Exploring red blood cells as a novel tolerogenic approach for factor VIII inhibitors employing immuno-dominant FVIII derived peptides presented on MHC class II

The main complication of hemophilia A treatment is the development of neutralizing antibodies (inhibitors) against factor VIII (FVIII). The eradication of anti-FVIII antibodies relies on immune tolerance induction (ITI).

Since ITI is efficient in only 60-80% of cases, within the EDUC8-consortium we are developing innovative methods to reduce the immunogenicity of biotherapeutics. Here we employed bioinformatics and proteomic-based peptide presentation assays to identify promiscuously presented FVIII peptides to use in pioneering immuno-tolerogenic approaches. To this end, we are exploring red blood cells (RBCs) as innovative antigen delivery system to induce tolerance.

A data-set of naturally processed FVIII peptides was generated by incubating human FVIII with immature monocytes-derived DCs from HLA-typed healthy donors. Specific attention was on the identification of HLA-DP4-FVIII derived peptides, since these alleles are highly prevalent in the Caucasian population.

We successfully developed a mass-spectrometry based protocol to study HLA-DR and HLA-DP antigen presentation utilizing specific monoclonal antibodies. Using this novel approach, approximately 2000 HLA-DR and 1000 HLA-DP presented peptides were identified. We detected over 40 HLA-DR and 14 HLA-DP4 presented FVIII peptides. The immunogenicity of the identified FVIII peptides was studied employing ELISPOT assays. Four HLA-DR presented FVIII peptide were fused to a cell-penetrating peptide and incubated with RBCs. Flow cytometry revealed their binding to RBCs in a dose-dependent manner. Binding of FVIII derived peptides to RBCs was confirmed by ImageStream analysis. FVIII peptide-treated RBCs were efficiently endocytosed by macrophages. Functional presentation of RBC bound peptides on MHC class II on the surface of macrophages was established employing immunopeptidomic approaches. We are currently exploring whether the presentation of immunodominant FVIII peptides on MHC class II results in tolerance induction.

Our data provide an inventory of promiscuously presented FVIII-derived peptides which will guide the development of novel tolerogenic approaches for FVIII inhibitors employing RBCs as carrier.

Bachelor or Master students, Duration: 6 months

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