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Optimizing signal strength and suppressive potential of FVIII specific CAR Tregs for tolerance induction in a murine model of hemophilia A

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Abstract

Objective: The development of inhibitory antibodies (inhibitors) against FVIII is a critical complication in the treatment of hemophilia A, as hemostasis can no longer be re-established by FVIII replacement therapy. Immune tolerance induction (ITI) for inhibitor eradication does not always have a successful treatment outcome and bypassing agents or alternatives like emicizumab and fitusiran are associated with their own risks or uncertainty about long-term outcomes. Inhibitor development has been shown to be dependent on CD4 + T cell help, which is in turn modulated by the regulatory T cell (Treg) subset. Cellular therapy with autologous Tregs is therefore a potential approach for tolerance induction to inhibitor development, either as stand-alone therapy or in combination with other established treatments. Engineering FVIII-specific specificity on Tregs can redirect Tregs to the antigen of interest without the risk of generalized immunosuppression. Here we achieved this objective by synthesizing a chimeric antigen receptor (CAR) molecule with specificity to human FVIII. Methods: We generated 2 nd and 3 rd generation FVIII-specific chimeric antigen receptors (FVIII CAR) with a single chain variable fragment (scFv) specific for the C2 domain of human FVIII fused to murine CD3 z , CD28 and 4-1BB primary and co-stimulatory signaling domains. This was packaged in a retroviral system (pMys-IRES-eGFP, pMys-IRES-mScarlet) and activated polyclonal Tregs were transduced to generate FVIII CAR Tregs. To tackle exhaustion and activation induced cell death (AICD) due to prolonged exposure of CAR T effector cells and CAR Tregs to FVIII, select point mutations were introduced into immunoreceptor tyrosine-based activation motifs in the primary CD3zsignaling domain. For cellular therapy, 1x10 6 GFP + FVIII CAR-Treg sorted cells were adoptively transferred into F8 e16 −/− hemophilia A mice, and recipients were challenged with weekly IV injections of BDD-FVIII for 8 weeks. Plasma was tested for inhibitor formation at 4 and 8 weeks using the Bethesda assay and FVIII IgG1 ELISA. Summary: FVIII CAR expressing murine Tregs were able to bind soluble FVIII as tested by flow cytometry. Antigen recognition via the scFv triggered specific transcription factor upregulation, FVIII CAR-Treg activation, cytokine secretion and cell proliferationindependent of the requirement for antigen presenting cells (APC) / MHC restriction. Adoptively transferred FVIII CAR Tregs were able to suppress inhibitor formation against frequent IV injections of BDD-FVIII, while control mice that did not receive cellular therapy developed high titer inhibitors. We are evaluating the suppressive ability of adoptively transferred FVIII CAR Tregs in mice with pre-established inhibitors, either alone or in combination with mouse CD20 antibody. Conclusions: We demonstrate that FVIII CAR Tregs represent an effective way to generate a large pool of antigen-specific cells, with no requirement for MHC restriction, which can effectively suppress an inhibitor response to FVIII in a preclinical model of hemophilia A.