limited treatment options are available outside clinical trials.

As in other pomalidomide trials, toxicity consisted primarily of myelosuppression, although it was more pronounced than what was previously reported with this agent. Reduced hematopoiesis related to both advanced refractory disease and extensive prior treatments justifies the higher rates of grade 3-4 neutropenia (66% and 51% in the 4- and 2-mg cohorts, respectively) and thrombocytopenia (30.5% in both cohorts) observed in the current study compared with others. Nevertheless, the overall frequency of febrile neutropenia was low (0% and 11% in the 2- and 4-mg cohorts, respectively). The most common nonhematologic toxicity was fatigue. With adequate thromboprophylaxis and use of reduced-dose dexamethasone, thrombotic complications were observed in 4% of all patients. Grade 2 peripheral neuropathy (PN) possibly related to pomalidomide therapy was 11% in both cohorts, while grade 3 PN was 3% in the 4-mg cohort. However, it is worthy of note that the majority of these patients had baseline PN that worsened during pomalidomide treatment.

The lack of superiority with the higher over the lower pomalidomide dose reported by Lacy et al might suggest that for this drug there is no dose-response effect. However, before concluding that higher pomalidomide doses are useless, it should be noted that the shorter exposure to the study drug in the 4-mg compared with the 2-mg cohort (median number of 28-day cycles administered per patient: 3 vs 6, respectively) and comparable total doses of pomalidomide actually delivered in the 2 subgroups (median dose per patient: 12 mg in both cohorts) might have ultimately affected similar rates of response. This issue was not addressed by Lacy and colleagues, who continue to investigate the optimal dosing schedule of pomalidomide. In this context, a regimen of 4 mg on days 1 through 21 of each 28-day cycle compared with 2 mg continuously for 28 days is actually under investigation.

Pomalidomide represents a leap forward in myeloma care and serves as an important platform on which to build future rationally based combination strategies. In addition to pomalidomide, carfilzomib (PR-171), a novel generation proteasome inhibitor of the epoxyketone class, has shown remarkable single-agent activity and holds great promise in the setting of advanced rel/ref MM. Complementary to the development of newer immunomodulators and proteasome inhibitors, including salinomycin (NPI-0052) and the novel orally bioavailable inhibitor MLN9708, a great number of investigational agents targeting novel pathways (such as histone deacetylase inhibitors, AKT/P13K/mTOR inhibitors, heat-shock-protein inhibitors, and monoclonal antibodies) have entered clinical testing. The armamentarium of available treatment options for MM continues to expand and it is likely that patient outcomes will continue to progressively improve in the coming years.

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Comment on Yahata et al, page 2941

HEMATOPOIESIS & STEM CELLS

HSCs: stressing out over ROS

Qishen Pang CINCINNATI CHILDREN’S HOSPITAL MEDICAL CENTER

In this issue of Blood, Yahata and colleagues demonstrate that reactive oxygen species (ROS)–induced DNA damage impairs the self–renewal capacity of human HSCs, and that oxidative DNA damage repair is less efficient in quiescent stem cells than in progenitor cells.1

HSCs are a rare population of pluripotent cells that can self-renew and produce various types of cells of the blood lineage. Under steady physiologic conditions, the most primitive HSCs are in a quiescent state and reside in the BM niche where they preserve the capacity to self-renew and to continue to produce all types of blood cells throughout a prolonged lifespan.2 In response to stress or stimulation, the HSCs can move out of the BM niche, entering cell cycle and undergoing division (see figure panel A). In addition, the cycling HSCs may return to the BM niche and regain their quiescent state.3 Disruption of HSC quiescence prematurely exhausts the HSC pool and causes hematologic failure under various stresses, such as oxidative, replicative, and metabolic, and DNA damage.4 HSCs are exposed to various ROS, which are routinely generated during metabolic or inflammatory process (reviewed in Naka and Hirao5). ROS induce a variety of responses in HSCs, including cellular proliferation and apoptosis. ROS can also cause DNA damage and drive HSCs into cell division, which appears to be essential for DNA repair processes.4 Recent studies in mice suggest that mechanisms involving antioxidant defense and DNA repair appear to be essential for the maintenance of HSC self-renewal capacity and the suppression of malignant transformation. Indeed, mice with mutations in several oxidative stress response (Atp1, Fance, Fancd2, FoxO) and DNA damage repair (Lig4, Dna-pk, Ku80, Xpd, mTR) genes exhibit premature exhaustion of HSCs because of accumulation of ROS or DNA damage.4,5,7

