The work of Sun et al demonstrates VKORC1 as a main regulator of the carboxylation reaction, which, in view of the multiple downstream pathways affected by this protein, suggests further exciting studies in the near future. ■

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HEMATOPOIESIS

Comment on Lin et al, page 3803

A green light for the thrombopoietic program

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The opportunity to study the dynamics of thrombopoeisis in real time has long been awaited.

In their new paper, Lin and colleagues have described the generation of transgenic zebrafish with green fluorescent thrombocytes. This fish was created using a zebrafish CD41 gene promoter that drives the expression of jellyfish green fluorescent protein (GFP). Using this fish, the authors have identified that there are 2 populations of thrombocytes that are made in kidney marrow. The authors claim that one population of cells that expresses low levels of GFP seems to be the precursor for the other cells that have high levels of GFP and that this invention will be useful in studying thrombopoiesis. Even though thrombocytes have been identified earlier in zebrafish development and in circulation,[1,2] the current report is important in following thrombocytes in real time in development and in circulation and thus merits attention.

The significance of the above work is the fact that the zebrafish thrombocytes have parallels to megakaryopoiesis and platelet production in mammals. Since almost all mammalian genes exist in fish, it would not be surprising that knockdown of zebrafish genes using antisense morpholinos and the above transgenic line could identify novel players in megakaryopoiesis. In this context it is interesting to note that the suggested precursor GFP^+ cells are large, similar to other known hematopoietic progenitor cells. GFP^+ cells appear in the ventral region of the aorta that roughly corresponds to aorta-gonads-mesonephros (AGM), which is the site of hematopoiesis in mammalian development. However, these GFP^+ cells appear after the dissolution of AGM and are too caudal. Thus, the authors may have unearthed yet another novel site for hematopoiesis. All the above point out that there will be novel unidentified programs in thrombocyte development and the transgenic tool will help in exploring such programs. However, one limitation of this study is that it will enhance the information on only precursor thrombocytes that are marked by the expression of CD41 promoter. Thus, whether these large GFP^+ cells are in fact thrombocyte precursors remains to be established. It is entirely possible that there is expression of CD41 in the nonthrombocytic lineage and the cells could be the multipotent hematopoietic precursors. Thus, caution must be exercised in extrapolating the current findings. In fact, the authors are aware of the expression of CD41 in cell types other than megakaryocytes in birds and mammals. Fluctuations in gene expression during development are not unprecedented and indeed the authors themselves identified expression of CD41 promoter in unfertilized zebrafish eggs. Even though this is not relevant to thrombopoiesis, this observation is novel and raises several questions. Is there maternally derived CD41 mRNA in unfertilized eggs? If so, what is the role for CD41 in early development? Is there a species-specific difference for the role of CD41 since lack of functional CD41 in mice and men seem to have no apparent developmental abnormalities?

In conclusion, the current work will initiate further studies on thrombocyte differentiation and will open new avenues to explore dynamics of thrombopoiesis in real time. ■

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IMMUNOBIOLOGY

Comment on Airoldi et al, page 3846

Cytokine receptor gene plays antioncogene

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Mice genetically deficient for the *IL12RB2* gene develop systemic lymphocyte activation, spontaneous autoimmunity, and malignancy, particularly plasmacytoma and lung carcinoma.

Interleukin-12 (IL-12) is a heterodimeric cytokine formed by 2 chains, IL-12 p35 or α-chain and IL-12 p40 or β-chain.[1] The IL-12 p40 chain can also associate with IL-23 p19 to form the IL-23 cytokine. The IL-12 receptor is formed by 2 chains, IL-12Rβ1 and IL-12Rβ2. IL-12Rβ2 is specific for the IL-12 receptor, whereas IL-12Rβ1 also associates with IL-23R to form the receptor for IL-23. IL-12 is considered a typical proinflammatory

cytokine produced mostly by myeloid cells and dendritic cells, and its biologic effects, in particular the ability to induce production of interferon γ (IFN-γ) and to support T-helper 1 (Th1) type T-cell responses, have been studied particularly on natural killer (NK) and T cells that constitutively or upon activation express functional IL-12 receptors. Although an activity of IL-12 on B-cell functions and immunoglobulin production has been described in early studies,[2] whether B cells express functional IL-12 receptors has long been a controversial issue. More recently, however, it was clearly established that normal B cells express both chains of the IL-12 receptor and that they respond to IL-12 with increased immunoglobulin secretion, expression of the IL-18 receptors, and, particularly in the presence of IL-18, production of a high level of IFN-γ.[3] However, Airoldi et al[4] have shown that in malignant B cells the *IL12RB2* gene was silenced, probably by hypermethylation. When the *IL12RB2* gene expression was reestablished either by treatment of the cells with a DNA methyltransferase inhibitor or by gene transfection, IL-12, both in vitro and in vivo, induced apoptosis and growth inhibition of the malignant B cells.[4]

