

not affect the response because the A anti-C-activated CTL precursors cannot recognize them. Bachar-Lustig et al have effectively done the same series of experiments in vivo. Lethally irradiated strain-B mice received strain-A BM transplants containing large numbers of strain-A T cells. Rapid death ensued unless cells from a B anti-D CTL line were also included. Addition of rapamycin could make these cells more effective. In principle, the problem of GVHD has been solved!

—Richard G. Miller

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Monosomy 7 and the myeloid malignancies

Cytogenetics has made an enormous contribution to our understanding of the pathophysiology and prognosis of childhood leukemia. In most cases of acute lymphocytic leukemia (ALL) various translocations within a pre-B cell that probably occurred in utero set the stage for another event or events that trigger unbridled growth. The individual translocations, though not solely responsible for the leukemia, have enormous prognostic significance. The tel-acute myeloid leukemia 1 (AML1) fusion associated with a (12:21) translocation has a standard chemotherapy-induced cure rate of between 90% and 100%. In contrast, ALL with the Philadelphia chromosome is only effectively treated with massive chemoradiation therapy and stem cell transplantation.

In this issue of *Blood*, Kardos and colleagues (page 1997) review a large European experience of refractory anemia in childhood. This heterogeneous collection of premyeloid and virtual myeloid leukemias is characterized by several different cytogenetic abnormalities, the most glaring of which is monosomy 7, a

disorder characterized by an extremely poor prognosis. It must be treated with stem cell transplantation. Even a matched unrelated donor transplant offers a better chance for survival than watchful waiting or chemotherapy. Adults with monosomy 7 usually have multiple cytogenetic abnormalities, whereas the disorder is usually unadulterated in children. The haploinsufficiency associated with loss of the short arm of the chromosome is probably responsible for the development of malignancy that occurs at a variable rate even in patients with familial loss of the chromosome. The collection of monosomy 7 cells established by Kardos et al provides an important opportunity to learn much more about this disorder. It is vital to study gene expression in monosomy 7 to learn how the chromosome disorder leads to malignancy. It is believed that loss of a key suppressor is responsible, but does this permit the unbridled expression of a tyrosine kinase that finally drives the leukemia? This is the next step of inquiry in this instructive disease. For now, Kardos and her colleagues have given us important information on the best treatment approaches.

—David G. Nathan

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Challenges and progress in gene therapy for hemophilia A

In this issue, Powell and colleagues (page 2038) report the results of a phase 1 gene therapy clinical trial for hemophilia A, based on intravenous injection of a retroviral vector encoding B-domain-deleted factor VIII (FVIIIΔB). This phase 1 trial was based on encouraging preclinical studies, mostly in rabbits,¹ and essentially confirms the safety of this approach in patients. The vectors could be detected in the peripheral blood mononuclear cells for at least a year. Although some participants had detectable circulating FVIII levels (>1%) on repeated occasions, experienced fewer bleeding episodes, and required fewer FVIII protein infusions compared with historic rates, the clinical benefits were overall rather limited and a dose response was lacking. It

appears therefore that the preclinical studies that constituted the basis of this trial may not have accurately predicted the vector doses required to achieve therapeutic FVIII levels. However, the limited efficacy of this particular gene therapy approach in adult patients was not entirely unexpected, due to the inability of retroviral vectors to transduce nondividing cells.

Previous studies had shown that stable therapeutic levels of FVIII or FIX could only be obtained in neonatal hemophilic mouse and dog models or in adult mice that received hepatocyte growth factor to stimulate hepatocyte cell division.^{2,3} This inherent limitation of retroviral vectors justifies the development of vectors that can also transduce nondividing cells, such as lentiviral⁴ adeno-associated viral vectors (AAV). In this same issue, Scallan and colleagues (page 2031) report stable expression (> 14 months) of therapeutic levels of FVIII (2%-4%) in 2 dogs with hemophilia A following liver-directed gene therapy using an AAV-based vector encoding FVIIIΔB. Although AAV has successfully been used for gene therapy in hemophilia B dogs⁵ and results from clinical trials for hemophilia B are encouraging, progress in hemophilia A gene therapy has been hampered by the inherent, limited packaging capacity of AAV and the relatively large size of the B-domain-deleted FVIIIΔB cDNA. Scallan and colleagues showed that this limitation could be overcome by using small regulatory elements to drive FVIII expression, in accordance with previous reports.⁶ Although recent studies had shown that therapeutic levels of FVIII could be achieved in hemophilia A dogs with no apparent toxicity following gene therapy,⁷ the work by Scallan and colleagues is an important step forward since it is the first demonstration that long-term phenotypic correction of the bleeding diathesis could be achieved, albeit partial, in a clinically relevant, large animal model of hemophilia A. However, the reason for the lack of a dose response is not clear and warrants further studies in larger cohorts. Additional improvements in vector design and increased gene transfer efficiencies will be required to further increase FVIII expression

levels, potentially allowing the use of lower, clinically acceptable vector doses. The simultaneous development of different gene therapy approaches is justified to bring a cure for hemophilia A one step closer to reality.

