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MYELOID NEOPLASIA

Comment on Lim et al, page 885

Why do Tregs suddenly disappear in aplastic anemia?

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In this issue of *Blood*, Lim et al show that regulatory T cells (Tregs) from aplastic anemia (AA) patients are susceptible to Fas ligand (FasL)-mediated apoptosis, which may explain, in part, their diminution at disease onset.[1] The cell death can be overcome in vitro and in vivo by interleukin-2 (IL-2) supplementation. This work builds on the previous observation of the same group, which showed 2 subpopulations of Tregs with distinct phenotypes in AA: Treg A, with a naive phenotype (low proliferative index), and Treg B, with a memory phenotype (moderate/high proliferative index) that correlated with response to immunosuppressive therapy (IST).[2]

It is well established that acquired AA is characterized by a proinflammatory state whereby activated $CD4^+$ (T helper 1 [Th1], Th17), $CD8^+$ T cells and associated proteins/cytokines result in stem and progenitor cell damage and their elimination.[3] Various degrees of bone marrow failure ensue, with pancytopenia a hallmark of disease. If severe, consequences of pancytopenia can be dire when untreated. Along with proinflammatory signals, lack of regulation constitutes an important disturbance in autoimmune diseases, in general, which is also seen in AA.[2,4] The mechanisms that result in this reduction are not well established.

In the current study, the investigators explore the mechanisms that underlie the imbalance and functional impairment between the Treg subsets in a small cohort of AA patients (n = 19) and healthy donors (n = 25). Apoptosis-related genes and pathways were preferentially upregulated in Treg B (compared with Treg A) and associated with a greater induction of cell death following FasL exposure. IL-2 rescued Treg B undergoing FasL-mediated apoptosis. These expanded cells maintained suppressive properties in proliferation assays in vitro and in a graft-versus-host disease (GVHD) animal model in vivo. Although Treg A was more resistant to FasL, it did not expand in low IL-2 concentrations in vitro; however, at higher doses, Treg A and Treg B expanded in culture and acquired a more memory phenotype. During this expansion, increased expression of phosphorylated BCL-2 was observed, providing a prosurvival signal abating FasL-mediated apoptosis for both Treg populations. These observations provide a mechanistic insight into Treg depletion in AA. But how can they be translated into the clinic?

For many decades, the primary focus in clinical protocol development in severe AA (SAA) has been to address the proinflammatory milieu and suppress it with lymphocytotoxic and immunomodulatory drugs. Nearly 30 years ago, the combination of horse anti-thymocyte globulin (h-ATG) and cyclosporine (CsA) was defined as standard IST.[5] Since then, several alternate immunosuppressive regimens have been developed; however, results have been disappointing because of inferior outcomes in response to h-ATG/CsA or prohibitive toxicity.[5] In the past decade, a nonimmunosuppressive approach, using stem cell stimulation with thrombopoietin receptor agonists, produced promising outcomes. Improvements in marrow function and blood counts were shown with eltrombopag as a single agent in IST-refractory SAA, as well as in the front line when combined with standard h-ATG/CsA.[5] Thus, the combination of IST and stem cell stimulation addresses the different facets of AA pathophysiology, producing a more complete hematologic recovery.

Could an intervention that improves immune regulation help to overcome the inhibitory signals to the marrow in AA? One possibility would be increasing Tregs in vivo. Toward this goal, different approaches have been sought in transplantation and in autoimmune disorders. One of the earlier promising strategies was to infuse autologous ex vivo–expanded Tregs; however, its development has been hindered by technical, scalability, and logistical problems, with no robust clinical data reported.[6] Exogenous administration of IL-2, as proposed by Lim et al, could be an alternate strategy. At lower doses, IL-2 activates and expands Tregs and has been investigated in several proof-of-concept, dose-finding, and phase 1/2 trials in vasculitis, refractory GVHD, type 1 diabetes, alopecia, and systemic lupus erythematosus.[7] In general, IL-2 was well tolerated, and an increase in Tregs was noted in most studies. Clinical benefit was observed; however, given the lack of a control group, IL-2's activity could not be determined. Its short half-life has been limiting in these earlier studies, and different formulations and associations with other Treg-promoting agents are being explored.

