Induction of tolerance to human factor VIII in mice

Hengjun Chao and Christopher E. Walsh

Introduction

Gene therapy for hemophilia has become an exciting prospect for long-term curative treatment. Advances in gene transfer for hemophilia A and B have shown the feasibility of this curative procedure. However, the immune response against human factor VIII may be a difficult barrier to successful gene therapy for hemophilia. Inhibitory anti-factor VIII antibodies (inhibitors) develop in up to 30% of patients with hemophilia A receiving infusions of factor VIII concentrates. The mechanism responsible for development of the anti-factor VIII inhibitor remains unclear, although several factors may affect its formation, including the type of factor VIII mutation, the factor VIII product used, and patients' immune response. Currently, patients with the inhibitor are treated with induction of immune tolerance or administration of porcine factor VIII, activated factor complexes, immunosuppressive agents, or activated factor VIIa. Successful induction of immune tolerance can be achieved by continuous infusion of a high-dose factor VIII concentrate to "desensitize" patients. Because of the desirability of achieving long-term factor expression through gene transfer, coupled with the potential of inhibitor development, we performed experiments addressing inhibitor formation and tolerance in gene transfer.

We previously reported sustained expression of factor VIII in mice with use of recombinant adenovirus-associated vector (rAAV). When rAAV vector carrying factor VIII complementary DNA was administered by the intraportal route into immunocompetent C57BL/6 mice, high-titer anti-factor VIII inhibitor was detected. These data are consistent with findings of earlier studies in which inhibitor against factor VIII was detected in immunocompetent mice receiving either repeated administration of purified recombinant factor VIII or gene transfer of the factor VIII gene. We here report that the antibody response to factor VIII expressed from rAAV vector diminishes over time.

Study design

Vector constructs, cell cultures, rAAV production and purification, the enzyme-linked immunosorbent assay (ELISA) for factor VIII antigen, and the assays for activated partial thromboplastin time, Coatest factor VIII activity, and Bethesda inhibitor for factor VIII were as described previously, as were the animal care and surgical procedures.

Results and discussion

A total of $10^{10}$ to $10^{11}$ rAAV/DLZ6 virions expressing functional B-domain–deleted (BDD) factor VIII were injected into the portal vein of 4- to 6-week-old male C57BL/6 mice or nonobese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice. Up to 27.5% (55 ng/mL) of the normal factor VIII antigen level was detected in the plasma of the NOD/SCID mice. However, maximum factor VIII levels in the C57BL/6 mice were only 6 to 10 ng/mL. High-titer factor VIII inhibitor was observed in plasma as early as 1 week after injection in all C57BL/6 mice given rAAV/DLZ6. Anti-factor VIII inhibitor titer increased to the maximum level 9 to 12 weeks after injection.

Six C57BL/6 mice were given an injection of rAAV/DLZ6. Three of the six mice were killed 9 months after administration of rAAV vectors for performance of histologic and molecular analyses. The 3 surviving mice were tested for factor VIII antigen at bimonthly intervals (it has now been 1.5 years since those mice received the rAAV/DLZ6 injection). At 10 months after injection, the factor VIII antigen level in 2 of the 3 mice increased from a range of 3% to 5% to 29% (58 ng/mL) of the normal level of factor VIII. Coincident with this rise in antigen levels was disappearance of the anti-factor VIII inhibitor in plasma in the 3 mice (Figure 1). These results are consistent with previous observations of development of anti-factor VIII inhibitor in either adult immunocompetent C57BL/6 mice.

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Funded by National Institutes of Health grant RO1 DK54419. C.E.W. is a recipient of the Lucille Markey Trust.
Effective. Recombinant adenovirus, which is well-known to elicit administration of either high- or low-dose FVIII regimens, is a range of doses of FVIII are used clinically to induce tolerance; immune tolerance in hemophilia patients with inhibitor. A wide range of neonates or the relatively high FVIII levels may have accounted for three animals.

Barbital-buffered saline or mouse plasma. Values (±SEM) represent the averages of three animals.

Mice or neonatal FVIII knockout mice (C57BL/6 background) after repeated infusions of hFVIII. The immature immune status of neonates or the relatively high FVIII levels may have accounted for the lower incidence of inhibitor formation in those investigations.

Our experimental data are consistent with observations of immune tolerance in hemophilia patients with inhibitor. A wide range of doses of FVIII are used clinically to induce tolerance; administration of either high- or low-dose FVIII regimens is effective. Recombinant adenovirus, which is well-known to elicit cell-mediated immune response, did not provoke an inhibitor response to hFVIII in C57Bl/6 mice. Adenoviral production of supratherapeutic amounts of hFVIII (5- to 10-fold greater than physiologic levels) may facilitate immediate desensitization and immune tolerance that occurs with repeated administration of high-dose FVIII to patients with hemophilia A who have high-titer inhibitor.

In another report, retroviral gene transfer and bone marrow transplantation induced immunologic unresponsiveness in 50% of FVIII knockout mice (C57BL/6 background). Although no hFVIII protein or activity was detected, it was postulated that low-level exposure of protein in antigen-presenting cells could induce tolerance. This induction of central tolerance produced by means of hematopoietic chimerism allows tolerance of developing lymphocytes in the bone marrow and thymus. In contrast, our results suggest that peripheral tolerance of mature lymphocytes was induced by constant exposure of hFVIII protein leading to B-cell and T-cell energy. The reemergence of FVIII also confirms that rAAV-mediated transduced hepatocytes are stably maintained and not eliminated by cytotoxic immune effector cells.

Gene therapy holds the promise of radically changing the way in which hemophilia is treated. Investigation of the mechanism responsible for development of anti-hFVIII inhibitor in animal models is important not only for preclinical evaluation of protocols for hemophilia A gene therapy but also because it will contribute to the elucidation of inhibitor formation and its treatment in patients with hemophilia. Our results demonstrate that persistent expression of hFVIII in immunocompetent mice mediates immune tolerance.

References

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