Rheumatoid Arthritis

Tocilizumab; a promising biological

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Abbreviations

ACPA	anti-citrullinated protein/peptide antibodies
ACR	The American College of Rheumatology
anti-CCP	anti-cyclic citrullinated proteins
CRP	C-reactive protein
DMARD'S	disease-modifying anti rheumatic drugs
ESR	erythrocyte sedimentation rate
EULAR	The European League Against Rheumatism
HLA	Human leukocyte antigen
ICs	immune complexes
IL-1	Interleukin-1
IL-6	Interleukin-6
MRA	myeloma receptor antibody
MTX	methotrexate
NREM	non-rapid eye movement
NSAID's	non-steroidal anti-inflammatory drugs
PAD	citrullinating peptidylarginine deiminase
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
SAE	Serious adverse events
SE	shared epitope
TB	tuberculosis
TGF-ß	transforming growth factor beta
VEGF	Vascular endothelial growth factor

1.Introduction

1.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a relatively common, systemic and chronic autoimmune disease that has a prevalence of 1% in the western world (Brennan et al.,2008). RA has a 3 times higher incidence in women and has a peek around the ages of 30 and 50, which implies that hormones play a role in development of the disease. (Lockshin MD.,2002) Joint inflammation is a hallmark of RA, but RA isn't only a disease of the Joints (figure 1).

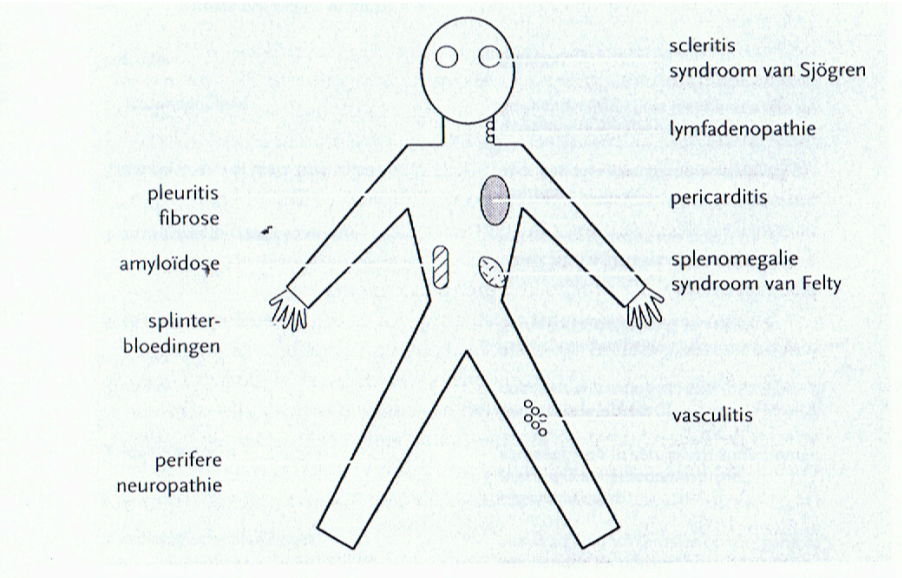


Figure 1: the extra articular features of RA.

It consists of a combination of a syndrome of pain, stiffness, and inflammation of the synovial membrane of freely moveable joints such as the hands, wrists, shoulders ankles and knee, which can ultimately lead to cartilage and bone destruction and functional decline (figure 2). Additionally, substantial co morbidity in the cardiovascular, neurologic, and metabolic systems can be detected (Brennan et al., 2008).

