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Dynamics of TNF during TNF inhibitor treatment

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General introduction



General introduction

The introduction of therapeutic monoclonal antibodies (biologics) has been a major step in the treatment of inflammatory autoimmune diseases, such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriatic arthritis (PsA) and ankylosing spondylitis (AS). One of the first biologics inhibited the pro-inflammatory cytokine tumor necrosis factor (TNF) and significantly improved disease status.¹⁻⁴ However, response rates to these TNF inhibitors (TNFi) vary between patients, with approximately one third of patients not responding. At present we are not able to predict the response to TNFi treatment, which forces clinicians to make treatment decisions without knowledge of whether a patient is likely to benefit or not. Despite this variation in clinical outcome, treatment regimens are generally fixed and treatment decisions in individual patients are mostly at random. Studying the factors that underlie (un)successful treatment with TNFi might result in a patient-tailored therapy. To date most studies focus on TNFi. Therapeutic drug monitoring (TDM) is a rapidly growing field, and applied to investigate serum drug concentrations during TNF inhibitor treatment. TDM, in relation with clinical response might help to identify the amount of TNFi that is needed for maintaining clinical response. In contrast, surprisingly little is known about the target, TNF, during TNFi treatment. The aim of this thesis is to provide insights in the dynamics of TNF, in relation with clinical response. This can potentially improve efficacy of and contribute to personalization of TNFi treatment.

Inflammatory autoimmune diseases

The immune system is a complex and potent defense system, consisting of a network of immune cells, proteins (including antibodies), and signaling molecules (like chemokines and cytokines). Together, they protect our body from harmful pathogens. One of the first line defense mechanisms is acute inflammation, a fast and non-specific response, which comprises increased infiltration of activated immune cells from the circulation into the affected tissue. Classical characteristics of inflammation are pain, redness, heat, swelling, and immobility. Inflammatory mediators are very potent, and can lead to tissue destruction at the inflammatory site. Chronic inflammation is associated with various diseases.

Overall, a dedicated balance of the immune system is of importance: an efficient reaction should be provided against invading pathogens (non-self), while leaving our own tissues (self) intact. Immune cells discriminate between self and non-self via pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) on invading pathogens. Immune cells that recognize self are removed, a process called clonal deletion. This negative selection of self-reactive immune cells is important to prevent

autoimmune disease. Characteristics of the autoimmune diseases RA, IBD, PsA and AS are briefly described below.

Rheumatoid arthritis

RA is a chronic inflammatory autoimmune disease with a global prevalence of around 1%.⁵ The disease is characterized by inflammation of the joints, with symmetric joint distribution at onset. Without adequate treatment cartilage and bone erosion result in irreversible bone destruction.^{6,7} In many patients other organ systems are affected as well, and RA is increasingly recognized as a syndrome that includes amongst others cardiovascular morbidity, psychological impairment and increased risk of osteoporosis.⁸

Inflammatory bowel disease

Chronic inflammation of the gastrointestinal tract in IBD can be divided in two types: ulcerative colitis (UC) and Crohn's disease (CD). There are overlapping symptoms, including (bloody) diarrhea, but key characteristics distinguishes between the two diseases. UC is restricted to the colon (large intestine) and only affects the top layers of the intestine. In contrast, CD can affect the entire gastrointestinal tract, from mouth to anus, and causes transmural inflammation. Furthermore, while CD typically shows discontinuous inflammation of the intestine, patients with UC have continuous inflammation of the colon.⁹ The prevalence of IBD varies globally. In Europe the prevalence of UC ranges between 2.4 – 294 cases per 100,000 persons and for CD between 1.5 – 213 cases per 100,000 persons.¹⁰

Ankylosing spondylitis

Long-term inflammation of the joints in the axial skeleton causes stiffness of the spine and back pain in AS. AS typically develops during adolescence. However, there is a diagnostic delay up to ten years, due to the progressive nature of AS.¹¹ The prevalence of AS is between 0.3 – 0.5% in Europe.¹²

