Immunogenicity of Therapeutic Antibodies. Immunological Mechanisms & Clinical Consequences
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Summary

Our immune system has evolved to protect us from the continuous exposure to pathogens. Antibodies play an important part in this system, and they ensure that pathogens are rapidly opsonized, lysed and cleared. The two Fab domains of an antibody bind with high specificity to their target, whereas the Fc domain exerts effector functions such as receptor binding and complement activation. Because of these properties, polyclonal antibody products have been used for over a century to treat all sorts of diseases. Over the last thirty years, monoclonal antibody therapy has greatly developed, and many different therapeutic antibody products are now approved to treat a whole spectrum of diseases.

One of the main disadvantages of antibody therapy is its ability to provoke an unwanted immune response in patients, leading to the formation of anti-drug antibodies (ADA). The immunogenic potential differs between antibody therapeutics, and is furthermore influenced by factors such as disease characteristics and genetic variations. Studies have shown a clear inverse correlation between ADA formation and free drug levels, and ADA may thus cause a reduced clinical response or even non-response. Furthermore, ADA formation increases the chance of adverse events for some therapeutics, while this effect is absent for other therapeutics. The immunological mechanisms involved in reducing the free drug concentration and inducing adverse events are yet to be determined. The focus of this thesis is to elucidate these mechanisms to provide a better understanding of the clinical consequences of ADA. The results might be used to optimize monoclonal antibody treatment and could furthermore prove valuable for the development of new therapeutics.

The majority of the research described in this thesis is performed with anti-TNF therapeutics, of which there are five in clinical use (infliximab, adalimumab, golimumab, certolizumab and etanercept). These drugs are used to treat inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Unfortunately, ADA are detectable in the majority of the infliximab and adalimumab treated patients, although often in low concentrations, and also golimumab and certolizumab are found to be immunogenic to some degree.

To quantify the ADA response, several assays have been developed, each with different characteristics. Since all assays include antibodies as reagents for capture and/or detection, and the ADA and the drug are also antibodies, several forms of interference may occur. This could lead to false-positive or false-negative results, possibly negatively affecting patient treatment or drug development. Chapter 2 describes the different assays used for ADA measurement as well as the factors that could cause interference.

Anti-TNF therapeutics reduce inflammation by binding TNF, thereby inhibiting its binding to TNF-receptors. In Chapter 3, TNF itself and the interaction of TNF with TNF-inhibitors is investigated. Biologically active TNF is an unstable trimeric protein that, under physiological conditions, rapidly dissociates into biologically inactive monomeric subunits. We demonstrate that on high concentrations TNF dissociates as well, but monomers can...
reassociate into the active trimeric form, a process called monomer exchange. Results furthermore show that adalimumab, infliximab and etanercept inhibit the monomer exchange and thus stabilize the trimeric form of TNF. In contrast, certolizumab and golimumab did not completely inhibit this process, but did slow the exchange down.

As described above, ADA formation is associated with a reduced free drug level. Although many have speculated that this could either occur via neutralization or through increased clearance, little solid evidence is available on this subject. In Chapter 4, the neutralizing capacity of ADA towards all anti-TNF therapeutic antibodies is investigated. We demonstrate that in all patients, more than 97% of all ADA towards adalimumab, golimumab and certolizumab are neutralizing, and that more than 90% of ADA towards the chimeric infliximab are neutralizing. This suggests that the vast majority of ADA compete with TNF for the TNF binding site, and that neutralization thus plays a significant role in reducing the free drug levels.

The findings described in Chapter 4 may be explained in two ways, I) the TNF binding site is very immunogenic, or II) the idiotype is the most immunogenic part of an antibody, regardless of its specificity. We therefore further examined the ADA response towards a different antibody, the anti-α4 integrin natalizumab, used to treat multiple sclerosis. As described in Chapter 5, the ADA response to natalizumab was also highly restricted to the antigen binding site. Together with the results from Chapter 4, we conclude that ADA predominantly target the idiotype of therapeutic antibodies.

Patients with ADA towards infliximab have an increased chance to experience adverse events called infusion reactions. Due to the allergic-like symptoms of these reactions, IgE-ADA is thought to play a role in these events, although contradicting results on the presence of IgE-ADA are published. This controversy is at least in part due to the lack of a robust assay to measure IgE-ADA and a positive control for assay validation. The study in Chapter 6 describes a novel assay including a recombinant human IgE anti-infliximab antibody as positive control. Using this assay, we established that in the majority of infusion reaction positive patients no IgE-ADA is detected. Only few patients were found positive for IgE-ADA, generally in low levels, whereas all these patients were also highly IgG-ADA positive. The results from this study therefore indicate that infusion reactions are not associated with IgE-ADA.

It was already established that IgG-ADA were associated with infusion reactions in infliximab and natalizumab treated patients. The mechanisms behind these clinical manifestations are unknown, but complex formation between ADA and drug has been proposed to play a role. In Chapter 7, the factors influencing complex formation between infliximab and ADA were investigated, as well as the immune activating potential of these complexes. Concentration and ratio were found to affect immune complex size independently. Immune activation was absent for dimers, tetramers and hexamers, likely due to their conformation. However, very large complexes did have some immune activating potential. These results indicate that
generally, immune complex formation between drug and ADA is not harmful. Only in rare occasions, when drug and ADA are in equal and high concentrations, large immune complexes may be formed and activation of the immune system may occur, possibly leading to the adverse events observed in the clinic.

The combined results described in this thesis provide new information on the mechanisms involved in the (unwanted) clinical effects as observed in ADA positive patients. These results may improve the clinical management of these patients, and can furthermore be used for the development of new antibody therapeutics.