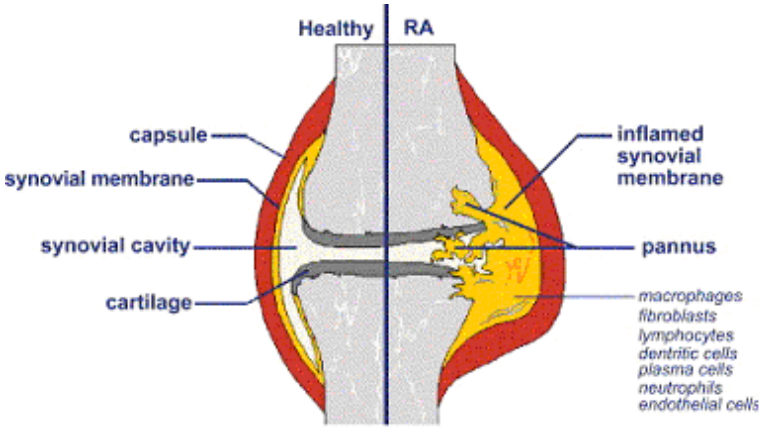
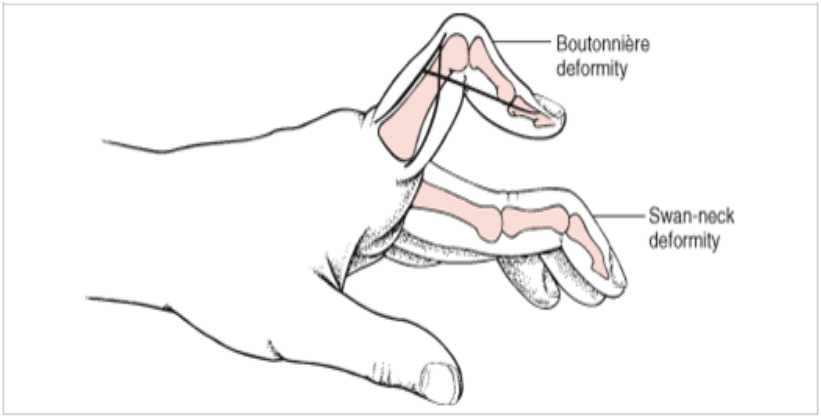


Figure 2: A healthy versus an inflamed joint. The features that occur in the inflamed synovial joint of RA patients is depicted. Inflammation is caused by an influx of inflammatory cells and proliferation of the synovial lining cells. When RA progresses, pannus tissue will develop. This pannus tissue can invasively grow into the cartilage and bone , which can lead to bone destruction (Nijenhuis et al.,2004).

1.1.1 The clinical course of RA

The clinical course of RA and therefore the severity of the symptoms differ between patients. (Klareskog et al.,2009) In the majority of patients, RA has a slow and insidious onset. At first, RA patients suffer from fatigue, malaise and musculoskeletal pain. The joints start to become involved after weeks or even months.
The involved joints, typically initially the small bones of the hand and feet followed by the knees, elbows, ankles and wrists, are swollen, warm, painful and stiff in the morning (or after inactivity). Destruction of tendons, ligaments and joint capsules leads to typical deformities, such as radial deviation of the wrist and subsequent ulnar deviation of the fingers, as depicted in figure 3.



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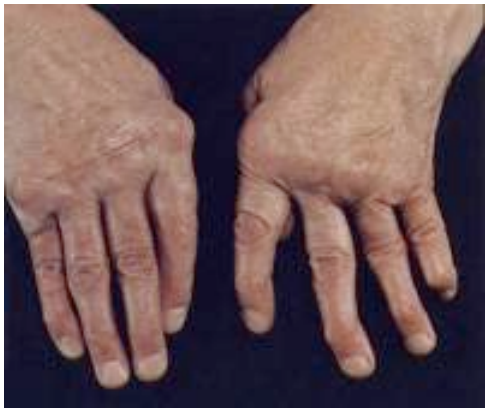

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*figure 3: D*eformities of the joints in the hand.

Eventually, RA will lead to deformed joints that lack stability and are almost immobile. Furthermore, do RA patients have a higher frequency of hart- and vascular diseases. RA has a heterogeneous image of disease. The course of the disease can be either slow, or rapid, but the most damage to the joints occurs within the first 4 or 5 years. (Cotran et al.,1999).

