Assessing immunogenicity of immunoglobulin variable regions

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Introduction

Immunoglobulin variable regions of therapeutic monoclonal antibodies are inherently immunogenic, and development of anti-drug antibodies is dependent on T-B cell collaboration involving B cell presentation of variable region fragments to specific T cells. We have suggested that a similar mechanism may operate in multiple sclerosis, where T cells are triggered by B cells presenting immunogenic variable region fragments from their own B cell receptor. This study demonstrates how *in silico* models can identify potentially immunogenic regions within monoclonal antibody variable regions and self-variable regions.

Materials and methods

In silico predicted HLA-DR affinity coupled with predicted endosomal processing by cysteine cathepsins S and B were used to analyze immunoglobulin variable regions in 16'000 curated human immunoglobulin heavy variable (IGHV) sequences. Nano liquid mass spectrometry was used to verify predicted cleavage sites after *in vitro* experiments where therapeutic mAbs alemtuzumab, natalizumab, rituximab and ocrelizumab were exposed to cathepsin S, L and B in simulated endolysosomal conditions.

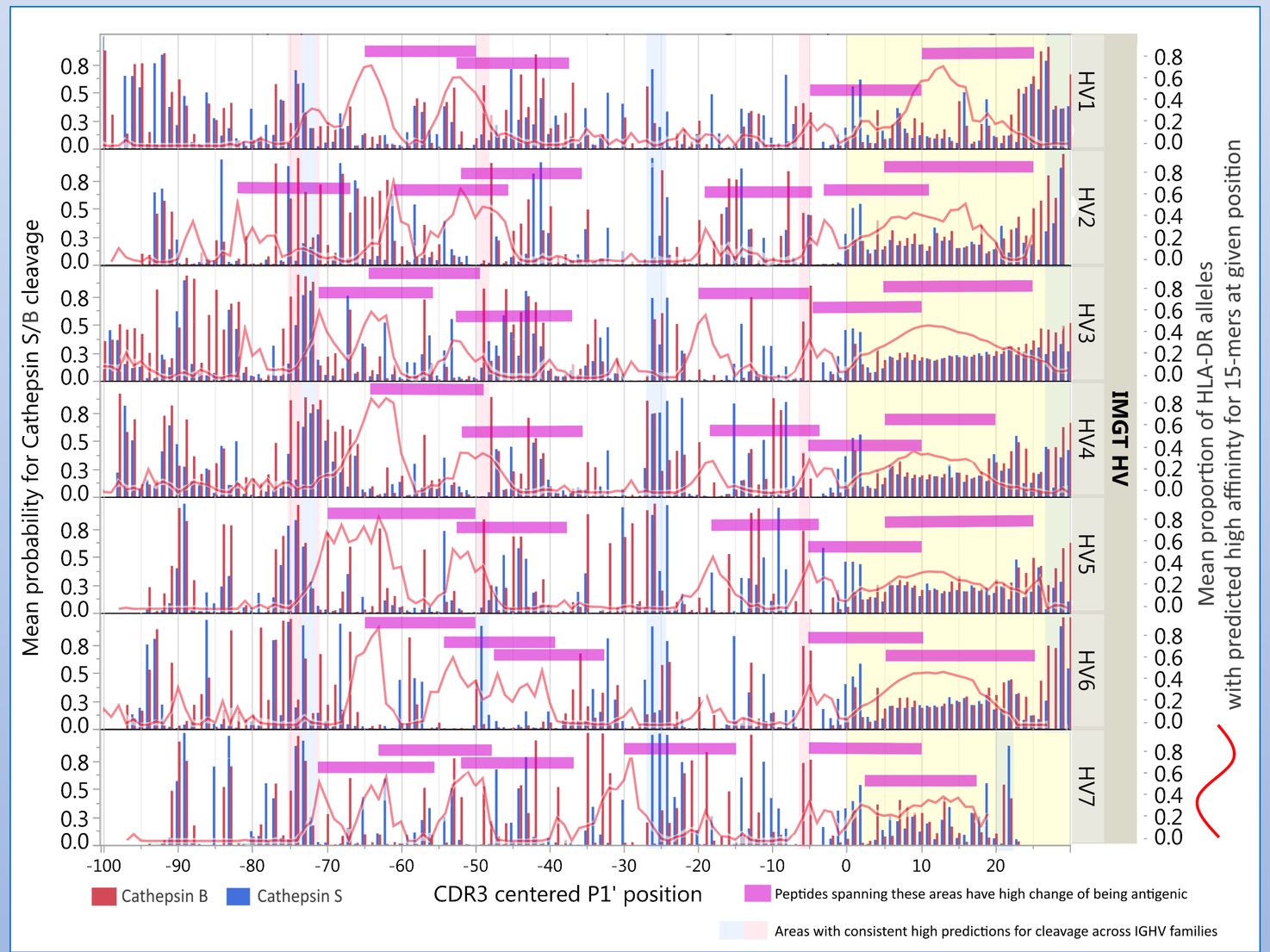

Figure 1. Mean predicted probability for cleavage by cathepsins S an B (bars, left y-axis), overlayed with the mean proportion of HLA-DR alleles with predicted high affinity binders (red line, right y-axis) at the given CDR3-relative position (x-axis). Areas with conserved high prediction for cleavage are indicated vertically. Areas with conserved high affinity peptides are indicated horizontally.

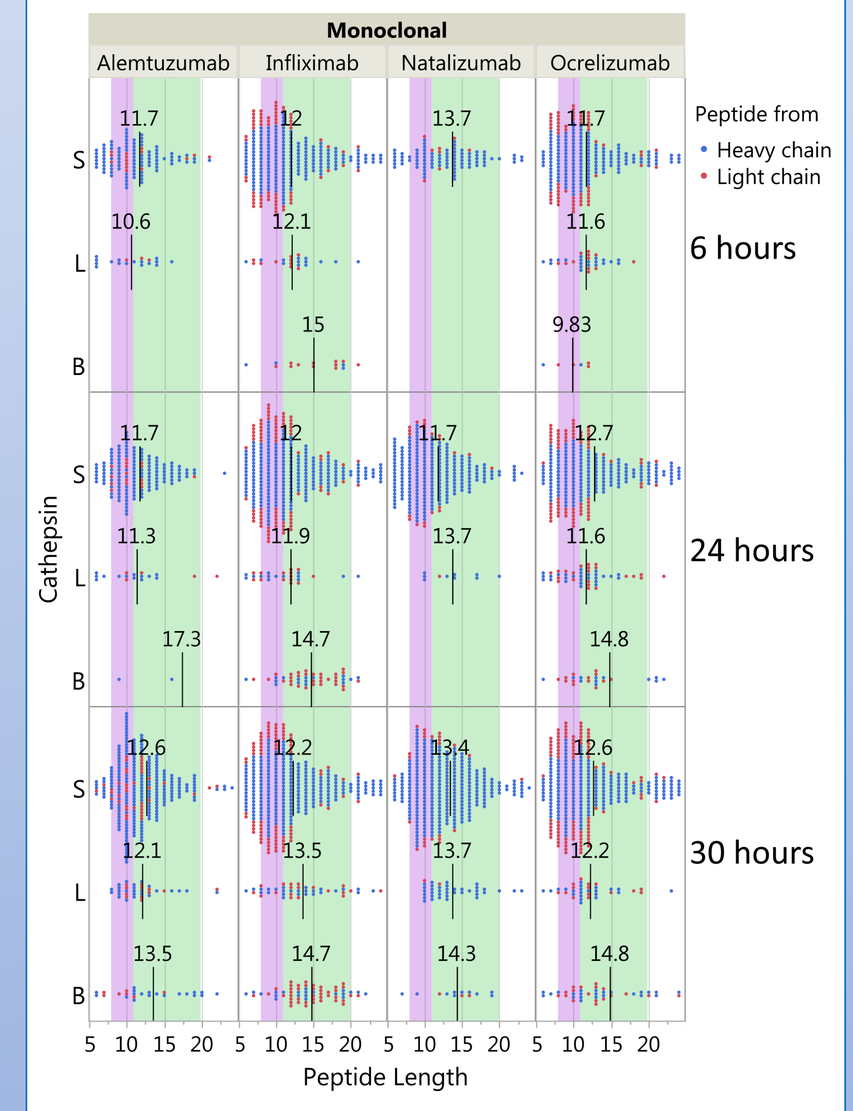

Figure 2. mAb peptides (dots) plotted by their length (x-axis) after digestion with cathepsin S, L or B at pH 6. Purple and green indicates peptides sized for HLA class I and II, respectively.

