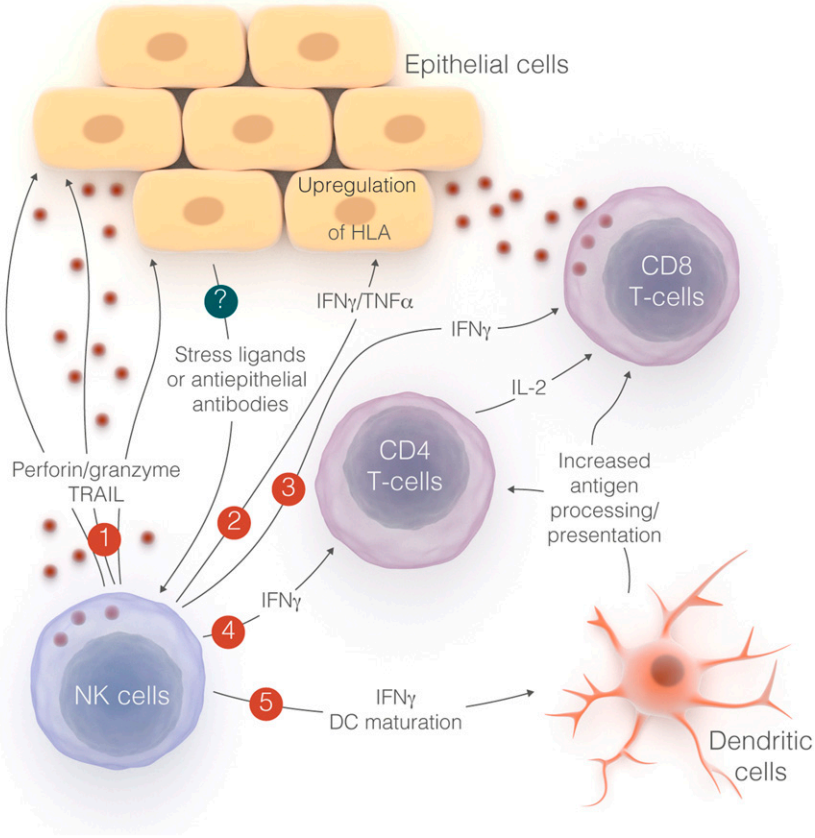

Possible mechanisms of NK cell–mediated acute GVHD. Subclinical GVHD triggers NK cell activation through unknown mechanisms (denoted by "?"), perhaps involving antiepithelial antibodies or expression of stress ligands induced by local inflammation. The resulting NK-cell responses may be targeted directly at epithelial cells (1), or may indirectly activate adaptive immune mechanisms that exacerbate T-cell–mediated GVHD (2-5). Professional illustration by Luk Cox.

Thus, the GVHD seen in this report is quite unexpected.

Possible factors in the current report that may help explain the unexpected GVHD rates include the timing of NK-cell infusion (8-35 days after transplant), the lack of posttransplant immunosuppression, or the hyperactivation of the NK cells, which were expanded on interleukin 15-secreting feeder cells. It is important to note that all 4 patients receiving unrelated donor transplants developed GVHD compared with only 1 of 5 patients receiving related donor transplants, further implicating a T-cell etiology mediated by minor antigens that was somehow exacerbated by the infused NK cells.

This then raises several possible mechanisms to explain the observed GVHD. As shown in the figure, subclinical dermal or mucosal inflammation may increase stress ligands, rendering epithelium susceptible to recognition by NK cells, causing (1) a direct effect via lysis or indirect activation of adaptive immunity through (2) cytokine-mediated upregulation of HLA for T-cell recognition, (3) stimulation of cytotoxic T cells, (4) activation of helper T cells, or (5) maturation of dendritic cells for enhanced antigen presentation or crosspresentation.

Whichever the case, these findings force us to recognize the potential potency of NK cells and to consider that GVL is no longer discretely separated from GVHD for NK cells. Further understanding of this mechanism is essential for understanding GVHD and the future of adoptive cell therapy with NK cells.

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

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● ● ● LYMPHOID NEOPLASIA

Comment on Pawlyn et al, page 831

Blind men and an elephant

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In this issue of *Blood*, Pawlyn et al examine the prognostic implications of overlapping chromosomal abnormalities in multiple myeloma (MM), demonstrating that coexistence of hyperdiploidy does not mitigate the impact of high-risk abnormalities.[1]

