression of MDS has been envisaged. Furthermore, innate immunity cells could offer an alternative to bone marrow transplantation in hematological malignancies including MDS, as promising results of cellular immunotherapy have been obtained in solid tumors.

Cellular components of the innate immune system, including natural killer (NK) and $\gamma\delta$ T cells have been shown to regulate cancer development and to play an important role in the lysis of transformed cells.[4] NK cells do not require prior immunization and are involved in the early phase of the immune response. Their activation relies on natural cytotoxicity receptors (NCR) that recognize yet unknown ligands on tumor cells. In addition, NK cells express NKG2D, an activating receptor that recognize danger signals including stress molecules (MICA/B molecules, UL-binding protein). NK also sense the level of HLA-I molecule expression through inhibitory receptors to discriminate normal from transformed cells.[5] Thus, the balance between activating signals and negative signals induced by the inhibitory receptors governs the final decision to induce or not the NK-cell activation.[6] NK cells differentiate from a bone marrow precursor belonging to the T/NK lymphoid lineage. Several laboratories, including ours, have shown that it was possible to reproduce *in vitro* the differentiation of mature NK cells from CD34+ hematopoietic progenitors cultured with a combination of SCF and IL-2 and/or IL-15.[7,8] In those conditions, CD34+ precursors proliferate and differentiate into cytotoxic NK cells leading to large expansion of NK cells.[8]

Different lines of evidence suggest that NK cells participate in the anti-leukemic immune response. Several reports have indicated an inverse relationship between NK-cell number or activity and prognosis in acute leukemia. Decreased NK-cell function along disease progression was reported in chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) patients.[9,10] Experimental results outline that Bcr/Abl leukemic targets are recognized and efficiently killed by NK cells[11,12] and outline the role of NKG2D-activating receptor.[13] In addition, recent clinical results support a role for alloreactive NK cells in the GvL effect observed in HLA-haploidentical transplants, and the therapeutic possibilities to manipulate NK receptor–ligand interaction to increase leukemia cell destruction are emerging.[14]

Non-classical T-cell receptor (TCR) $\gamma\delta$ T cells represent another important component of the innate immune system. As cytotoxic effectors, $\gamma\delta$ T cells may be involved in the anti-tumor potential against lymphoma and multiple myeloma.[15] In addition, $\gamma\delta$ T cells are regulatory cells through the secretion of pro-inflammatory cytokines [16] and their potential role as antigen presenting cells.[17] Peripheral $\gamma\delta$ T cells can be expanded *in vivo* and *in vitro* by a potent synthetic phosphoantigen-specific TCR $\gamma\delta$ agonist, the BrHPP molecule,[16] and acquire antitumor functions.[18]

The possibility to differentiate NK cells *in vitro* has raised the question of NK-cell differentiation in patients with myeloid disorders. We showed that the *in vitro* development of NK cells from CML progenitors was altered,[19] suggesting that such alterations may lead to the decreased function of peripheral NK cells described in CML patients. We also found a severe alteration in NK-cell differentiation from CD34+ cells derived from MDS patients.[20] None of the 14 cultures from bone marrow-purified MDS CD34+ cells resulted in NK-cell differentiation. Selection of more immature CD34+/CD38− progenitors and limiting dilution experiments showed that cloning frequency of NK progenitors was at least 10 times lower than that usually found in normal bone marrow. A decreased frequency of NK progenitors in MDS bone marrow might in part account for the dramatic alteration of NK-cell differentiation in response to SCF and IL-15 *in vitro*.

One year ago, we published the first comprehensive study on NK cells in MDS patients, based on recent developments made in NK-cell biology.[21] We studied expression of specific NK-activating and inhibitory receptors, which tightly control their cytolytic function in donor NK cells, as well as MDS-NK cell survival *ex vivo*, and their belonging to the myelodysplastic clone. Functional capacities of MDS NK cells were measured both in peripheral blood mononuclear cells (PBMC) and in immunoselected NK cells activated with interleukin (IL)-2. We showed that peripheral NK cells from MDS patients poorly killed K562 target cells as well as the MDS-derived cell line P39, and displayed decreased secretion of IFN$\gamma$ and TNF$\alpha$ in response to NCR triggering. Purified NK cells displayed deeply altered NK function in all MDS cases (regardless of subtype, cytogenetics or risk category), suggesting that this defect was a hallmark of MDS rather than a marker of more advanced disease.

