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Targeted Mutagenesis of a Therapeutic Human Monoclonal IgG1 Antibody Prevents Gelation at High Concentrations

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Et al.

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A common challenge encountered during development of high concentration monoclonal antibody formulations is preventing self-association. Depending on the antibody and its formulation, self-association can be seen as aggregation, precipitation, opalescence or phase separation. Here we report on an unusual manifestation of self-association, formation of a semi-solid gel or "gelation". Therapeutic monoclonal antibody C4 was isolated from human B cells based on its strong potency in neutralizing bacterial toxin in animal models. The purified antibody possessed the unusual property of forming a firm, opaque white gel when it was formulated at concentrations >40 mg/mL and the temperature was <6^{o}C. Gel formation was reversible and was affected by salt concentration or pH, suggesting a charge interaction between IgG monomers. However, formulation optimization could not completely prevent gelation at high concentrations so a protein engineering approach was sought to resolve the problem.

A comparison of the heavy and light chain amino acid sequences to consensus germline sequences revealed 16 amino acid sequence differences in the framework regions that could be involved with gelation. Restoring the C4 framework sequence to consensus germline residues by targeted mutagenesis resulted in no gel formation at 50 mg/ml at temperatures as low as 0^{o}C. Additional genetic analysis was used to identify the key residue(s) involved in the gelation. A single substitution in the native antibody, replacing heavy chain glutamate 23 with lysine, was found sufficient to prevent gelation, while a double mutation, replacing heavy chain serine 85 and threonine 87 with arginine, increased the temperature at which gel formation initiated. These results indicate that the temperature dependence of gelation may be related to conformational changes near the charged residues or the regions interact with. Our work provided a molecular strategy that can be applied to improve the solubility of other therapeutic antibodies.