The story of the blind men and an elephant originated in the Indian subcontinent and describes a group of blind men coming to different conclusions about how an elephant looks like by feeling different parts of the animal. The parable

implies that one's subjective experience may be true but is inherently limited by its failure to account for alternate possibilities or the sum total of facts. One faces a similar situation when trying to examine the genetic complexity in myeloma.[2,3] When only metaphase cytogenetics was available, an abnormality was detected in one-third of the patients, and it implied poor prognosis. With the introduction of interphase fluorescence in situ hybridization (iFISH), it became clear that nearly all myeloma cells carried 1 or more abnormalities.[4] Common abnormalities included translocations involving the heavy chain locus on chromosome 14 and a set of recurrent partner chromosomes (immunoglobulin heavy-chain locus [IgH] translocations) or trisomies of odd-numbered chromosomes (trisomic myeloma).[2,5,6] In addition, many had other abnormalities, mostly deletions involving chromosomes 1, 13, and 17, monosomies of chromosomes 13 and 17, and amplification of chromosome 1q. Because IgH translocations or trisomies are present in the majority of patients, these are considered early events in the development of myeloma. Many of these iFISH abnormalities have been consistently associated with poor outcomes, especially t(4;14), t(14;16), t(14;20), and del(17p).[5,6] Deletion of the short arm of chromosome 17 in particular is associated with poor survival. At the same time, certain therapies can mitigate the risk associated with specific abnormalities as is the case with t(4;14) or del(17p) and bortezomib-based therapies.[7,8] The recognition that specific abnormalities may benefit from particular drugs has also led to development of risk adapted treatment algorithms.

Over the years, we have classified the common iFISH abnormalities as standard, intermediate, or high risk, based primarily on its impact on overall survival with the available therapies. However, these abnormalities, with the exception of the different IgH translocations, are not mutually exclusive and can often coexist in the same plasma cell. This leads to additional complexity in interpreting the results of iFISH tests, and only recently has there been systematic evaluation of the implications of overlapping abnormalities, especially the overlap between high-risk and standard-risk lesions. Although the presence of multiple high-risk lesions is typically associated with worse outcomes, the interpretation of coexistent standard- and high-risk abnormalities can be confusing. The study presented by Pawlyn et al demonstrated superior outcome for patients with hyperdiploidy but also showed that the poor outcome seen in patients with high-risk lesions described above, as well as in those with 1q amplification, was not altered by the presence of concurrent hyperdiploidy. These results contrast sharply with a previous report from the Mayo Clinic examining the implications of trisomies among patients with newly diagnosed myeloma treated with modern therapies.[6] In that study, patients with high-risk lesions including del(17p) had a better survival when they coexisted with trisomies compared with the rest. The different results from the 2 studies can be related to several factors, the most important of which is the difference in the therapies used. The study presented by Pawlyn et al used induction regimens that combined cyclophosphamide with thalidomide and dexamethasone, or with vincristine, doxorubicin, and dexamethasone, whereas the Mayo Clinic study included patients treated mostly with lenalidomide- or bortezomib-based regimens. The differential impact of the regimens used is clear from the superior overall survival of the entire cohort in the Mayo study. The impact of coexistent abnormalities was also examined in a recent study by the Intergroupe Francophone du Myélome in 242 patients with t(4;14) or del(17p) abnormalities using a single nucleotide polymorphism array. As with the Mayo Clinic study, a protective effect of trisomies in patients with del(17p) was seen.[9] Although more work is required to define the impact of the different abnormalities, it is clear that the outcomes of patients depend on a complex interplay of factors including the specific combinations of lesions, the therapeutic approaches used, and the magnitude of treatment response. Thus, although the study by Pawlyn et al found no effect of trisomies in high-risk patients using thalidomide- or doxorubicin-based regimens, 2 other studies that used lenalidomide- and/or bortezomib-based therapies found that trisomies do ameliorate the adverse prognostic effect of high-risk cytogenetics in myeloma.

Coexistence of these abnormalities raises important biological questions; specifically, the chronology of development of the 2 types of abnormalities remains unclear. The current study, by performing single cell analysis of plasma cells, suggests that development of trisomies may precede development of IgH translocations. This sequence of development of genetic abnormalities had been suggested previously by Chng et al, who performed a comprehensive analysis of karyotypes in 469 patients with hyperdiploid MM.[10] However, in the current study, this assumption is based on analysis of cells from 5 patients and needs verification in a larger group of patients using similar techniques.

The challenge going forward is to develop a better understanding of the implications of the multiple abnormalities seen in MM, not only from a prognostic standpoint but also the selection of therapy. Unlike the blind men coming to different conclusions about the elephant, we need to develop methodologies that will allow us to integrate all of the available genetic information to better predict outcomes in MM. We also need to recognize that the impact of various prognostic factors will vary based on the specific therapy used, and thus generalizations are not possible if a significant change in treatment is present. The demonstration of numerous mutations in myeloma cells demonstrated by recent studies using whole genome sequencing has made this task more difficult.[3] Ongoing studies in the context of large clinical trials with uniform therapies will continue to shed more light on this complex problem and contribute to improving our understanding of the disease biology.

