Inhibitory Anti-Drug Antibody (ADA) responses interfere with Factor VIII replacement efficacy in 25-30% of Hemophilia A (HA) cases, greatly increasing patient morbidity and treatment costs. As an extension of previous work on tolerance-inducing peptides in iG (Tregpeps), we investigated whether there were peptides in other ubiquitous serum proteins that could have high homology to peptides found in Factor VIII. We hypothesized that tolerance to cross-conserved peptides in other prevalent proteins might explain why anti-FVIII antibodies fail to develop in some severely FVIII-deficient HA patients.

Using advanced computational modeling tools, we discovered in Factor VIII a potent recognition epitope (FVP4) with an immunological profile (HLA and TCR binding) that is homologous to a non-identical peptide in FVII. We postulated that treatment with a FVII peptide containing biologic may be induce tolerance to Factor VIII in patients who have anti-FVIII antibodies. In an ex vivo assay using human PBMCs, we found that the CD4 and CD8 T cell effector recall response was strongly inhibited by FVP4. However, other Factor V peptides with similar HLA-binding properties did not suppress the response. Using this assay, we are determining the evolution of markers on regulatory T cells, Antigen Presenting Cells, and effector T cells in order to identify specific parameters associated with cell populations and soluble factors mediating immune suppression in response to FVII.

Inhibition of CD4 T cells proliferation by FVP4

FVII peptides containing epitope-specific response peptides are inhibited by FVP4 peptides in vitro in the example of human PBMCs. FVII peptides responding to T cell stimulation, most showing inhibition of proliferation (CD3/CD28), is strongly inhibited by increasing concentrations of FVP4 peptides (25% of control).

Differential expression analysis was used to identify Factor VIII-related peptides for their regulation according to the following criteria: < 0.01 logFC for expression in FVP4 and > 0.01 logFC for expression in control. Multiple hypothesis test correction was performed by adjusting the p-value with Bonferroni correction.

FVII peptides contain epitope-specific recognition peptides that are inhibited by FVP4 peptides in vitro. FVII peptide (25%) of control.

Selection of tolerogenic FVII peptides

Table 1: F V peptides bind to HLA-DR molecules

<table>
<thead>
<tr>
<th>F V peptide</th>
<th>HLA-DR molecule</th>
<th>EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVII 1</td>
<td>DR1, DR2</td>
<td>5.78</td>
</tr>
<tr>
<td>FVII 2</td>
<td>DR1, DR2</td>
<td>5.78</td>
</tr>
<tr>
<td>FVII 3</td>
<td>DR1, DR2</td>
<td>5.78</td>
</tr>
<tr>
<td>FVII 4</td>
<td>DR1, DR2</td>
<td>5.78</td>
</tr>
</tbody>
</table>

Figure 1: Inhibition of activated T cell proliferation by FVP4. T cell proliferation was assessed by CFSE dilution in a mixed T cell expansion assay. T cells were stimulated with anti-CD3/CD28-coated beads and FVP4 peptides (25% of control)

FVII peptides exhibit inhibitory activity against T cell proliferation.

FVII peptides (25%) of control.

Conclusions

The purpose of the present work was to test the hypothesis that there might be Factor V regulatory peptides able to down-modulate the ADA response to FVIII in HA patients.

Using Epivax’s proprietary tools, FV was selected for regulatory peptide candidates. A human Factor V-derived peptide (FVP4) was found to consistently inhibit ex vivo CD4 and CD8 T cell effector responses in donors covering the nine major HLA-DRB1 supertypes.

Preliminary data on the mechanism of action points to the involvement of cytokine Tregs in the regulatory process.

These data support our hypothesis that HA patients with normal levels of Factor V may have Tregs with specificity for FV4 which could be used to induce tolerance to Factor VIII.

Peptide FV4 might be useful in the development of biotherapeutics able to stimulate regulatory T cells that will cross-inhibit an ADA response to FVIII.

References