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Fanconi anemia stem cells: going round and round

Fanconi anemia (FA) is a congenital form of aplastic anemia and is transmitted through an autosomal recessive mode. Inactivation of any of the 7 FA genes leads to progressive bone marrow (BM) failure, congenital abnormalities, and a predisposition to malignancy. Since a defect in any of the FA genes leads to a similar clinical phenotype, FA proteins appear to act together physically and functionally in a common pathway. However, the question remains: What role does each FA protein or the FA complex play in hematopoiesis?

Studies using the FA group C mouse model have shown that *Fancc*^{-/-} hematopoietic stem cells have impaired function shown by reduced repopulating ability and are found at lower numbers in *Fancc*^{-/-} BM. These results and the fact that BM aplasia in patients with FA is progressive suggest that the FA gene products are required for the maintenance of normal numbers of stem cells and/or for normal stem cell development.

In this issue, Li and colleagues (page 2081) have defined a new phenotype associated with *Fancc*^{-/-} stem cells. Using 2 simple assays, these authors have evaluated the cycling state of the hematopoietic stem/progenitor cell fraction from *Fancc*^{-/-} mice. They show that the stem/progenitor-enriched fraction is less quiescent than wild-type (WT) controls showing more bromodeoxyuridine (BrdU) incorporation and fewer cells in G0. They go on to show that the altered cell cycle kinetics in *Fancc*^{-/-} cells are, at least in part, cell autonomous and do not result from unscheduled DNA synthesis or increased damage and repair. In addition, the increased cycling activity found in *Fancc*^{-/-} hematopoietic cells does not seem to be a compensatory response related to their proapoptotic phenotype but may indeed contribute to the increased apoptotic response of these cells to cytokines. On the other hand, the defect in cytokine signaling in *Fancc*^{-/-} hematopoietic cells may contribute to the increased cycling activity. In any case, Li and colleagues clearly demonstrate that an accelerated cycling rate in *Fancc*^{-/-} cells, whether a direct or indirect consequence of absence of the *Fancc* gene, is a contributing factor to stem cell exhaustion in FA leading to BM failure.

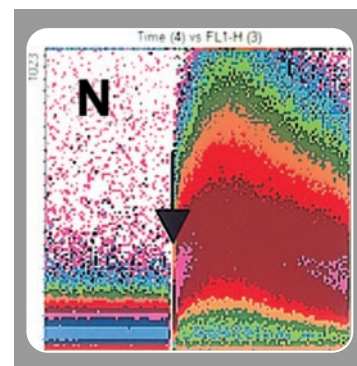
—**Madeleine Carreau**

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CD38: what is it there for?

CD38 is very much a molecule of the moment. Since it has been mentioned in well over 1000 articles in the past 5

years, we are entitled to ask, "What is it there for?" It is a type II transmembrane glycoprotein, the extracellular domain acting as an ectoenzyme, catalyzing the conversion of nicotinamide adenine dinucleotide (NAD⁺) into nicotinamide, aden-



osine diphosphate-ribose (ADPR), and cyclic ADPR. CD38 is expressed on many types of cells, but recent interest focuses on its role on B lymphocytes. Its expression during B-cell ontogeny is tightly regulated: it appears on bone marrow precursor cells but is lost on mature lymphocytes; on germinal center cells it protects against apoptosis, but on leaving the germinal center, memory cells lack the antigen; on terminally differentiated plasma cells it is one of the few surface antigens present. In chronic lymphocytic leukemia (CLL), expression of CD38 signifies a poor prognosis, though it does not correlate precisely with the presence of unmutated immunoglobulin variable region (*IgV*) genes and may vary during the course of the disease.¹

Is it more than a prognostic marker? Deaglio and colleagues (page 2146) suggest that CD38 is involved in signaling through the B-cell receptor (BCR). Unfortunately, even CD38⁺ CLL cells express the molecule at such low density that few cells show detectable signals on ligation by antibody. However, when the expression of CD38 was upregulated by exposing the cells to interleukin 2 (IL-2), incubation with anti-CD38 antibodies mediated a signal that could be detected by Ca⁺⁺ flux. Because CD38 patches on the