Less is known about how oxidative DNA damage affects the function and lifespan of human HSCs. Yahata and colleagues use a
strategy of detecting the in vivo repopulating dynamics of individual human HSCs to test the hypothesis that the continuous production of ROS during long-term repopulation induces an accumulation of DNA damage that leads to exhaustion of human HSCs. The authors have previously reported that the repopulating potential of human HSCs progressively deteriorated as they went through extensive repopulation process. In the present study, Yahata et al demonstrate that the decreased repopulating capacity of human HSCs during serial transplantation is accompanied by increased ROS and DNA damage in the repopulating donor HSCs (see figure panel B). Interestingly, the authors observed that higher DNA damage remained un repaired in quiescent HSCs than in cycling progenitors. In addition, the oxidative DNA damage increases expression of cell-cycle inhibitors, causing HSCs to undergo premature senescence and consequently leading to the functional impairment of HSCs in vivo. Finally, treatment with the antioxidant NAC can abrogate the effect of oxidative DNA damage on HSC function.

These results underscore the importance of oxidative DNA damage repair to maintaining the function of HSCs. One intriguing aspect of the findings in the study by Yahata et al is that actively cycling progenitors (Lin−CD34+CD38−) accumulate much less ROS or DNA damage than relatively quiescent HSCs (Lin−CD34+CD38+) do. In explaining why and how the HSCs and the progenitors respond differently to the same DNA damage, the authors argue that progenitor cells may either possess more efficient DNA repair mechanisms or simply purge the severely damaged cells. This would be consistent with a notion that compared with quiescent HSCs, proliferating progenitors are more resistant to DNA damage. Nevertheless, this finding is contrary to 2 recent reports showing that common myeloid progenitors in mice and flies produce significantly increased levels of ROS compared with HSCs. One possible explanation for this discrepancy is that the repopulated progenitors may have arisen from HSCs containing low ROS.

Another intriguing finding by Yahata et al is the observation that the cell-cycle status of human HSCs can reversibly change from quiescence to oxidative stress–induced activation during repopulation (see figure panel B). Analysis of BrdU incorporation shows that at 2 weeks after transplantation, Lin−CD34+CD38− cells exited quiescence and underwent activation and expansion. However, these proliferating Lin−CD34+CD38− cells were able to regain quiescence at the later phase of primary transplantation as well as in serial transplantation. This finding raises important questions as to whether activation is required for efficient repair of oxidative DNA damage in stressed HSCs and whether elimination of the DNA damage is sufficient for the activated HSCs to return to quiescent state. Although further evidence needs to be provided, it is tempting to speculate that reversibility between quiescence and activation may be a physiologic function of activated HSCs at the interface between damage repair and re-establishment of homeostasis. In this context, it is noteworthy that a recent study in mice shows that HSCs reversibly switch between dormancy and self-renewal at the interface between homeostasis and repair.

One major caveat associated with these findings is that the study used Lin−CD34+CD38− population, which may be not homogeneous but contain both stem cells and progenitors. Recent studies from the Dick group demonstrate that sorting based on CD49f identifies highly purified human HSCs. Nevertheless, the findings by Yahata and colleagues shed new light on oxidative DNA damage response in human HSCs and extend our understanding of HSC maintenance under stress. Moreover, the model of combined human xenotransplant with antioxidant supports the view that antioxidants could be of considerable therapeutic value in the clinical setting of HSC transplantation.