A recent paper by Epling-Burnette *et al.*[22] in a similar cohort of patients confirms the deeply altered NK cytolytic function we described in MDS. However, some results diverge between the two reports. For example, contrary to our study, the decreased lytic function measured in PBMC was correlated with high-risk disease and reduced NKG2D-activating receptor expression by NK cells. Although the authors used elegant correlations between percentages of NK cells in PBMC and cytotoxicity, a non-specific lysis due to other effectors could not be ascertained unless purified NK cells were used, and such assays were performed only in two patients. In our study, although more

severe defects were observed in high-risk MDS using resting PBMC, we observed no correlation between highly decreased cytolytic function and NCR expression or disease characteristics when we assessed the functions of immunoselected NK cells. In fact, we found that NK-cell function was severely altered in spite of normal NCR expression. We recently assessed the lytic potential of NK cells by measuring their degranulation capacities (externalization of CD107a by flow cytometry assays) in response to target stimulation.[23] This sensitive assay performed on PBMC but allowing specific analysis of degranulating resting NK cells confirmed our previous data. Such findings seem to be a characteristic of MDS, because in other hematological malignancies including CML and AML, decreased function was associated with low level of NCR expression on NK cells.[24] Finally, we showed that a significant proportion of MDS NK cells belonged to the myelodysplastic clone, as the chromosomal aberration was found in circulating NK cells from patients with either 5q−, −7 or +8.

Altogether, those two studies clearly show that cytotoxicity of NK cells is highly decreased in MDS. However, the mechanisms leading to such alterations are not fully explained. The decreased expression of NKG2D, found by Epling-Burnette *et al.*[22] but not in our study, cannot account by itself for the peripheral NK-cell anergy in MDS. Among other possible mechanisms, we found that a significant proportion of circulating NK cells in MDS were derived from myelodysplastic progenitors (bearing the same chromosomal abnormalities) and displayed high level of apoptosis. Finally, absence of MDS-NK proliferation after stimulation with IL-2 *in vitro* was described in the two studies.

Those alterations of NK cells in MDS prompted us to investigate other components of the innate immune system, the $\gamma\delta$ T cells. In a large cohort of MDS patients, we recently evaluated their phenotype, function and *in vitro* expansion. We found that this cell population was significantly decreased in peripheral blood of the subgroup of MDS patients presenting with associated autoimmune disorders (such as rheumatoid arthritis, systemic lupus erythematosus or several autoantibodies). However, *in vitro* stimulation with BrHPP induced expansion of V$\delta$2 $\gamma\delta$ T cells in 60% of MDS, including high-risk patients. Those expanded V$\delta$2 T cells exhibited normal cytolytic function toward leukemic and MDS cell lines, and FISH analysis indicated that they were not derived from the MDS clone. Although we could expand the $\gamma\delta$ T cell population in MDS, their subsequent proliferation in response to BrHPP and IL-2 was significantly decreased compared to normal donors. This common proliferation defect described in NK and $\gamma\delta$ T cells in MDS occurred despite normal expression level of IL-2 receptors (unpublished data), and suggests involvement of actors of IL-2 signaling pathway downstream of the IL-2 receptor.

Our study and that of Epling-Burnette *et al.*[22] suggest that alteration of NK-cell function may be an important factor in MDS pathophysiology and progression. Our recent results on $\gamma\delta$ T cells further support the hypothesis of defective immune surveillance in MDS, a concept now well established in solid tumors.[25] Those observations open new perspectives not only in the understanding of MDS pathophysiology, but also in the emerging area of immunotherapy. The basic advances made in the understanding of interactions between immune system and tumors have so far yielded only limited success using vaccines or adoptive immunotherapy. MDS are malignant diseases with several characteristics suggesting that they could be good candidates for immunotherapy, including a chronic phase with low tumor burden, a clearly demonstrated GvL effect, and the efficacy of immunosuppressive therapy in some cases. A comprehensive approach to define mechanisms of resistance or escape of myelodysplastic cells to the immune system may help in the search for effective therapies as alternatives to bone marrow transplantation or conventional chemotherapy.

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