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developed as inhibitors of the Ras oncoprotein. Their mechanism of action is considered to reside in the inhibition of Ras farnesylation that is required for its membrane association and signaling activity. Indeed, selective inhibition of FTase enzymatic activity and protein farnesylation by Zarnestra was shown in this study using patient peripheral blood and bone marrow samples. However, neither inhibition of FTase nor N-Ras and K-Ras mutation status of the myeloma cells correlated with response to treatment. These findings suggest that, even in Ras-mutated MM, deregulation of the Ras signaling cascade may be either a dominant transforming pathway or just one among redundant oncogenic events ensuring tumor growth and survival. Another likely explanation is that the biologic activity of FTase inhibitors is in fact more complex and involves proteins unrelated to Ras.[1,2]

Podar and colleagues (page 3474) provide the preclinical rationale for evaluation of the small molecule tyrosine kinase inhibitor GW654652 as novel antimyeloma agent. This indazolylpyrimidine inhibits vascular endothelial growth factor (VEGF) receptors 1 through 3. VEGF is known to be produced by both MM and bone marrow stroma cells and has been shown to optimize the micromilieu for MM tumors via autocrine and paracrine stimulatory loops. As a potent angiogenic cytokine, VEGF may also contribute to myeloma-associated marrow neoangiogenesis. The results of the in vitro studies reported demonstrate that GW654652 blocks VEGF-induced tyrosine phosphorylation of VEGF receptor-1 (Flt-1) and related downstream signaling in MM cells and inhibits MM cell migration, proliferation, and survival. It also acts on myeloma-stroma interactions, as shown by inhibition of VEGF and interleukin-6 production in a coculture model. Secondary to the interference with the production of the MM survival factor interleukin-6, GW654652 may also offer the potential to overcome drug resistance in MM. These findings are in line with and extend previous reports on in vitro activities of other VEGF receptor tyrosine kinase inhibitors.[3,4] Based on the promising preclinical data, first results of clinical evaluation of this novel class of drugs are eagerly awaited.

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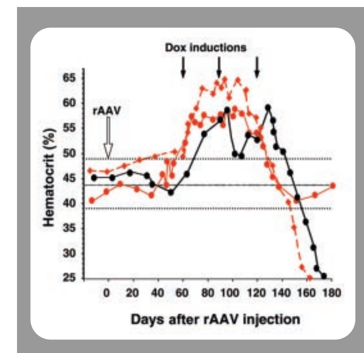
1. Gouill SL, Pellat-Deceunynck C, Harousseau J-L, et al. Farnesyl transferase inhibitor R115777 induces apoptosis of human myeloma cells. Leukemia. 2002;16:1664-1667.
2. Ochiai N, Uchida R, Fuchida S, et al. Effect of farnesyl transferase inhibitor R115777 on the growth of fresh and cloned myeloma cells in vitro. Blood. 2003;102:3349-3353.
3. Lin B, Podar K, Gupta D, et al. The vascular endothelial growth factor receptor tyrosine kinase inhibitor PTK787/ZK222584 inhibits growth and migration of multiple myeloma cells in the bone marrow microenvironment. Cancer Res. 2002;62:5019-5026.
4. Bisping G, Wenning D, Dreyer B, et al. In vitro effects of the novel indolinone derivative BIBF1000 in multiple myeloma [abstract]. Blood. 2003;102:190a.

GENE THERAPY

Inadvertent autoimmunity in EPO gene transfer

Several congenital deficiencies such as hemophilia B and anemia are currently treated by administration of recombinant proteins. However, repeated clinical administration of therapeutic proteins is cumbersome, and such genetic disorders could be cured via gene transfer vectors, allowing sustained and/or controlled expression of these proteins. In this respect, the intramuscular inoculation of adeno-associated viral (AAV) vectors expressing human blood coagulation factor IX seems extremely promising for treatment of patients with hemophilia B, and clinical trials have now progressed into phase 1/2.[1] Likewise, biologically active erythropoietin (EPO) can be secreted from skeletal muscle and was shown to improve erythropoiesis in beta-thalassemic mice.[2] So far, protein replacement therapy with recombinant human EPO (rhuEPO) produced from mammalian cells, biologically equivalent to the natural hormone, has been extremely effective to remedy several forms of anemia. Although approximately 3 million people throughout the world have now been treated, very few patients have developed pure red cell aplasia following rhuEPO therapy.[3] It is only in the last few years that a significant increase in the # of cases of absolute resistance to rhuEPO therapy due to anti–EPO antibodies was reported, most likely as a result of slight modifications in the production and formulation of the clinical grade rhuEPO. As for gene therapy, there is clear evidence for humoral and cellular immune responses against transgenic proteins—as well as absence of such responses, depending on vector design, gene dosage, or the underlying mutation in the dysfunctional gene.

