with clinicopathologic parameters for even better risk stratification.

Although RT is conventionally used in early-stage cases, it is not employed in advanced-stage patients. With current L-asparaginase–containing regimens, up to 50% of advanced-stage patients who have not received RT achieve durable remission,[7] clearly showing that RT is not mandatory for NK/T-cell lymphomas.

CT, however, is increasingly recognized as an indispensable treatment component. The beneficial effect of CT is also shown by Yang et al.[1] In high-risk patients, the 5-year overall survival for RT followed by CT was 72.8%, compared with 57.9% for RT alone. Yang et al reported that CT followed by RT gave poor results. This observation is undoubtedly from their upfront use of ineffective anthracycline-containing regimens. Furthermore, their results of L-asparaginase–containing regimens were surprisingly worse than other studies for early-stage NK/T-cell lymphomas (see table). This may be related to a small number of patients in their study who received heterogeneous L-asparaginase–containing regimens. In fact, prospective clinical trials have shown that, with effective CT, concurrent RT/CT or CT followed by RT gave very comparable results.[3,4,7-9] Therefore, the sequence of RT and CT may not be critical.

Although physicians will continue to give RT to early-stage patients, the high systemic failure rates of RT alone, even for low-risk patients in the study by Yang et al, mean that optimal schedules of additional CT to eradicate occult systemic spread should be determined. Intriguingly, it may even be possible to define in the future if RT can be safely omitted in specific groups of early-stage NK/T-cell lymphomas because short- and long-term toxicities of RT are considerable and may have permanent impacts on quality of life, particularly for elderly patients.

The results of Yang et al, which rely on simple clinicopathologic parameters to determine whether early-stage nasal NK/T-cell lymphoma patients may receive RT alone, are useful for resource-limited centers where more expensive investigations are not available and complicated CT regimens are not feasible.[10] In better-equipped centers, PET/CT scan and EBV DNA, neither used by Yang et al, should be employed for more exact staging and prognostication. These approaches more precisely identify patients with genuine early-stage limited disease, enabling clinical studies to establish the best modality of treatment that will lead to durable remissions in these patients.

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● ● ● LYMPHOID NEOPLASIA

Comment on Leone et al, page 1443

Myeloma escape from immunity: an "inside" job

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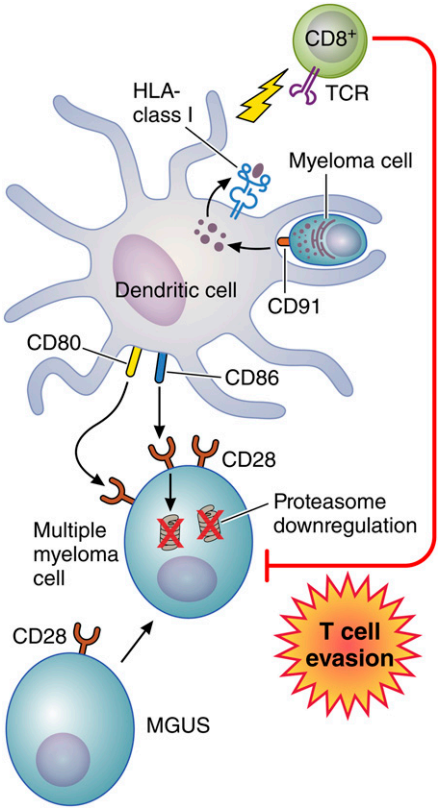
In this issue of *Blood*, Leone et al describe a novel mechanism mediated by bone marrow dendritic cells (DCs) that impairs T-cell recognition and killing of myeloma cells.[1]

A small but growing population of patients who remain in complete remission and are progression free at 10 years after diagnosis of multiple myeloma (MM) is emerging as a result of the use of the "total therapy" concept pioneered by Barlogie et al.[2] Total therapy uses highly active myeloma first-line therapies followed by 1 or 2 autologous stem cell transplantations (SCTs) and consolidation/maintenance therapy. Still only about 30% of MM patients are cured according to these criteria, and a much smaller fraction of patients with high-risk disease reach these milestones. Initial enthusiasm for an immunotherapeutic approach to MM based on evidence for a graft-versus-myeloma effect in the setting of allogeneic SCT has been tempered by the high risks of morbidity and mortality from graft-versus-host disease and by the higher-than-expected rate of relapse.[3,4] Mechanisms of immune evasion by MM cells are variable but are likely to include reduced expression of HLA molecules, reduced expression of tumor antigen peptides, enhanced expression of inhibitory ligands such as programmed cell death ligand 1 (PD-L1) and PD-L2, and recruitment of counterregulatory cells such as T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs).



