factor (VEGF) when cultured in hypoxic conditions. Addition of exogenous VEGF to ARNT null embryoid bodies restored CFU numbers to normal. Together, these studies suggested that embryonic hematopoiesis requires hypoxic ARNT activity and that physiologic hypoxia encountered by embryos is essential for the proliferation or survival of hematopoietic precursors during development. These studies did not determine whether HIF-1α or HIF-2α were necessary for this ARNT activity.

In the current issue, Scortegagna and colleagues (page 1634) have shown that HIF-2α (also known as endothelial PAS domain protein 1 [EPAS1]) is essential for normal hematopoiesis in mice. They generated viable adult EPAS1/HIF-2α null mice that were pancytopenic with hypocellular marrows. Multilineage hematopoietic maturation was normal but quantitatively reduced. Bone marrow from EPAS1/HIF-2α null mice functioned normally when transplanted into irradiated wild-type recipients. In contrast, transplantation of wild-type marrow into irradiated EPAS1/HIF-2α null recipients resulted in impaired hematopoietic reconstitution, indicating a defect in the marrow microenvironment. Interestingly, levels of VEGF and VEGF receptor mRNA in marrow from EPAS1/HIF-2α null mice did not differ from that of wild-type mice. On the other hand, expression of mRNA for a number of cell surface proteins, including vascular cell adhesion molecule 1 (VCAM-1), urokinase-type plasminogen activator receptor (uPAR), and fibronectin, were substantially altered in marrow from EPAS1/HIF-2α null mice. These studies reinforce both the importance of the HIF transcriptional complex in hematopoiesis and its function in providing the necessary marrow microenvironment for effective hematopoiesis. The different role of VEGF in embryonic and adult hematopoiesis is puzzling and may relate to a greater degree of hypoxia found in the embryonic hematopoietic compartment or be intrinsic to embryonic versus adult hematopoietic progenitors. Additional studies will be required to define whether EPAS1/HIF-2α is the ARNT partner required for embryonic as well as adult hematopoiesis. This study provides important insights into marrow stromal cell biology and opens potential avenues of exploration both into the role of stroma in marrow failure syndromes and into novel ways to support and expand hematopoietic progenitors in vitro.

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Use of CD28 agonists to refill the T-cell tank?

T-cell lymphopenia with a restricted T-cell diversity creates a long-lasting state of immunosuppression following autologous and, particularly, allogeneic hematopoietic stem cell transplantation. In addition, delayed T-cell reconstitution following control of viral replication in HIV-infected adults leads to continued susceptibility to opportunistic infections and is a barrier to immunization. In this issue, Elflein and colleagues (page 1764) report the induction of CD28-driven T-cell expansion in lymphopenic rats in vivo following anti-CD28 monoclonal antibody therapy. Two injections of an agonistic CD28 antibody were shown to dramatically accelerate repopulation of T cells, and the resulting T cells had a broad repertoire and were functional, suggesting that treatment with human CD28–specific agonists could protect T-lymphopenic patients from opportunistic infections.

CD28 ligation by natural ligands CD80 and CD86 is required for the induction of antigen-driven T-cell proliferation of naive T cells. Antibodies to CD28 may not always mimic the function of natural ligands—Nunès and colleagues previously showed that a panel of CD28 antibodies had distinct effects on T-cell signal transduction (Nunès et al, Int Immunol. 1993;5:311-315). CD28 antibodies that are mitogenic in vitro in the absence of antigen signals have been raised in several laboratories, and Elflein et al have shown that it is likely that the properties of mitogenic CD28 antibodies are governed by the epitope of CD28 recognized by the antibody, as well as the avidity.

In neonates, peripheral T cells are derived from thymic emigrants. In adults, early T-cell reconstitution following cytotoxic therapy is primarily the result of proliferation of peripheral T cells, a process referred to as homeostatic proliferation. The processes that regulate homeostatic T-cell proliferation are complex, but it is clear that the cytokines interleukin 7 (IL-7) and IL-15 have an essential role in this process. In addition, mice engineered to have increased or decreased CD80 or CD86 expression indicate that B7 costimulation plays a physiologic role in the regulation of $CD4^+$ and $CD8^+$ T-cell homeostasis (see Yu et al, J Immunol. 2000;164:3543-3553). Thus, the study by Elflein et al suggests that CD28 agonists could have therapeutic potential for lymphopenia.

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Aberrant somatic hypermutation and lymphomagenesis

Many non-Hodgkin lymphomas (NHLs) arise from B lymphocytes with chromosomal translocations that result in constitutive activation of oncogenes. In certain cases of NHL, mutations occur in regulatory regions of proto-oncogenes, and these may represent another mechanism for oncogene deregulation, independent of translocation. This latter process was first described in a subset of patients with diffuse large B-cell lymphoma (DLBCL; Pasqualucci et al, Nature. 2001;412:341-346). The characteristics of the mutations (eg, location on the chromosome and nucleotides affected) in these DLBCL cases resemble those found in the *IgV* and *BCL-6* genes of normal germinal center (GC) B lymphocytes undergoing the somatic hypermutation (SHM) process and are consistent with the derivation of some DLBCLs from GC B cells (Alizedah et al, Nature. 2000;403:503-511). However, since these proto-oncogenes are not mutated in normal GC B cells, a mistargeting of the SHM process during the GC reaction, "aberrant somatic hypermutation," was invoked as a cause for these potentially

dangerous gene alterations in immunocompetent patients with DLBCL (Pasqualucci et al).

