Unexpected Effect of Somatic Mutations on the Affinity of an Antibody by Altering Its Dynamics

Joachim D. Stump, Heinrich Sticht

Bioinformatik, Institut für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg

Human cytomegalovirus (HCMV) causes life-threatening infections in immunocompromised patients such as newborns, transplant recipients and HIV-infected patients. Spindler et al. recently characterized several neutralizing antibodies that bind Domain-II (DOM-II) of Glycoprotein B, which is an essential protein for the fusion machinery of HCMV. [1]

A recent crystal structure of the strongest binding antibody (SM5-1) in complex with DOM-II revealed that numerous residues emerging during affinity maturation do not directly interact with DOM-II. In particular, some polar amino acids in the CDR-H1 and CDR-H3 only form intramolecular interactions, thereby possibly playing a role in the stabilization of the antibody scaffold itself.

To investigate these interactions and the impact of somatic mutations on the dynamics of SM5-1, molecular dynamics (MD) simulations were performed for SM5-1 and a 6-fold mutant, in which 6 polar residues in CDR-H1 and CDR-H3 were exchanged to match less matured antibodies. This was done for the bound and unbound conformation of SM5-1 resulting in a total of 4 simulations. All MD simulations were performed with AMBER 11, the parm99SB force field, and in an octahedral box of explicit solvent for 100 ns.

Comparison of the dynamics of SM5-1 with the 6-fold mutant revealed that the mutations mainly enhance the flexibility of the long CDR-H3 loop both in the bound and unbound conformation. This higher flexibility in the 6-fold mutant can be attributed to the loss of important stabilizing interactions of the anchor region of CDR-H3.

Our studies show that somatic mutations within CDRs do not necessarily optimize only the direct antibody binding interface. In addition, such mutations can also have an indirect effect on the binding competent conformation of the antibody, thus increasing its affinity. Therefore, our findings have implications for other areas of computational research such as docking: It underscores the difficulty of predicting antibody-antigen structures since these approaches frequently consider residues, which have emerged during affinity maturation, as part of the interface. Consequently, in a case like SM5-1 and DOM-II they will fail to predict the right solution.

[1] N. Spindler, et al., *J Virol.*, **2013**, *87*, 8927-39.