1.1.2 The Diagnosis of RA

The Diagnosis of RA is based on classification criteria. RA is diagnosed when a patient shows at least 4 out of the 7 symptoms. The American College of Rheumatology (ACR) formulated their criteria for the classification of RA in the mid 1980s (Arnett et al.,1988). These criteria are the following:

1. morning stiffness in and around joints lasting at least 1 hour before maximal improvement (lasting at least 4 weeks)
2. Soft tissue swelling (arthritis) of three or more joint areas observed by a physician (lasting at least 4 weeks)
3. Swelling of the proximal interphalangeal, metacarpophanlangeal, or wrist joints (lasting at least 4 weeks)
4. Symmetric swelling (arthritis) (lasting at least 4 weeks)
5. Rheumatoid nodules
6. The presence of rheumatoid factor (RF)
7. Radiographic erosions and/or periarticular osteopenia in hand and/or wrist joints

These criteria are mainly based on clinical parameters. The main problem is that some of these criteria are only visible after the first damage to the tissue has already taken place. Therefore, the ACR and The European League Against Rheumatism (EULAR) are currently working together to come up with new guidelines for the diagnosis of early RA. This is necessary because of the fact that due to the new therapies some of the old criteria, such as nodules, are rarely seen early in the disease anymore (Klareskog et al.,2009).
It would be very useful to have a reliable serological test that is able to detect RA in a very early stage of the disease. In the ACR criteria the RF is mentioned. RF is an antibody that is directed to the Fc domain of IgG molecules and it has a prevalence of 75% in RA patients. The problem, however, is that it also can be found in patients suffering from other autoimmune diseases, infectious diseases and even in about 5% of the healthy (mainly elderly) population. (Mageed, 1996) This led to the search for better markers, which has been found in antibodies to citrullinated proteins, anti-CCP (anti-cyclic citrullinated proteins) or ACPA (anti-citrullinated protein/peptide antibodies) (De Rycke et al.,2004). These autoantibodies are very specific (+/- 95%) and very sensitive (up to 80%). In approximately 70% of the RA patients these autoantibodies are present. Moreover, they are present prior to disease onset (van Venrooij et al.,2008, Rantapää-Dahlqvist et al.,2003).
The presence or absence of ACPA also leads to a division of RA into (at least) 2 Subsets; ACPA positive an ACPA negative.

RA is known as a complex genetic disease, which means that a lot of different factors, such as; genes, environmental factors and immunologic triggers work together in the cause and development of the pathological process. It has been shown that the contribution of genetic factors for the whole syndrome of RA is almost 50% (MacGregor et al.,2000). A considerable amount of this 50% is due to the shared epitope (SE) which is carried by the vast majority of ACPA positive RA patients. The SE, is a 5-aminoacid sequence motif in the third allelic hypervariable region of the HLA-DRβ chain (Klareskog et al., 2009). Furthermore, it has been shown that in the 2 subsets of RA different gene - and environmental risk factors play a role (Table 1).

	ACPA-positive disease	ACPA-negative disease
Genetic risk factors		
HLA-*DRB1* alleles	Yes	No
PTPN22	Yes	No
TRAF1-C5 locus	Yes	No
OLIG3-AIP3 locus	Yes	..
STAT4	Yes	..
Non-*DRB1* MHC genes	Yes	No
IRF5	No	Yes
CLEC4A	No	Yes
HLA *DRB1*03*	No	Yes
PADI4	–	–
Genetic protective factors		
HLA-*DRB1* molecules containing aminoacid sequence DERAA	–	–
Non-inherited maternal HLA-*DR*	–	–
Host factors		
Female sex	–	–
Perinatal factors	–	–
Obesity	–	–
Environmental risk factors		
Cigarette smoking	Yes	No
Mineral oils	Yes	No
Environmental protective factors		
Alcohol	Yes	Yes

Table 1: The genetic and environmental factors associated with rheumatoid arthritis. - = no division made between subsets (Klareskog et al., 2009).

The knowledge about RA is constantly growing. Whereas in the past RA has been regarded as a disease of uncertain pathogenesis, nowadays RA has become a pioneer for the development of new treatments. (Klareskog et al.,2009) This doesn't mean that nowadays the process of RA is completely elucidated. Previously, the main focus was on care and rehabilitation of deformated handicaps, whereas nowadays the focus lays mainly on early treatment that can prevent disability in many patients.

1.2 The pathogenesis of rheumatoid arthritis

The pathology of RA is very complex. A lot of different cell types and cytokines are involved in the disease process. A schematic overview of the most important cytokine pathways involved in RA pathogenesis are depicted in figure 4.