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

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Comment on Bender et al, page 860

Proplatelets slip slidin' away

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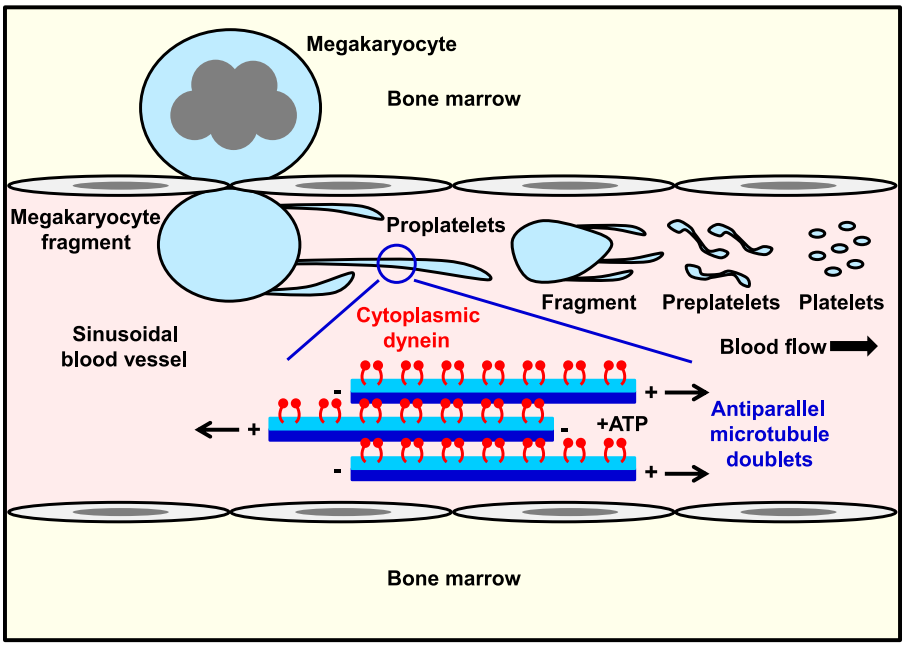
In this issue of *Blood*, Bender et al provide compelling evidence that the motor protein cytoplasmic dynein provides the necessary force for microtubule sliding and proplatelet elongation from megakaryocytes (see figure).[1]

Microtubule sliding has been proposed to drive proplatelet elongation, but direct proof of this mechanism has been lacking. Bender et al report a highly dynamic process involving repetitive phases of extension, pause, and retraction that is independent of de novo microtubule growth. They also show that physiological shear forces generated in a microfluidic platelet bioreactor accelerate proplatelet extension by reducing the pause phase.[1,2] Better understanding the molecular basis of platelet biogenesis will yield improved strategies for treating thrombocytopenia and thrombocythemia, and optimize conditions for culturing platelets in vitro for experimental and therapeutic purposes.



Cytoplasmic dynein drives microtubule sliding and proplatelet elongation. Dynein-mediated sliding of antiparallel microtubule doublets within proplatelet shafts drives elongation under static and physiological shear force in vitro. Presumably, the same occurs in vivo, where shear force applied to megakaryocyte fragments extruded into sinusoidal blood vessels triggers the highly dynamic process of proplatelet formation and platelet biogenesis.

Previous findings by Hartwig and colleagues led to the hypothesis that dynein-driven microtubule sliding underlies proplatelet elongation.[3] They showed that microtubules continuously polymerize in megakaryocytes in vitro at a rate that is considerably faster than the average rate of proplatelet formation in vitro.[3] Because this was not impaired by nocodazole, which blocks microtubule assembly, it was concluded that tubulin polymerization was unlikely to be the primary driver of this process. Evidence supporting the role of dynein was that this was an adenosine triphosphate (ATP)-dependent process, and disruption of dynein function by overexpression of the dynamitin subunit in megakaryocytes severely impaired proplatelet formation.[3] To address this hypothesis, Bender et al used fluorescence loss after photoconversion (FLAC) time lapse and fluorescence recovery after photobleaching (FRAP) to directly visualize and quantify the rates of proplatelet elongation in fetal liver-derived mouse megakaryocytes expressing β1-tubulin tagged with the photoconvertible fluorophore Dendra2 (β1-tubulin-Dendra2).[1] Experiments were performed in the presence and absence of structurally distinct inhibitors of dynein, under static and physiological shear stress. Bender et al establish that proplatelet elongation is not a continuous process once initiated, but rather undergoes repetitive phases of extension, pause, and retraction back to the megakaryocyte cell body, the rate of which is considerably increased when megakaryocytes are exposed to shear. Shear shortened the pause phase, but microtubule sliding remained essential for proplatelets to form. The same applies to released proplatelets, which subfragment in the circulation. The physiological significance of the pause and retraction phases remains unclear and warrants further investigation. Resolving the mechanisms underlying all 3 phases could lead to pharmacologically or genetically