Psoriatic arthritis

PsA is a chronic, progressive inflammatory autoimmune disease involving arthritis in patients with psoriasis, an inflammatory skin disease. In most patients psoriasis precedes the onset of arthritis on average with ten years.¹³ The global average prevalence of PsA is 133 per 100,000 and diagnosed in up to 30% of patients with psoriasis.¹⁴

Tumor necrosis factor

In the 1980s Marc Feldman and Ravinder Maini were one of the first who identified the pro-inflammatory cytokine TNF (also called TNF-alpha) as the main driver of inflammation

in above described chronic inflammatory conditions.¹⁵⁻¹⁷ TNF is a pleiotropic cytokine that belongs to the TNF superfamily. The TNF superfamily consists of 19 ligands, which signal through 29 receptors. TNF is initially produced as a transmembrane protein, but proteolytic cleavage by the metalloprotease TNF alpha converting enzyme (TACE, also known as ADAM17) results in the release of soluble TNF. Three monomeric TNF units associate via noncovalent interactions to form a homo-trimeric cytokine. Both membrane-bound and soluble TNF are biologically active and signal via two distinct transmembrane receptors: TNF receptor type 1 (TNFR1) and TNF receptor type 2 (TNFR2). A complex of three TNFRs is formed upon trimeric TNF binding, resulting in intracellular signaling.^{18,19} Of note, it is thought that transmembrane TNF not only functions as a ligand, but can also function as a receptor, mediating signals into the transmembrane TNF expressing cells. This process is referred to as reverse signaling.

TNFR1 is ubiquitously expressed in most tissues and can be activated by both membrane-bound and soluble trimeric TNF. In contrast, TNFR2 expression is restricted to specific cell types and is primarily activated by membrane-bound trimeric TNF.²⁰ The two TNFRs share homology in their extracellular domain, while their intracellular domain is different. Consequently, different intracellular signaling pathways are activated, resulting in distinct biologic effects (see below). Overall, TNF has both pro-inflammatory and immune regulatory effector functions, and plays a key role in mediating the host response to infection.

TNF is primarily produced by activated macrophages, but can be produced by various cell types, including natural killer (NK) cells, T- and B- cells, endothelial cells, mast cells, fibroblasts, cardiac muscle cells and neurons. Upon infection, lipopolysaccharide (LPS), other bacterial products and pro-inflammatory cytokines induce the release of large amounts of TNF in infected tissues. TNF activates vascular endothelium, leading to increased expression of adhesion molecules and subsequent recruitment and activation of immune cells to the site of infection. Furthermore, TNF increases blood flow and vascular permeability resulting in the classical inflammatory features of 'rubor', 'calor' and 'tumor' of the infected area.²¹ The importance of TNF in mediating the host response is confirmed in studies using TNF or TNFR deficient mice. These mice were highly susceptible to challenges with infectious agents, like *L. monocytogenes* and *M. tuberculosis*, but were resistant to lethal doses of LPS.^{22,23}

Dysregulated expression of TNF can be harmful: long lasting and systemic induction of TNF causes tissue destruction and might even lead to shock, respectively. This highlights the importance of tight regulation of this potent cytokine. Dysregulated expression

or function of TNF has indeed been linked to a wide variety of pathophysiologies, including cardiovascular diseases, neurologic diseases, cancer and chronic autoimmune diseases.^{15,18,24} In this thesis we focus on TNF in inflammatory autoimmune diseases; of which some have been described above.