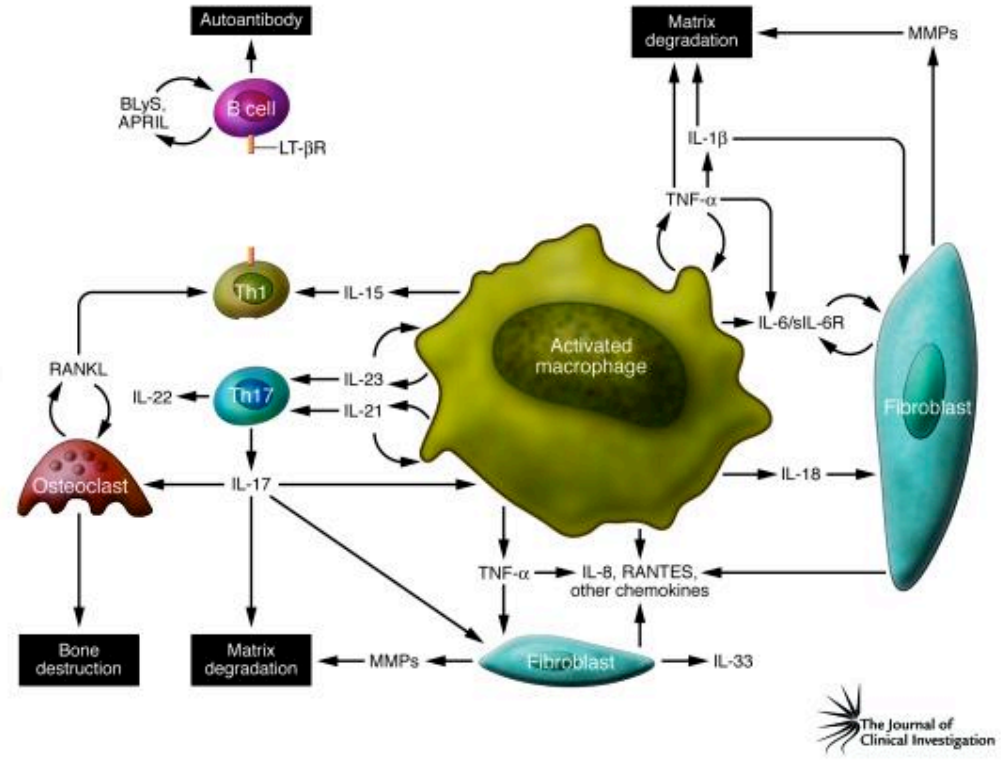

Figure 4: This picture is a summary of the different types of cells (hematopoietic as well as nonhematopoietic) involved in RA, and their interactions. These interactions are possible due to the depicted cytokines, which are released from the activated cells. These cytokines, in their turn can induce the production of other additional proinflammatory cytokines. Together these cytokines contribute to the pathogenesis of RA that ends up with joint destruction (Brennan et al., 2008).

Although the pathogenesis of RA is complex and a lot of different cells and cytokines (such as, macrophages T and B cells, fibroblasts, TNFα, IL1 and IL6) play a role, it is possible to hypothesize a model for RA pathogenesis. Several (animal) models illustrate the role of certain cytokines and immune cells in RA pathogenesis. Each model consist of the following steps:

- A trigger, such as a trauma, immunization, a chemical or stress, causes an inflammation of the joint
- Activation of T cells via antigen presentation
- B cell activation and antibody production
- This cycle keeps on repeating itself (normally this wouldn't be the case and the inflammation would stop)

One of the suggested models involves an important feature of rheumatoid arthritis, namely the generation of ACPA's. These autoantibodies are very specific and are already present prior to disease onset. The presence of these autoantibodies and citrullinated proteins in RA led to a model, which thus only counts for a subset of RA patients, suggested by Van Venrooij and colleagues (van Venrooij et al., 2008). The model for RA pathogenesis is shown in figure 5 and it involves 5 steps, which are discussed further below.

