(Anti-)TNF alpha matters in rheumatoid arthritis

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Chapter 1

General introduction
 Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovitis, destruction of joints through cartilage degradation and resorption of bone, ultimately leading to bone erosions. Patients present with symptoms of pain, swelling, stiffness and limited motion of the joints. Irreversible joint erosions and deformities together with clinical symptoms lead to the patients' disability. Despite early and aggressive treatment with anti-rheumatic drugs RA is associated with long term morbidity and mortality. Especially the risk for cardiovascular morbidity is increased in active RA (1;2), but also the process of osteoporosis is accelerated (3).

Extra-articular manifestations can also be observed, such as subcutaneous nodules, vasculitis, (epi-)scleritis, pericarditis, pleuritis, pulmonary nodules and interstitial pneumonitis. Although no test is 100% specific for RA, 70-80% of patients have elevated serum rheumatoid factors, which are auto-antibodies reacting with the Fc portion of IgG molecules. Recently, anticitrullinated peptide antibodies (ACPA) have been shown to be highly specific for RA (4-6). ACPA can be detected in early arthritis in rheumatoid factor positive and negative patients and their presence is associated with a more aggressive disease phenotype with early development of erosions (7).

In the synovial tissue of RA patients the intimal lining layer that normally consists of 1-3 cell layers and covers the non-cartilaginous parts of the joint cavity has proliferated. The tissue underneath the lining, also referred to as the sublining layer, shows an influx of a multitude of cell types such as macrophages, T cells, B cells, plasma cells and dendritic cells. The inflamed synovium is also characterized by neo-angiogenesis. Increased cytokine, chemokine, growth factor and adhesion molecule expression can be found during active inflammation. Activated macrophages are the predominant cells in the inflamed synovium and the most important source of TNF (8). In the synovial tissue of some patients clustering of lymphocytes into small or large aggregates, a few even resembling germinal centers, can be observed (9-11).

TUMOR NECROSIS FACTOR ALPHA

The basics

Tumor necrosis factor (TNF) is regarded a pro-inflammatory cytokine of which two forms were originally described. One is TNFβ also called lymphotoxin alpha (LTα), the other is TNFα also called cachectin which is the key cytokine discussed in this thesis and referred to as TNF. Macrophages, activated T cells, natural killer cells (NK), granulocytes and mast cells are producers of TNF-α, but also certain non-immune cells such as keratinocytes and neurons can produce TNF. Both LTα and TNF share 30% homology. LTα can be found as a homotrimer (LTα3) or a
heterotrimer with membrane bound LTβ (LTα1β2 or LTα2β1) whereas TNF can be found in monomer (17kDa), homodimer or homotrimer form. The homotrimer LTα and TNF interact with the same receptors TNFR1 (p55, CD120a) and TNFR2 (p75, CD120b) (12), (see Figure 1).

![Diagram](image.png)

**Figure 1.** Interaction of TNF and lymphotoxin (LT) with their receptors.

TNFR1 is expressed on almost all cells, whereas TNFR2 is mainly expressed on lymphocytes, other immune cells, endothelial cells and neurons. Both receptors can be shed from the cell surface to become soluble receptors. These soluble receptors can act as a reservoir for TNF and modulate the effect of this cytokine by acting as natural inhibitors. Binding to these soluble receptors can hinder the detection of active TNF. TNFR1 is capable of inducing caspase-mediated apoptosis and is also referred to as the death receptor, although the dominant response to TNFR1 stimulation seems to be gene induction. This gene induction occurs through activation of nuclear factor kappa B (NF-κB), which is a transcription factor that translocates to the nucleus and mediates the transcription of many different proteins involved in cell proliferation, survival, inflammation and inhibition of apoptosis (13). The cellular response in many cells is mainly regulated by TNFR1 signaling although TNFR2 is often co-expressed. Both receptors can bind both sTNF and tmTNF, however TNFR1 favorably binds sTNF and TNFR2 preferentially binds tmTNF rather than

Accordingly, TNFR2 is supposed to play an important role in signaling during cell-cell interaction. Transmembrane TNF is expressed mostly on activated cells like T cells and macrophages. More and more evidence suggests that tmTNF and other TNF superfamily ligands have the unique capability of receiving signals and act as receptors which can transmit positive and...
negative signals into the cell with the ligand presented on its surface. This “reverse signaling” enables two way (inside-out vs. outside-in) communication in cell-cell signaling (15).