TNF inhibitors

In a novel *in vitro* cell culture system, which consisted of cells extracted from diseased RA joint tissue, spontaneous production of cytokines, including interleukin-1 (IL-1), IL-6, TNF and granulocyte-macrophage colony stimulating factor (GM-CSF) was observed. Surprisingly, it has been demonstrated that neutralization of TNF with anti-TNF antibodies in this cell culture system not only inhibited TNF, but also the simultaneous synthesis of other pro-inflammatory cytokines.^{16,17} This was the start of TNFi treatment. Infliximab was the first TNFi that was approved for clinical use in 1998, followed by etanercept (1998), adalimumab (2002), golimumab (2008) and certolizumab pegol (2009). These five TNFi are structurally different: etanercept is a fusion protein consisting of two TNFRII linked to an Fc-tail, infliximab is a mouse-human chimeric antibody, adalimumab and golimumab are fully human antibodies and certolizumab pegol is a pegylated Fab fragment (Figure 1).^{25,26} Although different tissues and structures are affected in abovementioned autoimmune diseases, the treatment of all diseases has significantly improved since the introduction of TNFi treatment.¹⁻⁴

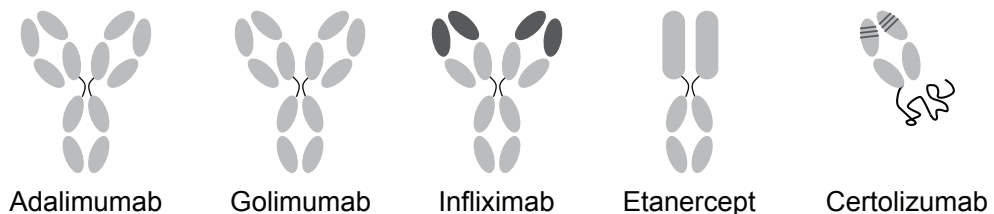


Figure 1. Schematic overview of the different TNF inhibitors. Adalimumab and golimumab are fully human IgG1 antibodies and infliximab is a mouse-human chimeric antibody. Etanercept is a TNF receptor-Fc fusion protein and certolizumab pegol a pegylated Fab fragment. Gray represents human origin, black mouse origin.

TNF inhibitors for the treatment of inflammatory autoimmune diseases

All five TNFi bind TNF and prevent the simultaneous interaction with TNFRs, resulting in neutralization of TNF and suppression of inflammation.²⁷ The introduction of TNFi revolutionarily improved the treatment of RA, and a few years later TNFi were also approved for PsA, AS and IBD.^{1,2,4,28-30} Target neutralization is supposed to be the critical and common mechanism of action of TNFi. However, given the complexity of TNF signaling in a complex immune environment, many studies propose additional effector functions.

This is supported by the fact that not all TNFi are similarly effective in the treatment of the different autoimmune diseases. While the five TNFi are equally effective in reducing RA symptoms,³ they do not work equally well in IBD: certolizumab shows varying clinical results³¹⁻³³ and for etanercept, studies failed to demonstrate clinical and endoscopic remission.³⁴ The structural differences of the five TNFi might largely contribute to differences in secondary mechanisms of action.^{35,36}

Secondary mechanisms of action of TNFi

TNFi can bind transmembrane TNF, which via different mechanisms can result in inflammatory cell depletion.³⁷ One of these mechanisms is antibody-dependent cell-mediated cytotoxicity (ADCC). The full length antibodies adalimumab, golimumab and infliximab, but also the fusion protein etanercept can bind via their Fc tail to Fc-gamma receptors (FcγRs). Certolizumab is the only TNFi lacking an Fc tail. Binding of the TNFi to transmembrane TNF results in FcγR cross-linking on effector cells. Cross-linking of FcγRs activates effector cells, primarily NK cells, which triggers the release of cytotoxic compounds resulting in lysis of the TNF expressing target cell. Numerous studies investigated induction of ADCC by TNFi. As expected, certolizumab did not have ADCC activity, but the results for the remaining TNFi vary, depending on the type of TNF expressing target cell and type of effector cell that has been studied.³⁸⁻⁴⁰

Next to ADCC can multimerization of antibodies on a cell surface (i.e. TNFi binding to transmembrane TNF) induce activation of the classical pathway of the complement system.⁴¹ The complement system consists of numerous proteins which through proteolytic cleavage form an enzymatic cascade. Activation of this enzymatic cascade will ultimately lead to the formation of a membrane attack complex, which results in cell lysis, a mechanism known as complement-dependent cytotoxicity (CDC). Again, depending on the type of target cell that has been used, the TNFi show varying CDC activity.^{38,42,43}