In this issue of *Blood*, 2 independent studies from Chenuaud and colleagues (page 3303) and Gao and colleagues (page 3300) describe for the first time an inadvertent autoimmune response in nonhuman primates resulting from transfer of a gene encoding a self-antigen. Their approach was to deliver the homologous EPO cDNA driven by ubiquitous and/or regulatable promoters via AAV vectors injected in muscle or aerosolized in lung, resulting in supra-physiologic serum levels of EPO, from 10- to 100 000-fold over the baseline. However, this genetic



intervention broke the immune tolerance, and, within a few weeks, some macaques suffered from severe autoimmune anemia, possibly induced by T/B lymphocytes and monocyte infiltrates in inoculated muscles as well as by neutralizing antibodies against both the transgenic and endogenous EPO. The reasons for the autoimmunity induced by homologous EPO gene transfer are not

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clear. Trivial mechanisms such as sequence differences between transgenic and endogenous EPO cDNAs as well as vector particle–induced inflammation could be ruled out a priori. Furthermore, the fact that anemia sporadically arose in 2 different macaque species upon gene transfer using several rAAV serotypes in 2 ectopic expression sites (muscle or lung versus kidney) rather suggests an EPO-specific effect. Thus, the autoimmune anemia might be due to the ectopic expression site of the transgene or to supraphysiologic expression levels that could induce posttranslational modifications; yet it may also be contributed by vector spreading in the draining lymph nodes and subsequent EPO expression in professional APCs. In addition, there was no clear correlation between elevated EPO levels and anemia, suggesting that the events occur in a haplotype-dependent manner.

These results are extremely important, not only because they teach us that in vivo gene delivery is effective, but also because they stress that the direct translation to the clinic of preclinical data obtained in inbred rodent models is not straightforward. Although the laboratory mouse has been invaluable in the preclinical gene therapy era, it shows limitations. Autoimmune responses to gene transfer have never been reported in these small animal models, possibly because they have not been identified or exhaustively assessed. In contrast, more relevant information in safety, efficiency, and immunologic consequences of gene transfer can be expected from wild and large animals. Thus, as vectors have now become efficient enough to allow effective gene delivery, experiments in nonhuman primates are more practical than before, and their more extensive implementation in preclinical studies may be a prerequisite before going to the clinic.

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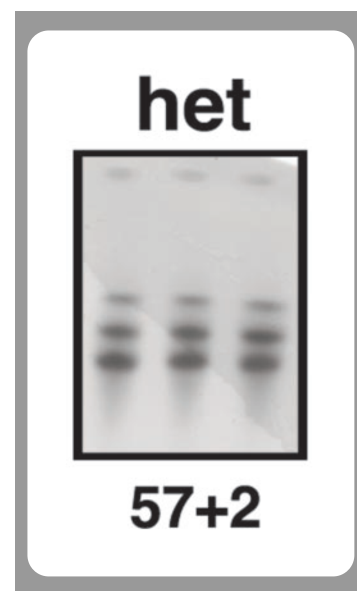
1. Manno CS, Chew AJ, Hutchison S, et al. AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. Blood. 2003;101:2963-2972.
2. Bohl D, Bosch A, Cardona A, Salvetti A, Heard JM. Improvement of erythropoiesis in beta-thalassemic mice by continuous erythropoietin delivery from muscle. Blood. 2000;95:2793-2798.
3. Casadevall N, Nataf J, Viron B, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med. 2002;346:469-475.

HEMATOPOIESIS

The "stem cell leukemia" gene: what's in a name?

The name "stem cell leukemia" (SCL) was assigned to a gene involved in a t(1;14) translocation associated with an acute leukemia with early T-cell phenotype and myeloid differentiation potential. SCL is a transcription factor of the basic helix-loop-helix (bHLH) family and normally functions as a critical regulator of hematopoiesis. It is expressed in hematopoietic stem cells (HSCs) and erythroid and megakaryocytic lineages, but not in granulocyte/monocytes or lymphocytes. It is expressed in endothelium and is critical for hemangioblast development in ontogeny. Forced expression of SCL in zebra fish embryos results in expansion of hematopoietic and endothelial progenitors at the expense of other mesodermal derivatives.[1] Enforced expression of SCL in $CD34^+$ cells enhances megakaryocyte and erythroid development and inhibits myelomonocytic differentiation. Mouse embryos lacking SCL die with complete absence of blood.[2] Rescue of defective embryos with SCL under a hematopoietic-specific promoter (GATA1) rescues erythropoiesis but embryos still die of defective angiogenesis.[2] SCL specifies hemangioblast development from primitive mesoderm in an embryonic stem cell model.[3] To overcome in utero lethality, a conditional knockout of SCL in adult mice has been achieved by inducible cre/lox technology.[4,5] Mikkola et al[4] have reported that adult HSCs retained long-term repopulating activity and multipotency in the absence of SCL. Thus, while SCL is critical for hematopoietic specification, it is not required for HSC self-renewal, or there may exist compensatory systems to sustain SCL-deficient HSCs. Hall et al[5] have shown that SCL deletion in adult mice perturbed megakaryopoiesis and erythropoiesis, with loss of early progenitors in both lineages and blunted response to hematopoietic stress. In addition, the spleen colony-forming unit day 12 ($CFU-S_{12}$), a population of short-term repopulating multipotent stem/progenitor cells, generated only myeloid but not erythroid or megakaryocytic colonies.

In this issue of *Blood*, Curtis and colleagues (page 3342) used the conditional Cre/Lox system to generate adult SCL null mice and observed a severe defect in the ability of null marrow to provide normal hematopoietic reconstitution by 4 weeks in a transplant model. In competitive repopulation with wild-type marrow, there was a significant reduction in contribution of the SCL null marrow to the myeloid lineage and absence of contribution to the erythroid lineage at 4 weeks. At 16 weeks there was



still some defect in contribution to myeloid and lymphoid lineages. How can this be reconciled with the lack of defects in the study by Mikkola et al? Hall et al[5] duplicated the Mikkola et al model and, consistent with the latter's report, found no defect with SCL-deleted cells at 4 weeks, although

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