The role of TNF in innate immunity

During an infection the physiological role of TNF in innate immunity becomes clear. This pro-inflammatory cytokine is released locally in large amounts in response to bacterial products such as the bacterial cell wall component lipopolysaccharide (LPS). The aim of this response is to contain the infection locally. However, when TNF is released systemically it can induce symptoms associated with sepsis such as vasodilatation, increased vascular permeability and subsequent shock. In addition, diffuse intravascular coagulation is triggered by TNF. TNF, as well as the down-stream cytokines such as IL-1 and IL-6 induce fever and initiate an acute phase response. The liver responds by releasing several acute phase proteins like C-reactive protein (CRP), serum amyloid protein and mannan binding lectin. Macrophages increase their cytokine production and enhance phagocytic capacities. Furthermore, neutrophils become activated and through cytokine and chemokine production become attracted to the site of inflammation where endothelial cells are activated and adhesion molecule expression is enhanced to facilitate trafficking of these cells into the infected tissue. Furthermore, TNF stimulates the migration of phagocytic cells into the regional lymph nodes containing pathogens to initiate adaptive immunity. Together, all these different mechanisms orchestrate a complex immune response to tackle the infection.

The role of TNF in rheumatoid arthritis

RA is characterized by chronic inflammation of the synovial tissue covering the joint cavity without evidence of an ongoing bacterial infection driving the inflammatory process. However, intensive investigation in the 1990’s into the potential role of TNF in the pathogenesis of this chronic inflammatory disease demonstrated increased synovial expression levels of this cytokine (8;16). In the joint TNF leads to enhanced expression of vascular adhesion molecules, cytokine and chemokine production, resulting in the influx of T cells and monocytes into the synovial tissue. Here the activated monocytes transform into tissue macrophages that produce an array of pro-inflammatory cytokines such as IL-6 and IL-1, and even more TNF. These macrophages also secrete chemokines such as IL-8 and CCL2 (MCP-1), hereby attracting even more immune cells to the site of inflammation. IL-1 and TNF activate fibroblast-like synoviocytes (FLS) which secrete matrix metalloproteinases (MMPs), causing cartilage degradation and activation of chondrocytes to produce MMPs and other mediators of inflammation and degradation. Furthermore, TNF activates osteoclasts causing erosion of bone (reviewed in (17)), (18) (Figure 2).
The introduction of TNF blocking therapy lead to a dramatic clinical effect; reducing joint pain, swelling, and an arrest in progression of joint erosions (19-22). By blocking TNF and inhibiting the binding of this cytokine to its receptor on effector cells the inflammatory process perpetuated by TNF is interrupted (12), (Figure 3). On the microscopic level blockade of TNF was shown to reduce the number of cells infiltrating the synovial tissue, especially macrophages, but also adhesion molecule expression and activation status of endothelial cells were down regulated (23-26). Together with a decrease in cytokine and chemokine expression this treatment resulted in silencing local, and potentially even systemic inflammation.

Figure 2. Schematic representation of inflammation in the RA joint.

**ANTI-TNF THERAPY**

**Mechanism of action**

The introduction of TNF blocking therapy lead to a dramatic clinical effect; reducing joint pain, swelling, and an arrest in progression of joint erosions (19-22). By blocking TNF and inhibiting the binding of this cytokine to its receptor on effector cells the inflammatory process perpetuated by TNF is interrupted (12), (Figure 3). On the microscopic level blockade of TNF was shown to reduce the number of cells infiltrating the synovial tissue, especially macrophages, but also adhesion molecule expression and activation status of endothelial cells were down regulated (23-26). Together with a decrease in cytokine and chemokine expression this treatment resulted in silencing local, and potentially even systemic inflammation.
It is still unclear whether the effect of reducing inflammation can all be explained by neutralization of TNF through binding to the TNF blocker. One could hypothesize that local cell death at the site of inflammation within the first days after TNF blocking therapy might in part be responsible for the observed reduction of the synovial infiltrate. In this regard, several mechanisms have been implicated, including antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) as demonstrated by in vitro cell death after binding of infliximab to murine myeloma cells which expressed uncleavable transmembrane TNFα (27). Alternatively, binding of a TNF blocking monoclonal antibody to tmTNF may even induce caspase mediated apoptosis through reverse signaling (28). It has been suggested that after more prolonged treatment increased apoptosis could occur (29). However, this may be a secondary phenomenon rather than a direct anti-apoptotic effect (12). Whether complement activation and apoptosis actually occur in vivo in RA patients will be discussed in this thesis.

