



Dr
Lingfei
Xu

16 Hemophilia A is an X-linked bleeding disorder resulting from a deficiency of factor VIII (FVIII). Approximately 30% of patients with severe hemophilia A develop anti-FVIII inhibitory antibodies, which reduce the response to FVIII replacement. We recently demonstrated that neonatal IV injection of a retroviral vector (RV) expressing canine FVIII (cFVIII) resulted in therapeutic levels of cFVIII in hemophilia A mice and dogs without development of inhibitory antibodies. The goal of this project is to further investigate immune responses after gene therapy for hemophilia A. For these studies, we will use a RV expressing human FVIII (hFVIII), as mice and dogs usually mount an immune response to hFVIII, and purified hFVIII is available.

The first aim is to determine if high-dose neonatal gene therapy with a RV expressing hFVIII can correct the clinical manifestations of hemophilia A in mice and dogs without inhibitor development. An amphotropic RV expressing hFVIII will be

Tolerance induction with neonatal gene therapy for hemophilia A

administered via IV injection at a dose of 10^{10} transducing units (TU)/kg shortly after birth, and hFVIII levels, anti-hFVIII antibodies, and bleeding manifestations will be evaluated. We predict that therapeutic levels of hFVIII will be achieved without inhibitor production.

The second aim is to determine the minimum level of hFVIII needed to induce tolerance in hemophilia A mice with low-dose neonatal gene therapy. This might result in injecting low doses of RV at birth for the sole purpose of inducing tolerance in patients that are at high risk of inhibitor development. Hemophilia A mice will receive 10^{10} , 10^9 , 10^8 , or 10^7 TU/kg of the hFVIII-expressing RV shortly after birth, and be tested for hFVIII activity and anti-hFVIII antibodies. RV-treated and naive mice that did not receive gene therapy will be challenged with hFVIII protein at 2 months, and anti-hFVIII antibodies levels will be determined. We predict that expression of $>1 \times 10^{-10}$ M of hFVIII will be necessary for inducing tolerance to hFVIII.

The final aim is to identify the immunologic mechanisms that result in tolerance with neonatal gene therapy in mice. Normal C3H mice will be used, as inbred mice are required, and this strain usually makes potent immune responses to foreign proteins. RV will be injected at the doses noted in aim II, and hFVIII expression and anti-hFVIII antibodies will be determined at two months. Animals will be stimulated with hFVIII or an irrelevant antigen [human Factor IX (FIX)] with adjuvant, and lymphocytes will be evaluated in vitro for the secretion

of cytokines and proliferation in response to hFVIII or hFIX, and by cytotoxic T cell assays. Neonatal tolerance could be caused by clonal deletion or induction of regulatory T cells that suppress immune responses. Lymphocytes from mice that received different doses of RV as newborns and were challenged with hFVIII at two months will be used in adoptive transfer experiments to investigate these possibilities. The recipients of different types of lymphocytes will be challenged by hFVIII protein to determine if they are tolerant. These studies should further our understanding of the efficacy and mechanisms of neonatal tolerance, and might result in a method for inducing tolerance in patients with hemophilia A.

“

The goal of this project is to further investigate immune responses after gene therapy for hemophilia A

”

Washington University School of Medicine
USA