Transmembrane TNF not only functions as a ligand, but it can also function as a receptor. Binding of TNFi to transmembrane TNF provides signals from outside to inside the cell, also referred to as reverse signaling. Reverse signaling by TNFi requires cross-linking of transmembrane TNF, resulting in phosphorylation of the cytoplasmic tail.⁴⁴ Etanercept and certolizumab bind TNF in a monomeric manner and are not able to cross-link TNF. Consequently, these two TNFi fail to induce reverse signaling and subsequent apoptosis. However, cross-linking of etanercept with anti-human IgG resulted in increased apoptosis.^{43,45} This highlights the importance of multimeric interactions with transmembrane TNF for reverse signaling.

Of note, most of the above mentioned studies made use of target cells overexpressing transmembrane TNF. These transmembrane TNF concentrations do not reflect the *in vivo* situation and might have affected ADCC and CDC activity and the efficiency of reverse signaling.^{37,38,44}

Formation of anti-drug antibodies

Immunogenicity of TNFi has been shown to be an important factor associated with treatment efficacy and safety. An immunogenic reaction in response to the non-self TNFi ultimately results in the formation of antibodies targeting the drug, also known as anti-drug antibodies (ADA). Not only the chimeric TNFi infliximab is immunogenic,⁴⁶⁻⁴⁹ also the fully human antibodies adalimumab and golimumab elicit an immunogenic response, resulting in the formation of ADAs.⁵⁰⁻⁵³ The latter can be explained by the fact that even fully human antibodies contain foreign determinants, restricted to the antigen binding site (idiotype). This makes the idiotype potentially immunogenic. Previous work by our group demonstrated that the antibody response to essentially all therapeutic antibodies is restricted to this idiotype.⁵⁴⁻⁵⁶ All therapeutic antibodies have therefore the potential to elicit an unwanted immune response. The fusion protein etanercept is the only TNFi which is essentially non-immunogenic. Some studies demonstrated immunogenicity, albeit of low incidence, low titer and transient ADA responses, which do not target the idiotype.⁵⁷⁻⁵⁹

Assessment of immunogenicity

Numerous studies focused on the quantification of ADAs. The reported incidence and titres, however, vary widely between different studies.⁶⁰ This depends e.g. on the follow-up time of patients, concomitant immunosuppressive treatment and the type of assay that has been used to detect ADAs.⁵⁴ The detection of ADAs is hampered by the presence of the drug, due to anti-idiotype complex formation. This is referred to as drug-interference and results in an underestimation of ADA formation. Most assays are sensitive for the presence of the drug, but the degree of drug-interference varies for the different assays. This contributes – in part – to the wide range of ADA formation.^{61,62} In recent years, drug-tolerant assays have been developed to measure ADAs in the presence of large amounts of TNFi.^{62,63} The use of these drug-tolerant assays revealed ADA formation in the vast majority of patients and questions therefore the clinical relevance of drug-tolerant assays.

Clinical consequences of ADAs

As mentioned, the antibody response is mostly restricted to the idiotype, predominantly neutralizing the TNFi.⁵⁴⁻⁵⁶ Neutralization, but also enhanced immune-mediated clearance of the TNFi, due to complex formation between drug and ADA, can significantly lower the free active drug concentration.⁶⁴ High titers of ADAs are indeed associated with significantly

lower serum drug concentrations.^{47,65,66} However, the impact of ADAs on clinical efficacy depends on the amount of ADAs relative to the amount of free TNFi. ADAs might therefore only influence clinical response when they reduce the amount of free TNFi to a noticeable degree.⁶⁷ When enough free active TNFi is left to bind its target TNF, despite the presence of ADAs, ADAs are unlikely to impair clinical response.