On the basis of the data mentioned above, Airoldi et al[4] have postulated that IL-12Rβ2 functions as a tumor suppressor in human B-cell malignancies. In a paper in the present issue of *Blood,* Airoldi and colleagues tested this hypothesis by analyzing the appearance of malignancies in aging *IL12rb2*–deficient mice. They observed not only a very significant incidence of plasmacytoma and lung carcinoma but also immune complex mesengial glomerulonephritis with serum antinuclear antibodies and multiorgan lymphoid infiltrates with systemic B- and T-cell activation in all aging animals. The observed autoimmune pathology may in part be secondary to an up-regulation of IL-6 in the *IL12rb2*–deficient animals, and the data presented suggest that there is a reciprocal down-regulation between IL-6 and IL-12. These results strongly support the conclusions that IL-12 may be important in controlling aberrant or excessive B-cell activation and that the absence of signaling of this proinflammatory cytokine paradoxically results in a state of systemic B- and T-cell activation. These findings open a new perspective on the physiologic role of IL-12. The high frequency of plasmacytoma observed in the aging *IL12rb2*–deficient animals may reflect either the inability of the animals to control aberrant B-cell activation or an effect of the chronic inflammatory environment on B-cell neoplastic transformation and tumor progression. The occurrence in some animals of lung adenocarcinoma may have an opposite mechanism and be linked to defective innate antitumor surveillance in the animals lacking IL-12 functions, possibly secondary to a reduced production of IFN-γ. Future studies analyzing the specific role of IL-12 in regulation of B-cell activation and transformation, autoimmunity, and solid tumor immunosurveillance will shed new light on the mechanisms of homeostatic regulation of B-cell activation and on the complex role of proinflammatory cytokines in either promoting or preventing tumor initiation and progression. ■

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TRANSPLANTATION

Comment on Burroughs et al, page 4002

Antagonizing CXCR4 accelerates CD34+ cell mobilization

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A novel bicyclam CXCR4 antagonist, AMD3100, appears capable of mobilizing a fully functional hematopoietic allograft within just 6 hours following a single injection.

Recent studies have demonstrated that the interaction between the chemokine stromal-derived factor 1 (SDF-1/CXCL12) and its only known receptor, CXCR4, serves as a key regulator of hematopoietic stem cell (HSC) trafficking.[1] In clinical practice, the hematopoietic cytokine granulocyte colony-stimulating factor (G-CSF) is widely used to induce the mobilization of HSCs and hematopoietic progenitor cells (HPCs) for reconstitution of hematopoiesis following myelosuppressive therapy. Several groups have demonstrated that G-CSF causes mobilization primarily through its indirect disruption of the SDF-1/CXCR4 interaction, inducing its cleavage by serine proteases or via down-regulation of SDF-1 mRNA.[2-4] Whatever the precise mechanism(s), this implies that agents that directly inhibit this interaction may be effective mobilizers. In this issue of *Blood,* Burroughs and colleagues have demonstrated that AMD3100, a direct antagonist of CXCR4, induces the rapid mobilization of hematopoietic cells with both short- and long-term repopulating capacity. Using a clinically relevant myeloablative canine transplantation model, they showed that a single dose of AMD3100 induces HSC and HPC mobilization within a few hours, allowing for apheresis to be performed on the same day of drug administration. Following both autologous and allogeneic transplantation, the cells collected following mobilization with AMD3100 alone were capable of reconstituting hematopoiesis with neutrophil and platelet recovery kinetics similar to those observed following transplantation of G-CSF–mobilized cells. One canine allogeneic recipient appeared to develop acute graft-versus-host disease (GVHD), but the overall incidence of this serious transplantation complication did not seem to be different from what would be expected following transplantation of G-CSF–mobilized cells. All dogs that received a transplant achieved full donor hematopoietic chimerism following myeloablative radiation.

These intriguing preliminary data suggest that by directly antagonizing CXCR4, a more rapid mobilization of clinically relevant $CD34^+$ cells can be induced. This stands in stark contrast to the 4 to 5 days of G-CSF treatment normally required to mobilize