EDITORIAL

Applying Fc-fusion proteins to tolerize perinatally and block inhibitor formation

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A significant challenge in the treatment of hemophilia A (HA) is the immune response to the therapeutic protein, factor VIII (FVIII). This response is primarily driven by the lack of immune tolerance to FVIII. Despite recent advances in HA therapies, the development of FVIII inhibitors remains a major issue for a subset of patients. Notably, patients with inhibitors who are on emicizumab prophylaxis face a therapeutic crisis if they experience major bleeds following injury or upon invasive surgery. Although immune tolerance induction (ITI)[1] is the most effective method for eliminating FVIII inhibitors, its high cost, complexity, and lack of success in up to 40% of patients have stimulated research into alternative strategies for inducing immune tolerance. In recent decades, various approaches have thus been explored in preclinical HA models.[2] These include non-specific immunosuppression using, for instance, monoclonal antibodies to CD3, CD20 or CD40-ligand, the use of FVIII nanoparticles,[3] oral tolerance,[4] and gene or cell therapies,[5] such as chimeric antigen receptor (CAR) T regulatory (Treg) cell immunotherapy.[6] However, few of these strategies, if any, have successfully translated to clinical practice.

A novel approach was recently validated in a mouse model of severe HA, in which pregnant mice were treated with Fc-fused immunodominant A2 and C2 domains of FVIII.[7] These domains were transferred across the placenta to the fetuses' circulation. This transfer was mediated by the binding of the Fc fragment of the Fc-fused FVIII domains to the neonatal Fc receptor (FcRn), leading to the induction of FVIII-specific Tregs and a reduction in the anti-FVIII immune response when the offspring were challenged with FVIII at six weeks of age.[7] The transplacental delivery of the A2 and C2 domains of FVIII, which together represent 20% of the FVIII protein, resulted in a 10-fold reduction in the extent of the anti-FVIII humoral response. These findings support the hypothesis that Fc fusion of FVIII domains could promote immune tolerance through the downregulation of B-cell receptor (BCR) signaling via Fc receptors (FcR) on B cells.[8] It is well established that BCR crosslinking induces rapid tyrosine kinase phosphorylation and initial B-cell activation; however, simultaneous crosslinking of FcR subverts this activation process through phosphatase activity.[9]

In this issue of *Haematologica*, Reyes-Ruiz *et al*. investigated whether the materno-fetal transfer of the complete Fc-fused B domain-deleted FVIII could confer complete immune tolerance to therapeutic FVIII in the offspring.[10] In contrast to the A2Fc and C2Fc domains, recombinant FVIIIFc (rFVIIIFc) was poorly delivered across the placenta, with the resulting fetal plasma levels representing only 3% of normal FVIII levels. While this is presumably sufficient to correct coagulation in the case of minor bleeds,[11] these low levels were insufficient to induce immune tolerance. A possible explanation for the poor transplacental delivery of rFVIIIFc was provided by a recent study from the same group,[12] which showed that the positive electrostatic potential of the C1 and C2 domains of FVIII in rFVIIIFc promotes interactions with FcRn at neutral pH, preventing efficient recycling of the molecule. In support of this, a mutant rFVIIIFc, in which positively charged amino acids in the C1 and C2 domains were substituted with alanine, demonstrated a 2.5-fold extended half-life in HA mice compared to native rFVIIIFc, although this was in the absence of endogenous von Willebrand factor (VWF).[12] Interestingly, in the current study,[10] the FVIII^{C1C2}Fc variant showed an 8-fold increase in transplacental delivery compared to rFVIIIFc. Despite this improvement, the achieved levels in the offspring remained too low to influence the immune repertoire and induce full tolerance to exogenous FVIII. Together, these observations highlight the importance of FVIII's potential in enhancing its binding promiscuity. Of note, the binding promiscuity of monoclonal antibodies has been identified as a key factor in promoting the catabolism of the molecules, which ultimately excludes them from the clinical development pipeline.[13] Binding promiscuity has also been proposed, though not yet formally demonstrated, to play a

role in their immunogenicity. Future research should focus on further modifying the electrostatic properties of FVIII, particularly in its light chain, to improve its pharmacokinetic and immunogenic profiles.

In the present study,[10] Reyes-Ruiz *et al*. also administered Fc-fused FVIII immunodominant peptides to pregnant HA mice to enhance fetal exposure to FVIII epitopes. When the offspring were challenged with FVIII as adults, FVIII-specific antibody titers were significantly reduced, although not completely eliminated. It is important to note that translating these results from murine models to clinical applications should be done with caution (see, for example, Königs *et al*.[14]). These findings nonetheless provide a potential protocol for functionally tolerizing human hemophilia patients during fetal development by injecting Fc-fusion proteins into pregnant women, possibly during the third trimester when the fetal immune system is beginning to develop. This approach would be safer than directly injecting the fetus but may require large quantities of Fc-fusions to ensure sufficient transfer to the fetus.

Disclosures

No conflicts of interest to disclose.

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