A more practical approach would be to use drugs with Treg-inducing properties. Unfortunately, this approach has not been encouraging in SAA. Sirolimus and rabbit anti-thymocyte globulin (ATG) are immunosuppressants that have shown to expand Tregs in vitro and in vivo[8,9];

however, when compared directly with h-ATG/CsA in randomized trials, outcomes were unchanged or inferior, respectively.[5] Combination with CsA (in the case of sirolimus) and the more potent overall and Treg absolute lymphocyte depletion (with rabbit ATG) could explain, in part, this lack of efficacy.[10] Thus, a successful strategy with this approach in AA may need to go beyond a simple increase in the frequency of Tregs. Interestingly, eltrombopag, which was developed to stimulate marrow progenitor cells, may have other beneficial effects, such as a decrease in proinflammatory signals, and promote immune regulation by stimulating Tregs.[5]

Could IL-2 be "added to the mix"? Possibly. One of the challenges in IST-refractory patients is to determine whether there is persistent autoimmune disease activity, a profound lack of progenitor cells sufficient for adequate hematopoiesis, or both. It is unlikely that single-agent IL-2 would be active if stem cell numbers are limiting. In combination with CsA, the Treg activity of low-dose IL-2 could be negatively affected.[7] In combination with agents that result in profound and prolonged lymphopenia (eg, rabbit ATG and alemtuzumab), the increase in Treg frequency provided by IL-2 could be curtailed by the absolute reduction in lymphocytes. Thus, the development of IL-2 would need to be well strategized in AA. The King's College group has provided the rationale for devising IL-2–based treatment protocols; I look forward to their follow-up work in AA, this time in the clinic.

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THROMBOSIS AND HEMOSTASIS

Comment on Chen et al, page 898

Vitamin K therapy to reduce bleeding

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In this issue of *Blood*, Chen et al[1] used a cell-based screening to identify 9 unrelated drugs that may cause bleeding by interfering with the vitamin K (VK) cycle that is required for the VK-dependent γ-carboxylation of blood coagulation proteins. Bleeding caused by drugs inhibiting the reduction of VK epoxide, but not VK, can be rescued by VK administration.

Adverse drug reactions, and, in particular, drug-induced bleeding, are among the leading causes of hospitalization and death. Bleeding is associated with the use of anticoagulants, as well as with nonsteroidal anti-inflammatory agents, serotonin reuptake inhibitors, and antibiotics. With regard to the last, the β-lactam antibiotic subgroup cephalosporins that contain an *N*-methylthiotetrazole side chain are well known to interfere with the VK cycle.[2] In this cycle, VK is reduced to VK hydroquinone by VK epoxide reductase (VKOR) and an unidentified VK reductase (VKR) (see figure).[3] VK hydroquinone serves as a cosubstrate for γ-glutamyl carboxylase (GGCX) that adds a carboxylic acid to specific Glu residues in VK-dependent (VKD) blood-coagulation proteins, an essential step to attain full clotting functionality. The generated VK epoxide (KO) is recycled back to VK by VKOR.

Previously, Chen et al were able to study the VK cycle in more detail by developing an elegant cell-based system that allowed for functional assessment of its components in the cell milieu. Using genome editing to knock out endogenous GGCX[4] or both VKOR and a VKOR-like homolog[5] and introducing mutant patient variants of these enzymes in HEK293 cells, they explained the clinical manifestations of specific mutations that included a VK-responsive bleeding phenotype and sensitivity to the VK antagonist warfarin. Taking advantage of the developed technology to identify VK cycle–targeting drugs, Chen and colleagues now performed high-throughput screening of a drug library in the GGCX-knockout cell line using KO as substrate. Twenty-two drugs were uncovered that impacted the biosynthesis of the VKD reporter protein, 9 of which appeared to be true inhibitors and were not associated with cytotoxic effects on the cells. Subsequent inhibitory efficacy assessment and high-performance liquid chromatography analysis to quantify VK and KO, using a clever combination of knockout cell lines and substrates, allowed for further dissection of the pathways involved (see figure). One set of drugs, including warfarin, was shown to inhibit VKOR, whereas another set of drugs inhibited VKR, and a third set affected cellular VK availability. The anticoagulant effect of drugs targeting VKOR could be completely restored by the administration of VK in vitro and in vivo, confirming that VKOR is primarily responsible for the reduction of KO to VK.