Many studies demonstrated a clear correlation between ADAs to adalimumab and infliximab and a lower likelihood of minimal disease activity or clinical remission in treated patients.^{46,48,68-70} Of note, studies often used assays that are drug-sensitive. These assays mainly detect the surplus of free ADAs, which is limited to those individuals in whom significant amounts of ADAs are formed, and in whom the drug level has been decreased, consequently. In contrast, ADAs detected with drug-tolerant assays are not necessarily associated with clinical response, since these assays can detect low concentrations of ADAs in almost all patients, which do not affect the pharmacokinetics (PK).

Studies focusing on anti-certolizumab antibodies showed mixed results. ADAs in RA patients did not correlate with clinical response,⁷¹ while Sandborn and colleagues demonstrated in CD patients that persistent ADAs, but not transient ADAs, were associated with reduced clinical efficacy.⁷² The impact of ADAs on TNF neutralization by certolizumab was therefore investigated in more detail in **Chapter 6**.

Reducing immunogenicity

Since ADA formation is one of the most important causes of clinical nonresponse, it is important to reduce immunogenicity of TNFi. Many different factors influence the immunogenic response. First of all, patient-related factors are associated with ADA formation. It is suggested that patients with higher baseline disease activity are more prone to develop an immunogenic response. Moreover, patients who develop ADAs against the first TNFi have an increased risk to produce ADAs against a second TNFi.^{54,68} This might, in part, be explained by the fact that specific human leukocyte antigen (HLA) alleles are associated with ADA formation.⁷³⁻⁷⁵ CD patients carrying the HLA-DQA1*05 allele have a significantly higher risk for both anti-adalimumab and anti-infliximab antibody development.⁷⁶ Pre-treatment genetic testing might be used to predict which patients are at increased risk of ADA development and thus has the potential to personalize TNFi treatment, e.g. by choosing a less immunogenic TNFi.

Next to patient-related factors, characteristics of the TNFi itself can influence immunogenicity. Although humanization of therapeutics successfully reduced immunogenicity, the human antibodies adalimumab and golimumab still elicit

an immunogenic response. Identification of drug-related factors prone to elicit an immunogenic response might help to further de-immunize therapeutics. Knowledge of the different steps involved in the formation of an immunogenic response is therefore of importance, and will be briefly described below. Therapeutic antibodies can be internalized through phagocytosis (by macrophages and dendritic cells) or by specific receptors on B-cells (B-cell receptor; BCR). These cells are referred to as antigen presenting cells (APC), because upon internalization the antibody will be processed and presented as peptides on their membrane to cognate T-helper cells. T-cells that recognize the same cognate antigen as the B-cell, will provide help to B-cells by expressing CD40L, a co-stimulatory molecule, and by secreting cytokines. This results in the survival, proliferation and differentiation of the activated B-cell into antibody secreting plasma cells.^{77,78}

Recently, Cassota and colleagues demonstrated that a single T-cell epitope was responsible for the neutralizing antibodies against natalizumab.⁷⁹ Although further studies should investigate whether this is a generic finding and thus also applies to TNFi as well, it might be a step forward in targeted de-immunization of therapeutics. Furthermore, it has been demonstrated that the size of TNF in complex with the TNFi is important. Large immune complexes are more efficiently taken up by APCs and can directly cross-link the B-cell receptor resulting in increased immunogenicity.⁸⁰