Clinical practice

In literature and clinical practice, TNF blocking therapy is also referred to as anti-TNF therapy. These drugs are not considered conventional Disease-Modifying Antirheumatic Drugs (DMARDs), such as methotrexate (MTX) or sulphasalazine, but they are referred to as “biologics or biologics”.

Since the first large clinical trials with TNF blockers in the 1990’s (19-22) and the introduction of these drugs in rheumatology practice the impact on patients’ lifes was enormous as was the change it brought for rheumatology as a medical specialty. Specialized rheumatology surgery and wheelchairs have almost disappeared from the day to day clinic.
At present there are 5 different TNF blockers; infliximab, adalimumab, etanercept, golimumab and certolizumab pegol, of which the last two are not yet registered. Infliximab is a chimeric IgG1 monoclonal antibody (mAb), adalimumab is a fully human IgG1 mAb, and etanercept is a TNF receptor (p75) Fc-fusion protein. Infliximab and adalimumab only bind TNF while etanercept can also bind LTα. Golimumab is also a fully human IgG1 mAb, while certolizumab is a Fab antibody fragment (the antigen binding fragment) which is linked to polyethylene glycol (PEG). This drug lacks the Fc-portion of a monoclonal antibody, and is therefore not able to induce effector functions such as ADCC or CDC.

Except for positive clinical effects the side effects are not to be discarded. During post marketing surveillance the high incidence of tuberculosis during treatment was noticed which was mostly due to reactivation of latent tuberculosis (30). The function of TNF in granuloma formation and subsequent granuloma breakdown after TNF blocking therapy was the explanation for this phenomenon (31). For this reason all patients are screened for tuberculosis prior to initiation of treatment (reviewed in (32)). Certain opportunistic infections, especially those that are macrophage (or granuloma) dependent have occurred, such as histoplasmosis, listeriosis, and coccidomycosis. Serious bacterial infections have been observed, but at an only slightly higher frequency than in controls (33) (reviewed in (34)). The use of TNF blockade has been contra-indicated in patients with severe (NYHA class III-IV) heart failure (35;36), but recent data in RA patients do not show an increased risk of worsening heart failure after anti-TNF therapy (37). The incidence of lymphomas seems to be increased in patients with RA, but was not shown to be increased specifically by TNF blockade (38). Whether TNF blockade is associated with an increased risk of developing malignancies is still a matter of debate as published data are inconsistent (39;40).

Clinical response

Although the majority of patients respond to TNF blocking therapy (60-70%), not all patients show clinical improvement (19-22). Potential explanations for the heterogeneous clinical response may be found in the individual variability in TNF expression among patients (8;41). Consistent with this notion, a recent study using an in vitro bioassay suggested that good responsiveness to anti-TNF therapy is associated with significantly higher TNF bioactivity at baseline compared to non-responding patients (42). In contrast, some genetic studies have suggested that individuals predisposed to high TNF production could show worse responses to anti-TNF therapy (43). Another explanation for the diversity in response may be that inflammatory mediators other than TNF drive different pathogenetic subsets of RA. Naturally, pharmacokinetic differences between drugs and adequate dosing can also determine response. After an initial good response a lack of efficacy occurs in some patients, also referred to as secondary loss of response. This may in part be due to the formation of antibodies against
the TNF blocking drugs (44). The concurrent use of methotrexate has been shown to reduce
antibody formation (45).

Co-morbidity associated with rheumatoid arthritis
An increased risk of cardiovascular morbidity and mortality exists for RA patients with chronic
active disease (1). Besides traditional risk factors for atherosclerosis (smoking, diabetes, dyslipidemia, obesity) the chronic systemic inflammation in RA is thought to enhance atherogenesis (46). Theoretically, the spill over of inflammatory mediators from the synovium into the circulation may induce pro-atherogenic changes such as insulin resistance, endothelial dysfunction and dyslipidemia (2). Furthermore, inflammatory cytokines may also induce the influx of leukocytes into the atherosclerotic plaque, hereby promoting plaque growth (47). The notion that inflammation in RA and atherogenesis are linked is supported by data suggesting that a reduction in disease activity by DMARD therapy may reduce cardiovascular mortality (48;49). Recent studies suggested that TNF blocking therapy reduces cardiovascular morbidity and mortality as well (50;51). Furthermore, previous work has shown that TNF blockade may influence lipid levels, insulin resistance, vascular adhesion molecule expression, and endothelial function (24;25;52-54).

