We delivered the homologous erythropoietin (Epo) cDNA driven from a doxycycline-regulated promoter via recombinant adeno-associated virus in skeletal muscle of 9 cynomolgus macaques. Upon induction, rapid supraphysiologic levels of Epo were obtained. Unexpectedly, some individuals developed a profound anemia that correlated with the appearance of neutralizing antibodies against the endogenous Epo. Both the endogenous erythropoietin and vector sequences were identical. This is the first example of the inadvertent development of an autoimmune disease in primates as a result of gene transfer of a gene expressing a self-antigen. It raises some concerns when a therapeutic protein is produced at high levels from an ectopic site. (Blood. 2004;103:3303-3304)

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the rAAV-injected muscles. Both Mac 10 and Mac 14 endogenous Epo were sequenced and found to be identical to the vector Epo, and the Epo protein secreted from in vitro rAAV-transduced cells had a similar molecular weight as the rhuEpo (not shown).

Gao et al. reports a similar result with rAAV serotype-1, -5, and -8, constitutively expressing homologous Epo cDNA injected in muscle or aerosolized in lung of macaques (see the accompanying article by Gao et al., beginning on page 3300). The fact that an autoimmune anemia can occasionally arise using 4 different rAAV serotypes (rAAV-1, -2, -5, and -8) in 2 different ectopic organs (skeletal muscle and lung) with respect to normal Epo synthesis and secretion suggests an Epo-specific adverse effect. The partial correlation that we observed between the amount of Epo secreted at peak 1 and the occurrence of anti-Epo antibodies also suggests that posttranslational modifications could take place as previously suggested for highly expressed myotube-derived factor IX, thus breaking tolerance to self-antigens in a haplotype-dependent manner (see Mac 13 that, despite high levels of Epo synthesis, remained healthy). An aggravating factor could be related to the previously published observation that rAAV serotype-2 spreads efficiently to draining lymph nodes after intramuscular delivery and is associated with long-term peripheral blood monocyte cell transduction. Whether rAAV serotype-1, -5, and -8 have similar transduction patterns in nonhuman primates after intramuscular administration remains to be determined. In any case, the occurrence of a life-threatening autoimmune response in nonhuman primates following in vivo transfer of a homologous cDNA underscores the need for extensive preclinical studies to understand the mechanism(s) involved, in particular when a therapeutic protein is produced at high levels from an ectopic site.

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References

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Figure 1. Maximum serum Epo concentration for each Dox induction cycle. (A) Dox-induced Epo secretion measured at the peak level in the serum of Mac 9 through Mac 17 after rAAV-2-mediated (red), rAAV-1-mediated (black), and rAAV-5-mediated (blue) gene transfer. Each induction (referred to as “Induction peaks”) lasted 5 days and was repeated every month for 5 months. (B) Amounts of rAAV-2 (red), rAAV-1 (black), and rAAV-5 (blue) vector administered expressed as vector genome/kg determined by dot blot. Mean Epo levels before vector injection are represented by error bars.

Figure 2. Hematocrit levels from Mac 9 and Mac 10 (red circles and diamonds, respectively), and Mac 14 (black circles) from the time of rAAV-2 (red) and rAAV-1 (black) injections (open arrow), until anemia occurred. Black arrows correspond to the Dox induction cycles. Dotted lines are normal hematocrit levels ± SD. Symbols in this figure are identical to those in Figure 1.

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Autoimmune anemia in macaques following erythropoietin gene therapy

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