In this issue, Gaidano and colleagues (page 1833) report similar somatic gene changes (mainly point mutations with infrequent nucleotide deletions or insertions) in regulatory and occasionally coding regions of several proto-oncogenes of NHL cells from patients with AIDS. They describe clonal abnormalities in one of these genes in approximately 50% of cases and in two genes in approximately 25% of patients. The point mutations identified had characteristics consistent with those occurring during SHM and similar to those previously defined in patients with DLBCL and intact immune systems. Thus, mistargeting of the adaptive SHM mechanism appears to represent a general phenomenon leading to clonal deregulation and lymphoma. Since these clonal abnormalities may have a broader spectrum than those initially reported in NHL patients without AIDS, this maladaptive process may be even more common in the setting of immune compromise.

The SHM process involves expression of the activation-induced cytidine deaminase gene, which is necessary and sufficient for this process (Muramatsu et al, Cell. 2000; 102:553-563). Although not analyzed in the studies of Gaidano et al, others recently reported the expression of activation-induced cytidine deaminase in NHL (Greeve et al, Blood. 2003;101:3574-3580). SHM and activation-induced cytidine deaminase may be linked to lymphomagenesis by generating double-strand DNA (dsDNA) breaks, which could initiate chromosomal translocations (Kuppers and Dalla-Favera, Oncogene. 2001;20:5580-5594) and the mistargeted and dangerous mutations described by Gaidano et al and Pasqualucci et al.

Thus, the seminal observations of the Dalla-Favera laboratory implicate aberrant targeting of the SHM process in the initiation of several aggressive B-cell lymphomas. Since the occurrence, over time, of new mutations in the same proto-oncogenes was occasionally identified, this mistargeting may also lead to the accumulation of additional genetic lesions and to the evolution of even more aggressive disease. If so, the SHM process and activation-induced cytidine deaminase may be therapeutic targets to limit lymphoma progression.

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Ara-G Fas L–itates T-cell death

Irreversible DNA damage is the hallmark of traditional cytotoxic chemotherapy. Antimetabolites exert their impact through interrupting the DNA replication and repair processes that are required for normal cellular function. Unfortunately, these processes are necessary for both normal and malignant cells and have resulted in fairly narrow therapeutic indexes for many agents. Efforts to find selectivity have focused on differences between normal and malignant cell populations in terms of rates of proliferation, regulation of apoptosis and cell cycle, and ability to repair DNA damage. However, the redundancy of these critical growth and survival pathways in all cell types continues to thwart our abilities to discriminate and target the malignant clone.

Rodriguez and colleagues (page 1842) offer an important model to better understand the mechanisms of arabinosylguanine (ara-G)–induced lymphocyte cell death, specifically the death of T cells, and they have shed important light on the mechanisms that contribute to the drug's selectivity. Provocatively, ara-G works by at least 2 complementary mechanisms. One is the classical inhibition of DNA synthesis for all nucleoside analogs. Ara-G also exploits a unique feature of T-cell biology, the autoregulation (in part through Fas/FasL-induced apoptosis) that is fundamental to the development of a normal, functioning immune system. However, the authors' insight creates important new questions needed in order to further exploit and broaden the applicability of these findings: (1) Does ara-G trigger expression and/or liberation of multiple proapoptotic factors by additional mechanisms? (2) Do other nucleoside analogs trigger similar bystander effects? How? Is this also restricted to T cells or other lineages as well? (3) How can the selectivity of T-cell death through ara-G be translated clinically?

T-cell malignancies and inherited disorders remain a small fraction of malignancies, even within their respective rare disease categories. Our bias is that broader application of this finding may be found as a treatment approach for other T-cell regulation disorders such as autoimmunity and graft-versus-host disease. We also suggest that ara-G's full potential will be realized through the development of rational combinations and sequences of drugs that exploit discrete pathways in selective and complementary fashions.

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AML: clustering genes to predict outcome

Acute myeloid leukemia (AML) is a heterogeneous group of hematopoietic malignancies with diverse genetic abnormalities and phenotypes. Currently, treatment decisions are based on the French-American-British (FAB) classification scheme, which uses largely morphologic characteristics, as well as immunophenotyping and cytogenetic analyses to identify different subtypes of the disease associated with better or worse prognosis. In this issue, Yagi and colleagues (page 1849) have used microarray-based assays to identify gene expression patterns that correlate with prognosis in a collection of pediatric AML patients. The authors assayed the expression of more than 12 000 genes in bone marrow and blood samples and used various data analysis methods to identify groups, or clusters, of patients with distinct phenotypes. Although the study was performed with only 54 patients divided amongst several FAB subgroups, the results have several important implications for the development of new prognostic tests and for the analysis of microarray data in patient samples. First, the simplistic approach of