Bone marrow DCs engulf apoptotic myeloma cells via CD91. Myeloma tumor antigens are then processed and presented on class I HLA molecules to activate infiltrating $CD8^+$ T cells. At the same time, using CD80/CD86, these DCs interact with nonapoptotic myeloma cells that express higher levels of CD28 compared with plasma cells from patients with monoclonal gammopathy of undetermined significance (MGUS). As a result, proteasome degradation occurs, which impairs tumor antigen expression on the myeloma cells thereby rendering them more resistant to T-cell recognition and killing. Professional illustration by Patrick Lane, ScEYEnce Studios.

More recent work on immunotherapy for MM has centered on strategies for generating an autologous graft-versus-myeloma effect and is based on observations that myeloma-reactive T cells with cytotoxic potential after activation are present at low frequencies in the marrow and blood of MM patients.[5] Promising approaches include administration of MM-DC fusion vaccines, adoptive transfer of activated marrow-infiltrating lymphocytes, and infusions of genetically modified autologous T cells engineered to express an affinity-enhanced T-cell receptor (TCR) for myeloma tumor antigens, including the cancer-testis antigens NY-ESO-1 and LAGE-1.[6-8] These approaches have generally used autologous SCT as a platform because the lymphopenia and lower tumor burden following high-dose chemotherapy may promote homeostatic proliferation of myeloma-reactive lymphocytes while reducing the burden of immunosuppressive cells such as Tregs and MDSCs.

The bone marrow microenvironment plays a critical role in support of myeloma cell growth and resistance to apoptosis and homing as a result of the elaboration of cytokines such as interleukin-6 (IL-6), C-X-C motif chemokine 12 (CXCL12), insulinlike growth factor 1 (IGF-1), vascular endothelial growth factor A (VEGF-A), and many others by mesenchymal stem cells, osteoblasts, osteoclasts, vascular endothelial cells, adipocytes, and fibroblasts.[9] In addition, the marrow represents a complex immunologic milieu that both attracts and modulates the function of tumor-reactive immune cells.

The success of many of these novel immunotherapeutic approaches for myeloma depends on effective trafficking of myeloma-specific T cells to the marrow and on their persistence and functionality. Leone et al[1] provide insight into a novel mechanism whereby the marrow microenvironment may promote immune evasion (see figure). By using marrow samples from 20 patients with MGUS and 20 patients with symptomatic MM, they found that both myeloid and plasmacytoid DCs accumulate in the marrow during the transition from MGUS to MM. Furthermore, these DCs engulf apoptotic myeloma cells through recognition of calreticulin (CD91), an "eat me" signal on the myeloma cells, and then, through tumor antigen processing, they activate myeloma-specific marrow-infiltrating $CD8^+$ T cells. The latter was demonstrated by analyzing the subset of samples from 4 patients carrying HLA-A*0201 for the level of $CD8^+$ T cells specific for the HLA-A*0201-restricted NY-ESO-$1_{157-165}$ epitope. The authors showed that the level of NY-ESO-$1_{157-165}$-specific $CD8^+$ T cells found in the marrow of all 4 of these patients became significantly lower after CD91 blockade, which prevented the DCs from engulfing the myeloma cells and presenting tumor antigen peptides to the $CD8^+$ T cells. Conversely, by using surface CD80/CD86, these same DCs interacted with nonapoptotic myeloma cells through CD28, a major T-cell costimulatory molecule that is expressed at higher levels in plasma cells from MM patients than from MGUS patients and that mediated a significant downregulation of expression of proteasome subunits. Because the proteasomes are critical for tumor antigen processing, this would be expected to decrease expression of tumor antigen peptides on the myeloma cells, enabling them to evade $CD8^+$ T-cell recognition and killing. Indeed, by using flow-based cytotoxicity assays, the $CD8^+$ T cells that expanded in the presence of autologous DCs preloaded with apoptotic myeloma cells successfully killed myeloma cells that were preincubated with DCs across a Transwell barrier, not exposed to DCs, or exposed to DCs in the presence of a CD28-blocking antibody. However, these primed and expanded $CD8^+$ T cells did not kill the myeloma targets if the myeloma cells were preincubated directly with bone marrow DCs. Importantly, this mechanism adds to earlier observations suggesting that CD28 expression on MM cells also induces chemotherapeutic resistance.[10]

