

response and had 100% donor-derived CD3- and CD33-positive cells in the blood by day 28. Seven of the 58 patients died of non-relapse-related causes by day 100. The estimated probability of recurrent AML was 40% at 1 year, and the 1-year survival rate estimate was 41%.

The use of reduced-intensity preparative regimens is now widely accepted and used for older and infirm patients.<sup>4-6</sup> The Seattle group has been in the vanguard of the development of reduced-intensity transplantation preparative regimens.<sup>7</sup> It is notable that the patients treated by Pagel et al were deemed ineligible for their standard reduced-intensity preparative regimen of fludarabine and TBI alone. The survival rate of 41% is quite remarkable, given that background. Toxicity to extramedullary organs was low, and treatment-related mortality was reasonable and low.

Targeting CD45<sup>+</sup> hematopoietic cells is not the only way to target the marrow space with additional radiation. Other hematopoietic antigens can also be targeted.<sup>8</sup> Alternative methods include the use of external beam radiation using intensity-modulated radiotherapy techniques<sup>9</sup> and bone-seeking radionuclide therapy.<sup>10</sup> These approaches are promising but have not been studied in substantial numbers of patients. The advantage of these alternative approaches is their exportability, and potentially greater reliability, with higher dosing to the marrow compared with antibody-delivered therapy. However, there may be possible advantages to protocols combining radioimmunotherapy (RIT) and external beam therapy. RIT has the advantage of targeting extramedullary disease as well as medullary disease.

AML in the older patient is a pressing medical problem. Chemotherapy alone results in few cures. Approaches to augmenting the efficacy of hematopoietic cell transplantation without unduly increasing toxicity are urgently needed. Reducing the intensity of therapy to uninvolved organs while boosting treatments to the sites of disease is a promising approach to achieving more cures for patients with this lethal disease.

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## ● ● ● MYELOID NEOPLASIA

Comment on Luesink et al, page 5512

# CCL2/CCR2: push/pull for migration

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In this issue of *Blood*, Luesink and colleagues report that in APL, the induction of massive production CC-chemokines (CCLs) and their receptors (CCRs) in APL cells by differentiating therapy with ATRA or ATO may play an important role in the development of the DS,<sup>1</sup> formerly known as retinoic acid syndrome.<sup>2</sup>

**T**he full-blown differentiation syndrome (DS) is characterized by unexplained fever, weight gain, dyspnea with interstitial pulmonary infiltrates, pleural or pericardial effusions, hypotension, and acute renal failure.<sup>2</sup> However, the pathophysiology of DS is not fully understood, and detailed knowledge about its molecular mechanism remains largely unknown. Previously, it has been observed that differentiation therapy in acute promyelocytic leukemia (APL) (1) increases the release of inflammatory cytokines from differentiating APL cells,<sup>3</sup> (2) increases the expression of cellular adhesion molecules on leukocytes,<sup>4</sup> and (3) up-regulates specific chemokines.<sup>5</sup> The simultaneous production of these proteins after exposure to all-*trans* retinoic acid (ATRA) may exacerbate the hyperinflammation observed in DS. The incidence of DS in patients receiving ATRA/arsenic trioxide (ATO) treatment has been reported to range from 2% to 27% with an associated mortality of about 2%.

The paper by Luesink et al in this issue of *Blood* presents in vitro evidence that chemokines may have a role in the development of DS.<sup>1</sup> Chemokines, together with their receptors, play a crucial role in directing the movement of mononuclear cells throughout the body, contributing to the pathogenesis of a variety of diseases.<sup>6</sup> These investigators, using ATRA-stimulated of NB4 cells, were able to induce mRNA expression of multiple CC-chemokines (CCLs) and their receptors (CCRs), resulting in increased chemokine production in supernatant of these cells and increased chemotaxis. Two of these chemokines (CCL2 and CCL24) were up-regulated early and, despite the addition of cycloheximide (a protein translation inhibitor), up-regulation of the mRNA expression was confirmed even though protein levels were not increased. This indicated that their induction was directly mediated by retinoic acid receptors. The addition of dexamethasone to ATRA did not inhibit chemokine induction in

ATRA-stimulated NB4 cells. Moreover, as opposed to what was observed with NB4 cells, in primary leukemia cells derived from 5 newly diagnosed APL patients, only CCL2 was consistently up-regulated at mRNA and protein levels. Furthermore, increased levels of CCL2, CCL4, CCL7, and CCL24 were found in plasma of an APL patient with DS and not in 2 APL patients without DS. Finally, by adding ATO alone in NB4-cultured cells, only CCL2 and CCL7 were up-regulated more than 5-fold.