Besides an increased risk of cardiovascular disease, systemic bone loss resulting in osteoporosis is more frequent in RA patients than in the general population (3). This is again due to chronic active inflammation, but the use of corticosteroids and diminished mobility add to the risk of developing osteoporosis. Osteoporosis can lead to fractures after falling which together with associated co-morbidity can lead to disability, or even be life-threatening in elderly patients. Interestingly, TNF not only plays a role in inflammation, but is also involved in postmenopausal bone loss (55;56). Studies in animal models of RA suggested that blocking TNF may inhibit systemic bone loss. In agreement with the results, recent studies in RA patients treated with infliximab suggested beneficial effects of TNF blockade on bone metabolism and bone mineral density (57;58).

OUTLINE OF THIS THESIS
This thesis has been divided into three main sections.

Section I: focuses on the mechanism of action of TNF blockade, the identification of factors associated with clinical response and the effects on co-morbidity.

Chapter 2 discusses a study aimed at the prediction of clinical response to TNF blocking therapy with infliximab by analysis of synovial tissue obtained through mini-arthroscopy prior to the
initiation of treatment. The main focus was to distinguish responders from non-responders based on synovial cell infiltration as well as cytokine, adhesion molecule and growth factor expression studied by immunohistochemistry. We hypothesized that patients with high synovial TNF expression levels would respond better to TNF blockade than patients with low TNF expression levels.

In Chapter 3 micro-array analysis of RNA extracted from synovial tissue of RA patients was used to study gene expression levels in the joint. In line with the previous study, the aim was to extend the investigation on predictors of clinical response by identifying unique pathogenic gene expression signatures in responders and non-responders before start of treatment.

Chapter 4 investigates the immunogenicity of adalimumab by determining the incidence of anti-adalimumab antibody formation. Furthermore, the effect of antibody formation on clinical efficacy is discussed as well as the association with low serum drug levels and the importance of concomitant methotrexate use.

Chapter 5 looks into the question whether apoptosis is part of the primary mechanism of action of anti-TNF therapy. This was studied in peripheral blood and the synovium of RA patients before, and within 1 hour or 24 hours after the first infliximab infusion.

Chapter 6 describes both the short (16 weeks) and longterm (52 weeks) effects of TNF blockade by adalimumab on lipid profiles and macrophage migration inhibitory factor (MIF) levels (a key cytokine associated with atherogenesis). This study was performed in light of the accelerated atherogenesis found in RA patients and the potential beneficial effect of TNF blockade on cardiovascular mortality (50).

As patients with RA are also at risk of developing osteoporosis Chapter 7 discusses the effect of adalimumab therapy on bone mineral density one year after treatment. The effect of additional prednisone use was taken into account.

Since TNF blockade is not efficacious in all patients and clinical efficacy is lost over time in some patients Chapter 8 describes the clinical response in patients switching from infliximab to adalimumab (n=52) in comparison to TNF naïve patients receiving adalimumab for the first time (n=183). Furthermore, the role of immunogenicity resulting in anti-infliximab and anti-adalimumab antibody formation is studied in detail.

Section II: since the variable clinical response to anti-TNF treatment highlights the heterogeneity of the disease, this section focuses on subtype analyses of synovial tissue and blood from RA patients with the aim to distinguish potential pathogenetic subsets.
Chapter 9 describes a study in 35 RA patients and 15 healthy controls with the objective of identifying potential pathogenic disease subtypes. This was done by micro-analysis to determine peripheral blood gene expression profiles followed by subsequent pathway analysis. In Chapter 10 a more detailed microscopic analysis of lymphocyte aggregates in the synovial tissue of RA patients was performed in light of the ongoing ambition of identifying both clinical and pathogenic disease subtypes.

Secion III: as outcome measures are critical in the evaluation of new therapies, this section focuses on two investigations describing new clinical and molecular outcome measures in RA research.

Previous research demonstrated that on the group level the mean improvement in disease activity is associated with the mean decrease in the number of synovial macrophages (59). The investigation in Chapter 11 was an extension of the previous study now aimed at answering the question whether the change in sublining macrophages could also be used as a biomarker to distinguish clinically effective from ineffective anti-rheumatic therapies at an early stage of drug development (Phase I trials).

In Chapter 12 we introduce a new questionnaire in the rheumatology field, the Academic Medical Center Linear Disability Scale (ALDS) (60). The scale was designed to measure disability in patients with various conditions. The present study describes the psychometric properties of this new instrument and sensitivity to change after effective treatment in comparison to the extensively used and validated Health Assessment Questionnaire-Disability Index (HAQ-DI).

In Chapter 13 the most important findings of these studies are summarized and discussed in light of recent literature.
REFERENCES