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NRP-1 is a neuronal surface receptor involved in axon guidance. In the past few years, it has also been implicated in dendritic cell–T cell interactions. In this issue of Blood, Milpied et al find that NRP-1 is a marker of recently generated IL-17–producing natural killer (NK) T cells.1

Neuropilin-1 (NRP-1) is a transmembrane glycoprotein and was first described as a neuronal surface receptor involved in axon guidance.2 It functions by binding semaphorins, a family of chemorepulsive molecules.3 However, the role of NRP-1 extends outside the nervous system, and many of the functions have yet to be defined. NRP-1 is a vascular receptor expressed on endothelial cells and was found to bind selectively to the 165 amino acid form of VEGF (VEGF165).4 The neuropilins also interact directly with the classic receptors for VEGF, VEGF-R1 and -R2, and function as co-receptors by mediating signal transduction.4,5 Their role in angiogenesis has been shown both in physiologic and pathologic situations.4 Manipulating neuropilin function can regulate tumor growth and metastasis through effects on vascular biology in the case of neuropilin-1, and lymphatic biology in the case of neuropilin-2.6 More recently, NRP-1 was shown to be a part of the HTLV1–Receptor complex.7

The involvement of NRP-1 in the immune system was first shown in 2002 in the immune synapse.8 NRP-1 expression on activated T cells and dendritic cells (DCs) mediates homotypic interactions promoting the initiation of the primary immune response.8 Parallel studies showed that semaphorins are important molecules in the cross-talk between T cells and DCs.8 Since then, our knowledge of NRP-1 function in the immune system has progressed slowly. NRP-1 was shown to be expressed on plasmacytoid predendritic cells (pDCs).9 Recent work has shown that mouse regulatory T cells (Tregs) express NRP-1, which enhances their interaction with DCs and subsequently favors a tolerogenic response to low-dose antigens in the absence of danger signals.9 However, human Tregs were not found to express NRP-1.10 pDCs are the only cell type expressing NRP-1 in the steady state in human blood and secondary lymphoid tissue.10 However, semaphorins may promote regulatory responses by binding NRP-1 on activated T cells and participate in the termination of the immune synapse through their chemorepulsive action.11 Hence, major questions remain regarding the expression profile of NRP-1 on immune cells in humans and mice, as well as the functions of NRP-1 in vivo and in vitro.

Milpied et al found that NRP-1 was expressed on recent thymic NK T cell emigrants, but not long-lived mature NK T cells.11 Using NRP-1 as a biomarker, they were able for the first time to comparatively study the function and cytokine production of these 2 NK T cell populations. They could show that recent thymic emigrant NK T cells predominantly produced IL-17, but not IFN-γ and IL-4, a classic feature of mature NK T cells. This suggested that a continuous thymic output is required for in vivo IL-17 production by recent thymic emigrant NK T cells. Equally important, this study identifies a novel biomarker for IL-17–producing NK T cells and extends the expression pattern of NRP-1 to NK T cells.

The work by Milpied et al raises many important questions. In terms of NK T cell biology, why is IL-17 production restricted to recent thymic emigrants and lost on mature NK T cells? Does it constitute a regulatory mechanism to avoid excessive proinflammatory IL-17 production? In terms of NRP-1 biology, what is the function of NRP-1 on these recent thymic emigrant NKT cells? What is the nature of putative endogenous ligands for NRP-1 in the thymus? NRP-1 can function through both homotypic and heterotypic ligand–receptor interactions. Thus, NRP-1–expressing NK T cells may interact with other NRP-1–expressing cells, including NKT and T cells, as well as DCs. Alternatively, NRP-1 may bind to other known ligands, such as VEGF. TGF-β is also an interesting candidate, because it is produced in the thymus and NRP-1 can function as a TGF-β co-receptor.

In summary, the identification of NRP-1 as a novel biomarker for IL-17–producing NK T cells will enable the purification of this cell population, and will certainly speed up its functional characterization. The function of NRP-1 on NKT cells remains a major question. It will be important to pursue the study of NRP-1 biology in a systematic manner, in both humans and mice, taking into account the various possible ligands as well as species-related differences, to solve the many remaining mysteries around this multifaceted molecule.

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Qishen Pang