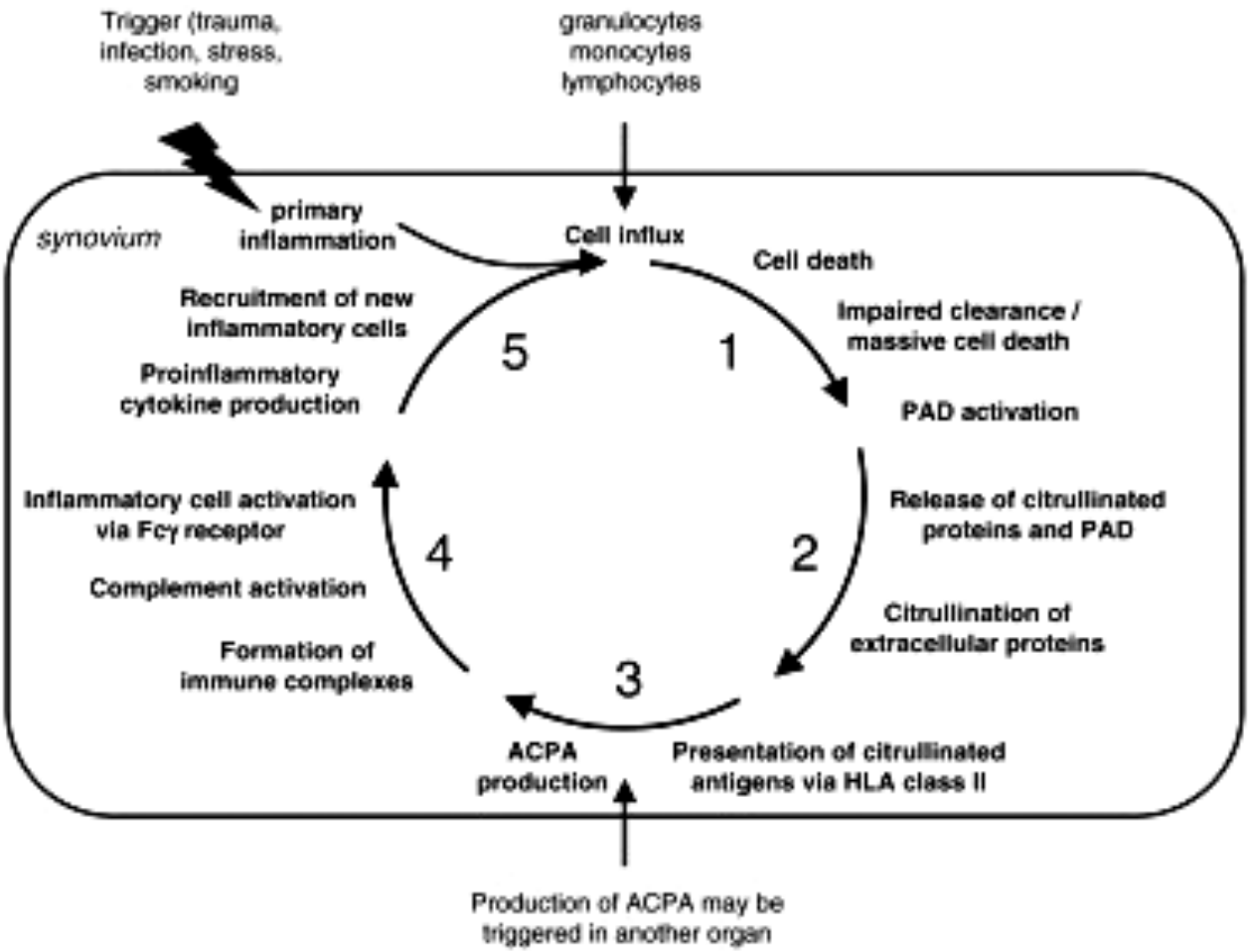

Figure 5: The RA cycle suggested by Van Venrooij and colleagues. Depicted are the various aspects of the 5 major steps. Step 1, entry and death of inflammatory cells in the synovium. Step 2, PAD activation and protein citrullination. Step 3, immune response to citrullinated antigens. Step 4, formation of citrullinated immune complexes and their effects. Step 5, recruitment of new inflammatory cells (van Venrooij et al., 2008).

Step 1

The pathology of RA starts with an in itself innocent inflammation of the joint that is caused by a trigger. (This trigger can for example be; a trauma, stress, smoking or an infection). This inflammation leads to the infiltration of inflammatory cells, such as granulocytes, monocytes and lymphocytes, into the synovium of the joint. Normally most of these cells will die due to apoptosis. These apoptotic cells will be removed by

phagocytes. However, in RA patients, there is massive apoptosis and a couple of these apoptotic cells may not be removed and become necrotic.
Granulocytes, monocytes and macrophages contain citrullinating peptidylarginine deiminase (PAD) enzymes. PADs are activated by high Ca^{2+} concentrations. This is for example the case when cells undergo apoptosis (Cotran et al.,1999).