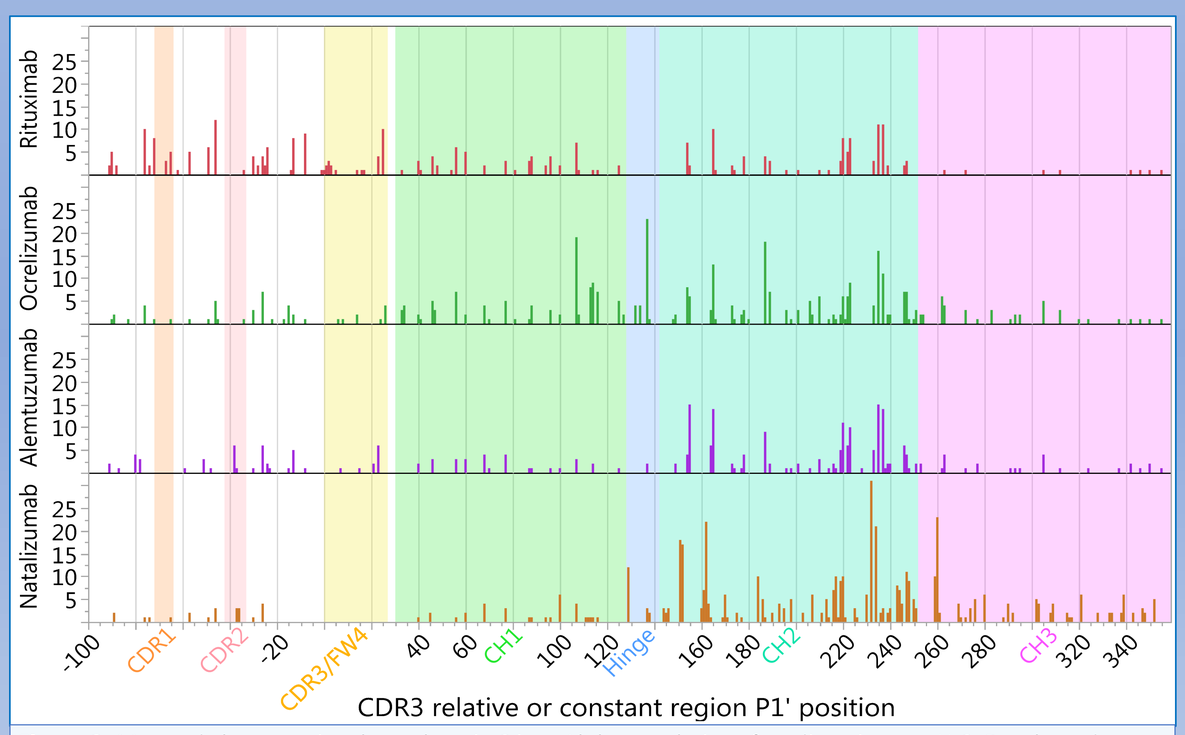

Figure 3. Mapped cleavage sites by cathepsin S in mAb heavy chains after digestion at pH 6. Purple and green indicates peptides sized for HLA class I and II respectively. Note a conserved cleavage pattern in constant region and differing pattern in variable regions. Natalizumab is IgG4, with a slightly different constant region.

Results

For each IGHV family we identified up to 4 areas of combined predicted high affinity for HLA class II molecules and probability for cathepsins cleavage among the 16'000 curated IGHV sequences, compatible with presentation for T cells. We further confirmed *in vitro* that cathepsins S, L and B cleavage patterns of alemtuzumab, natalizumab, rituximab and ocrelizumab are determined by IGHV differences. Additionally, we identified several cleavage-derived peptides with potential of being immunogenic epitopes sized for HLA class II presentation.

Conclusions

Here we predicted and confirmed that cathepsins S and B, present in professional antigen presenting cells including B cells, degrade IgG into peptides that fit in HLA class I and II. This knowledge may improve our understanding immune responses against self-IgG and therapeutic mAbs in MS.

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Høglund RA, Torsetnes SB, Lossius A, Bogen B, Homan EJ, Bremel RD and Holmøy T. **Human Cysteine Cathepsins Degrade Immunoglobulin G In Vitro in a Predictable Manner.** *Int. J. Mol. Sci.* 2019, https://doi.org/10.3390/ijms20194843

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