These results indicate that CCL2 and CCL7 are elevated in a single patient with DS studied and also in the NB4-cultured cells stimulated with ATRA and/or ATO. However, as only CCL2 was consistently up-regulated by ATRA addition at mRNA and protein levels in the primary leukemia cells derived from 5 newly diagnosed APL patients, it is this chemokine that may play an important role in the development of DS. CCL2 or monocyte chemoattractant protein 1 (MCP-1) is a potent agonist for monocytes, dendritic cells, memory T cells, and basophils.<sup>6</sup> Moreover, when secreted from alveolar epithelial cells, it has an important role in the cell-to-cell interaction involved in the chemotactic transmigration of differentiated APL leukemic cells toward alveolar epithelial cells.<sup>7</sup> Previous studies have indicated that IL-8<sup>3</sup> and adhesion molecules<sup>4</sup> may also have a role in DS. Because dexamethasone does not efficiently reduce leukemic chemokine production and pulmonary infiltration of leukemic cells may induce an uncontrollable hyperinflammatory reaction in the lung, the therapeutic use of chemokine-receptor antagonists may be a more efficient approach than the use of steroids to treat DS in APL. Among these chemokine-receptor antagonists, CCR2 (receptor for CCL2) and CXCR1 (receptor for IL-8) antagonists, used in phase 1 and 2 studies for treating rheumatoid arthritis and chronic obstructive pulmonary disease, respectively, are the most interesting. Despite the strength of in vitro experimental data, a potential limitation of this paper is that the studies are limited to only one APL patient with DS. Therefore, more APL patients with DS should be studied to evaluate more precisely the role of these chemokines in the pathophysiology of DS.

As in the setting of human migration,<sup>8</sup> the chemokine and chemokine receptor production in APL treated with differentiation therapy may act as push (chemokine) and pull factors (chemokine receptors) for migration of leukemic cells from the bloodstream to the

tissues, contributing to the development of DS. Moreover, the increased levels of CCL2 during differentiation therapy may become a marker of DS.

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## ● ● ● PLATELETS & THROMBOPOIESIS

Comment on Kunert et al, page 5532

# Getting in shape with RanBP10

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In this issue of *Blood*, Kunert and colleagues have characterized mice lacking RanBP10, demonstrating an essential role for this  $\beta$ 1-tubulin binding protein in platelet microtubule organization. Although not thrombocytopenic, RanBP10<sup>-/-</sup> mice have a bleeding diathesis and abnormal platelet aggregation and secretion.

**M**egakaryocytes are charged with the task of generating  $0.4$  to  $2 \times 10^{11}$  platelets each day. They assemble platelets along pseudopodial extensions termed proplatelets, which are generated by the outflow and evagination of an extensive internal membrane system.<sup>1</sup> Several lines of evidence indicate that microtubules drive proplatelet development and form the critical scaffold required for faithful production of platelets. Dynamic microtubule assembly must be tightly controlled to enable the orderly production of nearly identical platelets. Yet the mechanisms that organize microtubules during proplatelet formation are not well understood.

$\beta$ 1-Tubulin is the dominant structural constituent of platelet microtubules. To address the question of how  $\beta$ 1-tubulin polymerization is regulated during proplatelet formation, Schulze et al previously used a 2-hybrid system to identify proteins that interact with  $\beta$ 1-tubulin.<sup>2</sup> RanBP10, which also binds the GTPase Ran, was identified. This was a fascinating result considering that Ran orchestrates mitotic spindle formation,<sup>3</sup> another process characterized by a delicate and deliberate dance of microtubules. Further studies showed that RanBP10 serves as a

guanine nucleotide exchange factor (GEF) for Ran.<sup>2</sup> However, these studies did not directly address whether RanBP10 is important for platelet function.

Schulze's group has now generated a RanBP10-deficient mouse to determine the role of this binding protein in platelet morphogenesis and function.<sup>4</sup> Nearly half of all megakaryocytes isolated from these mice demonstrated shortened, discontinuous microtubulin filaments. Proplatelet formation in RanBP10<sup>-/-</sup> megakaryocytes cultured in vitro was slightly impaired. This minor defect was compensated in vivo, because RanBP10<sup>-/-</sup> mice had normal platelet counts. However, electron microscopy and quantitative analysis of the length-versus-width ratio demonstrated that these platelets were more spherical than wild-type platelets. Numbers of microtubule filaments, which vary from 8 to 12 in wild-type platelets, varied from 5 to 26 in RanBP10<sup>-/-</sup> platelets. These microtubule bundles were disorganized and did not demonstrate the typical cortical localization, giving RanBP10<sup>-/-</sup> platelets an abnormal morphology. These results showed that RanBP10 functions to prevent platelet anisocytosis.