

inhibitors after neonatal transduction of a vesicular stomatitis virus G glycoprotein (VSV-G) pseudotyped RV expressing human FVIII. However, in our study, none of 12 mice that were transduced as neonates produced cFVIII inhibitors, which was determined by Bethesda assay. To verify the capability of making inhibitors in hemophilia A mice, a single dose of RV was given IV into 6-week old mice at 3.3×10^9 TU/kg (N=3). All three mice made cFVIII inhibitors at 2-3 weeks after transduction. We conclude that completely therapeutic levels of cFVIII can be achieved in mice after neonatal transduction, and neonatal gene therapy with an amphotropic RV expressing cFVIII is not immunogenic. Based upon the above results, we performed neonatal RV transduction in two Chapel Hill hemophilia A dogs. RV was given IV at 3 days after birth at 9×10^9 TU/kg and 7×10^9 TU/kg, respectively. The whole blood clotting time (WBCT) in both dogs has been normalized since day 4 after RV transduction, and remained in the normal range for 4 months thus far. The plasma cFVIII activity in one RV-treated dog was 417% of normal by COATEST assay and 430% of normal by aPTT assay, and the aPTT time was normalized in this dog. The plasma cFVIII activity in another dog was 353% of normal by COATEST assay and 145% of normal by aPTT assay, and the aPTT time was shortened, but not normalized, in this dog. The plasma cFVIII activities have remained stable in both dogs for 4 months thus far. No cFVIII inhibitors were detected. No bleeding has occurred. We conclude that stable expression of therapeutic levels of cFVIII have been achieved in hemophilia A mice and dogs with neonatal transduction of an MLV-based RV.

35. Downregulation of Immune Responses Induced by Oral DNA Administration

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Protein replacement treatments often trigger unwanted immune responses in the host. Induction of immune tolerance would be a desirable goal to mitigate such events, and would eliminate the need for life-long immunosuppressive therapy in affected individuals. Hemophilia B is an X-linked bleeding disorder caused by blood coagulation factor IX (FIX) deficiency. Some hemophiliacs develop neutralizing antibodies to FIX (inhibitors) that render the infused FIX ineffective. In this study we evaluated whether oral delivery of hFIX-DNA nanoparticles can downregulate the immune response against hFIX in immunized mice. A group of C57BL/6 mice was immunized with recombinant hFIX in Freund's adjuvant followed by three boost at day 7, 14, and 21. As expected, all mice developed high titer of anti-hFIX antibodies as measured by day 28. At this time, half the mice were treated with a single oral dose of plasmid DNA nanoparticles containing hFIX cDNA under the control of the b-actin promoter. The other half was left untreated, and served as control. By ELISA, we detected circulating levels of hFIX in the treated mice by day 3 after the oral treatment. In contrast, control mice had undetectable levels of circulating hFIX. Importantly, the titer of inhibitors against hFIX was measured by a Bethesda assay. Control mice had inhibitors, on average, of 40 BU/ml on day 21 after the oral administration of DNA. In contrast, the level of inhibitors became undetectable in the treated group by day 21. The cellular immune response was also assessed. Stimulation of splenocytes from orally treated mice with hFIX resulted in increased secretion of transforming growth factor- β , but no detectable interferon- γ production. In contrast, stimulation of splenocytes from immunized control mice with hFIX showed an increase in the production of interferon- γ , and no secretion of transforming growth factor- β . Additionally, a hFIX-specific cytotoxic T-lymphocyte (CTL) response was detected in the control, but not in the orally treated

mice. Thus, our results suggest that the oral delivery of DNA nanoparticles downregulated the pre-existing immune responses against human FIX. This downregulation may result from the induction of a shift from a proinflammatory T helper cell type 1 to an anti-inflammatory T helper cell type 2 immune response. In conclusion, we have shown that pre-existing immune responses against hFIX can be overcome. This strategy may facilitate the delivery of proteins in a pre-sensitized host. Similar approaches may have potential applications for the induction of immune tolerance in autoimmune diseases. This strategy may also have implications for the prevention of unwanted immune responses elicited by the expression of novel transgenes.

The authors have a significant financial interest in a company commercializing this technology.

36. Permanent Phenotypic Correction of Haemophilia B in Immunocompetent Mice by Prenatal Gene Therapy

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Haemophilia B arises from mutations in the *factor IX* gene. Its treatment in humans, by recombinant protein substitution, is expensive thus limiting its application to intermittent treatment in bleeding episodes and prophylaxis during surgery; development of inhibitory antibodies is an associated hazard. This study demonstrates permanent therapeutic correction of this disease without development of immune reactions by introduction of HIV-based lentiviral vector encoding the human factor IX protein into the fetal circulation of immunocompetent haemophiliac and normal outbred mice. Plasma factor IX persists at therapeutic concentrations in all treated mice to date (ten months). Functional normalisation of the blood clotting has been achieved in two haemophiliac mice and significant correction in a third. In comparison, administration of an adenoviral vector encoding human factor IX resulted in transient and diminishing factor IX expression which was nearly undetectable by 8 months. Following lentiviral administration, no anti-factor IX antibodies were detected by ELISA and no cellular immunity was detected by immunohistochemical staining of macrophages, neutrophils, CD4-positive or CD8-positive cells. There was no elevation of serum liver enzymes or evidence of vector spread to the maternal circulation. No vector spread to sperm was detected by TaqMan analysis. This is the first demonstration of complete phenotypic correction of a severe genetic disease by *in utero* gene therapy with no evidence of toxicity.

