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# Structural and biophysical characterization of antigen : antibody binding interfaces and their potential biological implications in GM-CSF and interleukin-1β signaling

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Investigation of the molecular recognition between the paratope of the antibody and the epitope of its antigen exhibit profound significance in developing novel, targeted therapeutic molecules for a wide range of human diseases. The most profound significance of antibodies lies in their specific mechanism of action to their antigens and its corresponding biological background. In this thesis structural, biophysical and computational approaches were combined to present antigenic determinants of the respective antibodies and the molecular basis for their potential therapeutic relevance in two different projects.

**Structure prediction of GM-CSF in complex with a disease-associated autoantibody**

Many autoimmune disorders or diseases such as Graves´ disease, insulin-dependent diabetes mellitus (IDDM), and rheumatoid arthritis (RA) are associated with autoantibodies which target and damage certain organs or tissues. To date, the causes of autoantibody production are not well understood and the physiological role of their presence remains unclear.

Polyclonal autoantibodies against human granulocyte macrophage colony- stimulating factor (GM-CSF) are a hallmark of pulmonary alveolar proteinosis (PAP) affection. Idiopathic PAP is associated with high levels of autoantibodies against the cytokine GM-CSF, which regulates survival, differentiation and proliferation of macrophages. Interference with GM-CSF signaling by neutralizing autoantibodies which block GM-CSF bioactivity, thus inhibiting alveolar macrophage maturation and consequently leading to accumulation of lipoproteinaceous material within alveoli results in pulmonary surfactant accumulation, impaired gas exchange, and respiratory insufficiency.

MB007 is a high-affinity anti-human GM-CSF autoantibody (MB007) isolated from a patient suffering from PAP which shows only modest neutralization of GM-CSF bioactivity. This work describes the first crystal structure of a cytokine directed human IgG1λ disease-associated autoantibody binding fragment (Fab) at 1.9 Å (1 Å = 0.1 nm) resolution. To study binding interactions with its related antigen, human GM-CSF and 22 variants thereof containing either single or multiple amino acid substitutions were prepared. These GM-CSF constructs were characterized by a novel biophysical method termed Microscale Thermophoresis (MST), and measured binding affinities were validated by surface plasmon resonance (SPR) experiments. Despite substantial efforts co-crystallization of MB007 Fab in complex with GM-CSF resulting in acceptable diffraction properties was unsuccessful. However, a sequentially discontinuous three-dimensional epitope could be identified by nuclear magnetic resonance (NMR) using amide backbone chemical shift perturbation analysis (NMR epitope mapping).
Based on NMR-derived epitope information and measured binding affinities of GM-CSF variants the antigen was pre-orientated relative to the MB007 Fab crystal structure for a computational *ab initio* protein:protein complex structure prediction. The concerted application of experimental data combined with computational methods accounted in a reliable GM-CSF:MB007 complex model consistent with obtained biophysical and *in vitro* cell-based assay data. The simulated binary complex represents the first cytokine:autoantibody complex structure reported so far (Protein Data Bank; dated September

2012). Previously published autoantibody complexes are either associated with receptors, DNA, or peptides.

Finally, this study provides a structural basis for understanding the mode-of-action of the MB007 autoantibody. Superimposition of the modeled GM-CSF:MB007 binary complex structure with the human GM-CSF/GM-CSF ternary receptor complex reveals only little overlap between receptor and Fab when bound to GM-CSF. Based on this structural information the results help to rationalize the observed modest neutralization of the MB007 autoantibody that reduces GM-CSF activity by exhibiting high binding affinity. It might serve as a model case describing a generally relevant regulatory role of autoantibodies in cytokine homeostasis.

**Structural analysis of two anti-human IL-1β antibody interactions to IL-1β**

Interleukin-1β (IL-1β) is a key orchestrator in inflammatory and several immune responses, and is in the focus of pharmaceutical research since decades. IL-1β exerts its effects through interleukin-1 receptor type I (IL-1RI) and interleukin-1 receptor accessory protein (IL-1RAcP) which together form a heterotrimeric signaling-competent complex.

Canakinumab and gevokizumab are highly specific IL-1β monoclonal antibodies. Canakinumab is known to neutralize IL-1β by competing for binding to IL-1R and therefore blocking signaling by the antigen:antibody complex. Gevokizumab is claimed to be a regulatory therapeutic antibody that modulates IL-1β bioactivity by reducing the affinity for its IL-1RI:IL-1RAcP signaling complex. How IL-1β signaling is affected by both canakinumab and gevokizumab was yet not experimentally determined.

In this study the crystal structures of canakinumab and gevokizumab Fab as well as of their binary complexes with IL-1β were analyzed at the atomic level. Further, the epitopes on IL-1β employed by the antibodies by NMR epitope mapping studies were characterized. The direct comparison of NMR and X-ray experimental data was used to derive a set of rules that show how to reliably perform NMR epitope mapping based on chemical shift perturbation data.
The antigen:Fab co-structures confirm the previously identified key contact residues and provide insight into the mechanisms leading to their distinct modulation of IL-1β signaling.
A significant steric overlap of the binding interfaces of IL-1R, and canakinumab on IL-1β causes competitive inhibition of the association of IL-1β and its receptor. In contrast, gevokizumab occupies an allosteric site on IL-1β. Complex formation results in a minor reduction of binding affinity. This suggests two different mechanisms of IL-1β pathway attenuation.