# 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; involving self-immunoglobulin heavy chain variable region (IGHV), may cause autoimmunity observed in multiple sclerosis, where T cells are triggered by B cells by currently unknown antigens. This study demonstrates how *in silico* models can identify potentially immunogenic regions within both monoclonal antibody variable regions as well as self-variable regions.

**Methods***: In silico* predicted HLA class II affinity coupled with predicted endosomal processing by cysteine cathepsins S and B were used to analyze immunoglobulin variable regions of alemtuzumab, natalizumab, rituximab and ocrelizumab used in therapy of multiple sclerosis, as well as in 16'000 curated human IGVH. Nano liquid mass spectrometry was used to verify predicted cleavage sites after *in vitro* experiments where therapeutic mAbs were exposed to cathepsin S, L and B in simulated endolysosomal conditions.

**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 monoclonal antibodies is determined by IGHV differences. Additionally, we identified several cleavage derived peptides with potential of being immunogenic IGHV epitopes sized for HLA class II presentation.

**Conclusion**:
Here we demonstrated that cathepsins S, L and B, present in professional antigen presenting cells, degrade IgGs into peptides that fit in HLA class II under conditions resembling the endolysosomal compartments. This knowledge may be essential for understanding immune responses against both endogenous Igs and BCRs as well as therapeutic mAbs.

Disclosure: RB and EJH holds equity in ioGenetics LLC, the company responsible for designing the bioinformatics models used in this project. All other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.