Important questions remain, including whether this finding of CD28-CD80/CD86-mediated immune evasion can be translated to the clinic by using CD28 blocking agents such as CTLA4-immunoglobulin or inhibitors of CD28 downstream signaling through the PI3K-AKT pathway to restore immune sensitivity. Conceivably, T cells engineered to express myeloma-directed chimeric antigen receptors (CARs) may be able to bypass this specific immuno-inhibitory pathway if the expression of the specific CAR target is not affected by proteasome loss. T cells engineered to express large numbers of affinity-enhanced TCRs may also compensate for reduced expression of tumor antigen peptides. Finally, checkpoint inhibitors such as programmed cell death 1 (PD-1), PD-L1, or CTLA-4 antibodies may partially offset immunosuppressive signals from the marrow microenvironment. Nonetheless, the study by Leone et al[1] highlights the necessity to address defects in both the effectors and the myeloma targets to optimize the efficacy of immunotherapy for MM. It is likely that the most effective immunotherapeutic approach for MM will include strategies for expanding the repertoire, function, and persistence of myeloma-directed T cells as well as enhancing the sensitivity of MM cells to immune attack.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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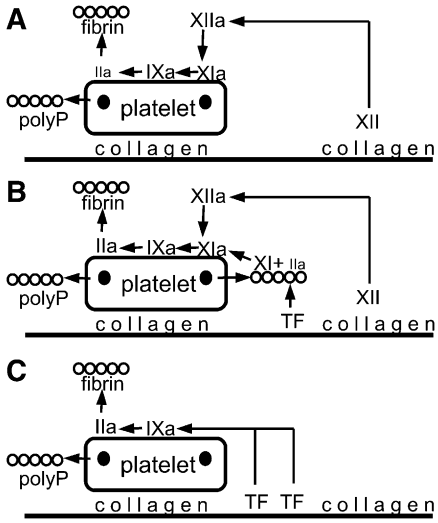

A broad overview of the components that are subject to inhibition under 3 sets of conditions (many of the coagulation steps are omitted). For an accurate analysis, see supplemental Figure 6 in the article by Zhu et al that begins on page 1494. Platelets adhere to collagen and are partially activated releasing polyphosphate from dense granules (dark spots on the platelets). During fibrin formation, fibrin structure is altered by association with polyphosphate, leading to a structure that is more resistant to lysis. (A) Factor XII is activated to factor XIIa. Factor XIIa activates factor XI to factor XIa, which activates factor IX to factor IXa, leading to thrombin (IIa) generation and fibrin formation. (B) Tissue factor initiates the generation of a small amount of thrombin (IIa) that binds to polyphosphate and activates factor XI to factor XIa. Additional factor XIa is generated from factor XIIa formed by contact activation. Factor XIa activates factor IX to factor IXa, leading to thrombin (IIa) generation and fibrin formation. (C) At high tissue factor, sufficient factor IX is activated to factor IXa to drive thrombin (IIa) generation and fibrin formation. polyP, polyphosphate; TF, tissue factor.

● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Zhu et al, page 1494

Polyphosphates rock! A role in thrombosis?

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In this issue of *Blood*, Zhu et al have established, in human blood, that factor XIa and polyphosphate make significant contributions to thrombus formation.[1] This makes these molecules good targets for therapeutic intervention.

The emphasis on human blood is important, as many thrombosis studies have been conducted in mice. Mouse studies take advantage of 2 things: (1) robust imaging tools that allow in vivo thrombus formation to be monitored and quantified, and (2) genetic manipulation of the mice to generate mechanistic information. However, there are data to suggest that mice may have significant differences in thrombus formation relative to primates and humans.[2] Thus, it is critical to test potential antithrombotic mechanisms in human blood.

Directed antithrombotics in current use are targeted inhibitors of thrombin and factor Xa. However, Zhu et al went in a different direction and studied the role of factor XI. Factor XI is an especially interesting molecule, because it lies at the critical junction of the classical contact pathway (factor XIIa and high-molecular-weight kininogen activation of factor XI) and the platelet-driven thrombin feedback amplification loop. This feedback was suggested by studies showing that sulfated glycans could promote thrombin activation of factor XI.[3,4] Subsequent work provided a physiological basis by showing that activated platelets could sustain this reaction.[5,6] The laboratory of Dr Morrissey established the mechanism of this activation by showing that polyphosphate released from platelet-dense granules was the agent that promoted thrombin activation of factor XI.[7]

A logical extension of those studies on the biochemistry of polyphosphate is to examine the antithrombotic properties of agents that target factor XI and polyphosphate. In this study, Zhu et al used sophisticated flow models of thrombosis to generate nuanced results.[1] They used whole blood to incorporate the critical contribution of platelets. They included the role of flow by passing the blood over collagen to give platelet adherence and contact activation. They studied the results of 3 inhibitors: (1) an antibody that selectively blocks factor XIIa activation of factor XI (without interfering with thrombin activation of factor XI), (2) an antibody that blocks factor XIa activation of factor IX, and (3) a compound that binds and neutralizes polyphosphate (see figure). With these 3 inhibitors, they used low, medium, and high tissue factor to modulate the procoagulant signal. The study is complicated by the fact that merely drawing blood can result in nonphysiological contact activation. Zhu et al get around this by using a small amount of an inhibitor of factor XIIa. This allows them to suppress background effects while continuing to measure the contribution of factor XII and the contact pathway.

Unsurprisingly, in the absence of tissue factor, platelet surface factor IX activation was purely driven by contact factors. Similarly, at high tissue factor, sufficient factor IXa was generated by a tissue factor reaction in which the contribution of contact factor was less significant. At intermediate TF levels, the inhibitors of factor XI activation and activity slowed thrombus formation and reduced thrombus size. This effect was not related to platelet accumulation into the thrombus but