inhibition of ribonucleotide reductase, an iron-dependent enzyme. CPX was significantly more potent that hydroxyurea, a commonly used ribonucleotide reductase inhibitor that acts through a different mechanism. CPX was also synergistic with cytarabine in inducing leukemic cell death. So intracellular iron chelation, and thus CPX, could represent a novel approach to inhibit this enzyme. Another group has successfully used an antisense oligonucleotide approach to the R2 subunit of this enzyme. Whether ribonucleotide reductase inhibitors like CPX will be a viable antileukemic approach alone or in combination with other cytotoxic agents remains to be seen.

The Eberhard paper has important implications beyond the derivation of a potentially interesting novel antileukemic agent. First, it suggests that screening large libraries of available compounds could yield potentially clinically effective agents at low developmental cost. Second, the notion that intracellular iron depletion might have a therapeutic role in acute myeloid leukemia (AML) is a relatively novel idea, possibly suggesting a pathway for rational drug development. Furthermore, the investigators chose a potentially relevant screen, survivin inhibition. Survivin is preferentially expressed in malignant cells compared with normal cells, caused by transactivation of the promoter rather than gene mutation. Perhaps a more elegant screening strategy uses expression-based screening to identify compounds that engender a desired pattern of gene expression. This approach has the advantage of looking at many genes simultaneously. Such screens have identified drugs that might be useful in the treatment of AML. For example, Stegmaier and colleagues have shown that dexamethasone, gefitinib, and dihydro-folate reductase inhibitors might be effective agents.

Before prescribing CPX for patients with leukemia, a great deal of work has to be done. There are concerns that the therapeutic index might be too narrow because the drug also appears to suppress normal stem cells at slightly higher doses. Second, the drug could turn out to be significantly toxic in humans despite being able to achieve therapeutic concentrations that are tolerable in rodents and canines. Nonetheless, the general approach of Eberhard et al is to be applauded. Despite the many ongoing clinical trials with a variety of drugs in AML (eg, nucleoside analogs, alkylating agents, and inhibitors of FLT3, c-Kit, farnesyl transferase, MDR, cyclin-dependent kinase, DNA methyl transferases, histone deacetylases), we are in dire need of novel agents. This is especially true as we still commonly use only 2 drugs, anthracyclines and cytarabine, for the management of patients with this difficult disease.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES

T cells helping GVHD: take-away lessons

Daniel Fowler NATIONAL INSTITUTES OF HEALTH

Using double cytokine knockout donor CD4 cells, in this issue of Blood, Yi and colleagues help clarify the complex role of IFN-γ and the tissue specificity of Th1, Th17, and Th2 subsets in murine GVHD.

Murine models investigating the role of functionally defined donor T-cell subsets in graft-versus-host disease (GVHD) have in general taken 1 of 2 approaches: either adding back purified or ex vivo expanded donor T-cells or using donor T-cells deficient in a subset-related cytokine. In the article by Yi et al, this latter “take-away” approach is taken to a higher state by use of double knockout T cells, specifically IFN-γ−/− IL-4−/− and IFN-γ−/− IL-17−/− CD4+ T cells consequently deficient in Th1/Th2 function and Th1/Th2 function, respectively. The investigators conclude that wild-type T cells by default develop into Th1 cells that primarily mediate gut and liver GVHD. By comparison, Th1/Th2 takeaway predisposes to Th17-cell outgrowth, which manifests primarily as skin GVHD. Association of Th17 cells with skin GVHD has been reported, but the current work reserves lung GVHD for Th2-cell pathology, which is generated after Th1/Th2 takeover. As such, the 3 T helper subsets appear to be “reciprocal” or stated otherwise, perhaps opportunistic, whereby the absence of two facilitates predominance of the third. The work is particularly interesting because it quantifies CD4 cell transcription factor expression to account for reciprocity at the single-cell level. For example, IFN-γ−/− CD4 cell infusion led to an approximately 10-fold increase in Th2 cells that expressed GATA-3 and an approximately 20-fold increase in Th17 cells that expressed RORγt. To further test this reciprocity model, it would be interesting to know whether simultaneous deficiency of IL-4 and IL-17 would further increase Th1 cells expressing t-bet and thereby exacerbate Th1-mediated gut and liver GVHD.

Although the article understandably focuses on the reciprocal balance of Th1, Th2, and Th17 cells, a fourth donor CD4+ T-cell subset, regulatory T cells, which are known to down-regulate GVHD, were not taken away or added back to this already dizzying array of immune interventions. Presumably, based on the literature, regulatory T cells would be capable of suppressing each of the Th1-, Th2-, and Th17-mediated GVHD pathologies described. The experiments also focused on CD4-mediated GVHD. Because others have described a differential role of Th17 cells in GVHD models that were CD4-mediated or...
both CD4- and CD8-mediated, further research should address reciprocal Th1/Th2/Th17 effects in the conventional CD8 cell replete transplantation context.

The experiments in particular shed light onto the biology of Th2-mediated lung GVHD that was uncovered with Th1/Th17 deficiency. The mechanistic aspects were relatively classic for a Th2 immune response because recipients of IFN-γ−/− IL-17−/− CD4+ T cells treated with anti–IL-4 or genetically deficient in IL-4 receptor signaling had reduced lung GVHD. Interestingly, the pathogenic Th2 cells that arose in this model secreted TNF-α in preference to IL-10 at a 10:1 ratio. Such Th2 cells likely represent a different brand than the population of Th2 cells that we found to down-regulate established lung GVHD. Furthermore, it was found that IFN-γ therapy operated by up-regulating host tissue expression of the counterregulatory molecule, programmed death ligand-1. In sum, in this model, Th2-mediated lung GVHD occurred as a result of Th1- and Th17-cell deficiency, was dependent on donor IL-4 and either IL-4 or IL-13 signaling on host tissue, and was reversed by IFN-γ via a host-dependent PD-L1 pathway. These findings are an example of the complicated circuitry of GVHD that can be uncovered with meticulous investigation, thereby identifying cellular and molecular targets for treating GVHD in a tissue-specific manner. Furthermore, these results improve an understanding of the somewhat paradoxical role of IFN-γ in GVHD and speak to the important role of the PD-1/PD-L1 pathway for immune regulation after allogeneic hematopoietic cell transplantation.

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

REFERENCES
T cells helping GVHD: take-away lessons

Daniel Fowler