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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1. Greengard JS, Jolly DJ. Animal testing of retroviral-mediated gene therapy for factor VIII deficiency. Thromb Haemost. 1999;82:555-561.

2. VandenDriessche T, Vanslembrouck V, Goovaerts I, et al. Long-term expression of human coagulation factor VIII and correction of hemophilia A after in vivo retroviral gene transfer in factor VIII-deficient mice. Proc Natl Acad Sci U S A. 1999;96:10379-10384.

3. Xu L, Gao C, Sands MS, et al. Neonatal or hepatocyte growth factor-potentiated adult gene therapy with a retroviral vector results in therapeutic levels of canine factor IX for hemophilia B. Blood. 2003;101:3924-3932.

4. Vanden Driessche T, Thorrez L, Naldini L, et al. Lentiviral vectors containing the human immunodeficiency virus type-1 central polypurine tract can efficiently transduce nondividing hepatocytes and antigen-presenting cells in vivo. Blood. 2002;100:813-822.

5. Mount JD, Herzog RW, Tillson DM, et al. Sustained phenotypic correction of hemophilia B dogs with a factor IX null mutation by liver-directed gene therapy. Blood. 2002;99:2670-2676.

6. Chao H, Walsh CE. Induction of tolerance to human factor VIII in mice. Blood. 2001;97:3311-3312.

7. Chuah MKL, Schiedner G, Thorrez L, et al. Therapeutic factor VIII levels and negligible toxicity in mouse and dog models of hemophilia A following gene therapy with high-capacity adenoviral vectors. Blood. 2003;101:1734-1743.

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.
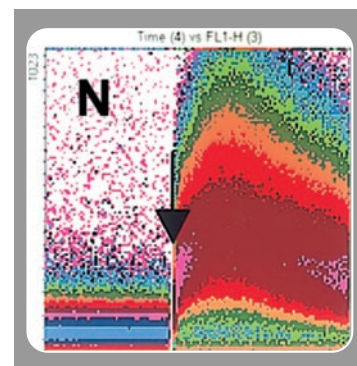
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CD38: what is it there for?

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

surface also contained the GM1 ganglio-side and CD81 with significant lateral associations with the immunoglobulin accessory molecules CD79a and CD79b and with CD19, they have suggested that CD38 forms part of the positive receptor complex on the cell membrane.

Does this paper advance our understanding of the nature of CLL? Some of the characteristics of the CLL cell such as CD5 and CD23 positivity and its resistance to apoptosis in vivo seem to be intrinsic to its nature, whereas other characteristics vary between benign and aggressive disease. Whether or not there are somatic mutations of the *IgV* genes seems to be hardwired in CLL and seems to predetermine the likelihood that subsequent adverse events will occur. The up-regulation of CD38 and its incorporation into lipid rafts may be one of these adverse events. Its function there may be to enhance signals transduced by the BCR with consequent increased proliferation and risk of genetic error.[2]

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1. Hamblin TJ, Orchard JA, Ibbotson RE, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood. 2002;99:1023-1029.
2. Lanham S, Hamblin TJ, Oscier DG, Stevenson FK, Packham G. B-cell receptor signaling responsiveness correlates with V_H gene mutational status on chronic lymphocytic leukemia. Blood. 2003;101:1087-1093.

A stand-off between rhAPC and LPS in healthy human subjects

Recombinant activated protein C (rhAPC) was recently tested in a large clinical trial for the management of severe sepsis.[1] The study revealed that infusion of rhAPC for 96 hours reduced the 28-day mortality rate from 30.8% to 24.7%. In this study group, 70% of patients were in shock and 75% were assisted with mechanical venti-lation. This significant advance in the management of sepsis, which afflicts some 750 000 hospitalized patients per year in the United States, has evoked acclaim and new expectations. Indeed, sepsis is the most common cause of death in noncoronary intensive care units (ICUs). Management of patients admitted with severe sepsis consumes an estimated 52% of the ICU total budget.

Severe sepsis consists of local or generalized invasion of the body by pathogenic microorganisms or their toxins. These agents can cause organ dysfunction, hypoperfusion, or hypotension associated with microvascular thrombosis as a consequence of systemic inflammation. One of the agents responsible for this constellation of microvascular changes is the lipopolysaccharide (LPS) component of the outer membrane on Gram-negative bacteria. Systemic response to LPS, also known as endotoxin, fulfills the criteria for an acute inflammatory reaction. This experimental model elicited in humans with injection of exceedingly small amounts of LPS (2-5 ng/kg) provided key insights into the temporal appearance and importance of mediators in the process of inflammation.[2]

In this issue, Derhaschnig and colleagues (page 2093) report the effects of rhAPC on coagulation and inflammation parameters in 24 healthy volunteers following acute perturbation with LPS. When infusion of rhAPC reaches steady-state levels (60- to 70-fold higher than baseline), basal levels of tissue factor transcripts and thrombin generation are suppressed. Subsequent challenge with LPS (2 ng/kg) induces a 15-fold increase in tissue factor transcripts and a significant increase in thrombin generation. Surprisingly, these elevated coagulation parameters remain untouched in rhAPC-treated subjects. Similarly, LPS-induced activation and subsequent inhibition of fibrinolysis and a wave of inflammatory cytokines (tumor necrosis factor α [TNFα] and interleukin 6 [IL-6]) remain unchanged by rhAPC. Thus, in this model of LPS-induced acute inflammation and coagulation, beefing up normal levels of APC with recombinant protein seems to leave coagulation and inflammation responses unshaken.

Does this pattern of nonresponsiveness to rhAPC raise questions regarding severe sepsis with shock and microvascular thrombosis? The answer seems to be "no" because LPS-producing Gram-negative bacteria were isolated from 64 of 107 patients with severe sepsis. The remainder of microorganisms was Gram-positive bacteria and *Candida albicans.*[3] These isolated pathogens are known to trigger a large set of distinct Toll-like receptors that function as vanguards of innate immunity. These receptors exhibit genetic polymorphism linked to differences in intracellular signaling required for activation of genes that encode mediators and suppressors of inflammation.[4] Furthermore, progression of microvascular changes in severe sepsis usually continues for days and weeks. Thus, it is likely that APC and its receptor, as well as the endothelial thrombin binder and regulator thrombomodulin, undergo dynamic changes induced by multiple waves of inflammatory cytokines and chemokines.

With these considerations in mind, it is difficult to extrapolate the results reported by Derhaschnig and colleagues to critically ill and hemodynamically compromised patients with severe sepsis. To wit, we need comprehensive studies of inflammation and coagulation parameters over the course of sepsis from its early to advanced stages, using genotypic and phenotypic analysis of the host response to a specific pathogen or its product. In such a context, the mechanism of in vivo action of rhAPC in etiologically diverse cases of severe sepsis can be delineated.

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1. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med. 2001; 344:699-709.
2. Gallin JI, Snyderman R, eds; Fearon DT, Haynes BF, Nathan J, assoc eds. Inflammation: Basic Principles and Clinical Correlates. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1999.
3. Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis