Induction of tolerance to human factor VIII in mice

Hengjun Chao and Christopher E. Walsh

This paper reports loss of human factor VIII (hFVIII) inhibitory antibody in immunocompetent C57BL/6 mice. High-titer anti-hFVIII antibody developed in the mice within 7 to 14 days of intraportal administration of adeno-associated virus (AAV) carrying FVIII that coincided with a reduction in plasma hFVIII antigen. Bethesda titers (> 100 units) persisted relatively unchanged for 9 to 10 months. Unexpectedly, at 10 months after injection of the virus, hFVIII protein (up to 59 ng/mL) was detected in 3 mice at the same time as disappearance of hFVIII inhibitor. The level of hFVIII was similar to that found in immunodeficient mice receiving the same dose of recombinant AAV carrying hFVIII without hFVIII inhibitor. These results suggest that tolerance to hFVIII can be induced by sustained expression of hFVIII in a mouse model. Further elucidation of this observation may affect use of FVIII gene transfer in the treatment of inhibitor-positive patients with hemophilia A. (Blood. 2001;97:3311-3312)
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 adenoavirus, 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 tolerization 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 anergy. 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

Induction of tolerance to human factor VIII in mice

Hengjun Chao and Christopher E. Walsh