Finally, it is suggested that also the treatment regimen is associated with the likelihood of ADA formation. Several high doses at treatment initiation might induce a state of tolerance through exhaustion of the immune response.⁸¹ High concentrations of circulating antigen (TNFi) can activate large numbers of T-cell receptors (TCRs). Once TCR activation is raised beyond a certain death threshold, these activated T-cells will be primed for apoptosis and removed from the circulation before proliferation and differentiation into effector T-cells.⁸² Higher doses of infliximab are indeed associated with a lower frequency of ADA formation.⁸³ Reduced ADA formation might partly be explained by the fact that high amounts of drug interfere with the detection of ADAs in drug-intolerant assays. In order to rapidly increase the serum drug concentration, dosing of TNFi sometimes starts with a loading dose during the induction phase. Thereafter, lower doses or longer dosing intervals are used during the maintenance phase. Furthermore, concomitant immunosuppressant treatment, including azathioprine or methotrexate, reduces ADA formation. ADAs are indeed less frequently detected in RA and IBD patients treated with a combination of adalimumab or infliximab and methotrexate. These patients had higher serum drug concentrations, which was associated with better disease outcome.⁸⁴⁻⁸⁶ Methotrexate in combination with etanercept does not have an improved clinical effect compared with etanercept alone, but this could be explained by the fact that etanercept is essentially not immunogenic.

Outline of this thesis

TNFi are efficacious and widely used in the treatment of inflammatory autoimmune diseases. It has been shown that, once in remission, a proportion of patients can successfully discontinue TNFi treatment.^{87,88} Furthermore, Charles and colleagues showed that TNF concentrations gradually declined after a single infliximab infusion.⁸⁹ This decline was associated with reduced inflammation, as demonstrated by a significant reduction of the inflammatory marker C-reactive protein (CRP).¹ Together, this suggests that there is reduced TNF production in patients in remission, thereby alleviating the need for blocking TNF. We therefore expected a decrease in TNF in patients who are in clinical remission, and monitoring TNF during TNFi treatment could be a potential biomarker in predicting successful treatment discontinuation. Within this thesis we focused on the dynamics of TNF during TNFi treatment and studied the relation with clinical response.

Although TNF concentrations during TNFi treatment have been studied before,⁸⁹⁻⁹³ all of these studies most likely underestimate the TNF concentration. This is due to the fact that TNFi interfere with the quantification of TNF.⁹¹ To overcome this limitation and be able to quantify TNF independent of the presence of large amounts of TNFi during treatment we developed drug-tolerant assays. In **Chapter 2** we describe the development of a drug-tolerant assay for adalimumab. We used this assay to quantify longitudinal TNF concentrations in a prospective cohort of adalimumab-treated RA patients and investigated the relation between TNF concentrations and clinical response.

The results of Chapter 2 raised new questions regarding a potential direct effect of methotrexate on TNF concentrations, in the context of ADAs. The effect of methotrexate on TNF concentrations, independent of ADAs, was investigated in more detail in **Chapter 3** in etanercept-treated RA patients, since etanercept is essentially a non-immunogenic TNFi.

In **Chapter 4** we comment on a recently published paper by Tanaka and colleagues.⁹⁴ They adjusted infliximab dose based on TNF concentrations at baseline, under the assumptions that baseline TNF concentrations can be accurately measured, and that high disease activity (i.e. more inflammation) is associated with higher TNF concentrations. However, our results obtained in Chapter 2 and 3 argue against these assumptions.

Chapter 5 provides an overview of the dynamics of TNF during treatment with the five different TNFi. We investigated potential mechanisms that could explain the variation in steady-state drug-bound TNF concentrations. We characterized TNF-drug complexes *in vitro* and *ex vivo* in more detail and studied whether differences in complex formation

were associated with differences in FcγR-mediated clearance. Finally, we investigated the impact of the five TNFi on TNF production.

As mentioned above, one important factor contributing to clinical non-response is the immunogenicity of TNFi. Although numerous studies demonstrate a clear correlation between ADA formation to adalimumab and infliximab and a lower likelihood of clinical remission, the impact of anti-certolizumab antibodies on clinical outcome showed mixed results. In **Chapter 6** we describe the incidence of anti-certolizumab antibodies in RA patients, as well as their impact on TNF neutralization. We studied the relationship between serum certolizumab concentrations and the TNF neutralizing capacity in presence and absence of ADAs, and compared this with adalimumab.

The findings of this thesis are summarized and discussed in **Chapter 7**.

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