Step 2

When the apoptotic inflammatory cells aren't removed and become necrotic, they release their intracellular citrullinated proteins, such as histones, as well as the activated PAD enzymes, into the synovium. These enzymes are then capable of citrullinating extracellular proteins in the synovium such as fibrinogen (Vossenaar et al., 2004).

Step 3

Some people, expecially those we carry the SE are capable of presenting citrullinated pieces of proteins to T cells via the HLA (human leukocyte antigen) molecules. This may trigger an immune response to citrullinated antigens. This immune response will result in the production of ACPAs (Hill et al., 2003).
The activation of the autoreactive B cells can either take place in the joint, but can also occur in other inflamed tissues. The ACPAs, or the cells producing them, will enter the joint via the circulation (van Gaalen et al., 2005).
It has been shown that independent of the site of B cell activation ACPAs are produced in the joint of RA patients and therefore may be able to mediate tissue injury (van Venrooij et al., 2008).

Step 4

Once the ACPAs are in the inflamed synovium they are able to react with the citrullinated antigens and form immune complexes (ICs) (Zhao et al., 2008). The inflammatory process is activated by these formed ICs because they activate the complement system. Furthermore, they recruit and activate new granulocytes, monocytes and macrophages. Via this way, ACPAs play an important role in the continuation of the joint inflammation (Zhao et al., 2008).

Step 5

New inflammatory cells will be recruited and enter the synovial fluid and the circle will start all over again. This leads to a new flare of inflammation, which will eventually create a chronic disease process. The constantly continuation of this circle will eventually lead to the symptoms that characterize RA.

1.3 Therapy

Because there is no definitive cure for RA, the major goals of RA treatment are to relieve pain, preserve joint function, and minimize the toxicity from medications. It is very important to diagnose RA in an early phase to make sure that treatment can be started. Both pharmacologic and nonpharmacologic treatments are used, the latter consisting of physical therapy, occupational therapy, and surgical treatments. The pharmacologic treatments can be divided into 3 classes, namely:

1. The first class includes painkillers, such as paracetamol, and non-steroidal anti-inflammatory drugs (NSAID's). This group of drugs provide symptomatic relief but do not reverse or prevent joint damage. Common side effects are the formation of gastritis and ulcers, furthermore they can cause hypertension, edema, and myocardial infarction.

2. The second class consists of the disease-modifying anti rheumatic drugs (DMARD'S), which are a group of drugs that are able to specifically retard the progression of joint disease by lowering the acute phase response (Weinblatt et al., 1985). Members of this class are o.a. Leflunomide, hydroxychloriquine and Sulfasalazine. But the most common used member of this group is methotrexate (MTX). MTX is a folium acid antagonist. It inhibits the biosynthesis of purine and therefore the cell division (Doan et al., 2005). The most common side effect of MTX is nausea. Furthermore, stomatitis and raised levels of liver enzymes are often seen.

3. The third class is perhaps the most promising one; namely the biologicals, which is actually a subgroup of DMARD'S. The reason that biologicals are seen as a different group is due to the fact that they are biological agens, in contrast to the previously mentioned pharmalogical treatments, that are able to target specific immune mediated processes. Biologicals are produced in a laboratory with advanced biotechnological techniques (Doan et al., 2005). In this way they can inflict the whole immune system. During the last decade the research at and the development of these biologicals have taken a huge flight. All this interest lead to the discovery and development of several biologicals, depicted in figure 6. The first successful (and therefore best known) biologicals used in RA patients, are TNF-blocking agents, namely infliximab (chimeric anti-TNF), etanercept (soluble TNF receptor) and adalimumab (humanised anti-TNF). All three of these drugs act by partly neutralising circulating and synovial TNF (Klareskog et al.,2009). Furthermore rituximab, a monoclonal antibody that binds to CD20 on the surface of pre-B as well as mature B cells, and depletes them from the circulation. Due to this depletion, the formation of autoantibodies will be reduced significantly (Edwards et al., 2004).

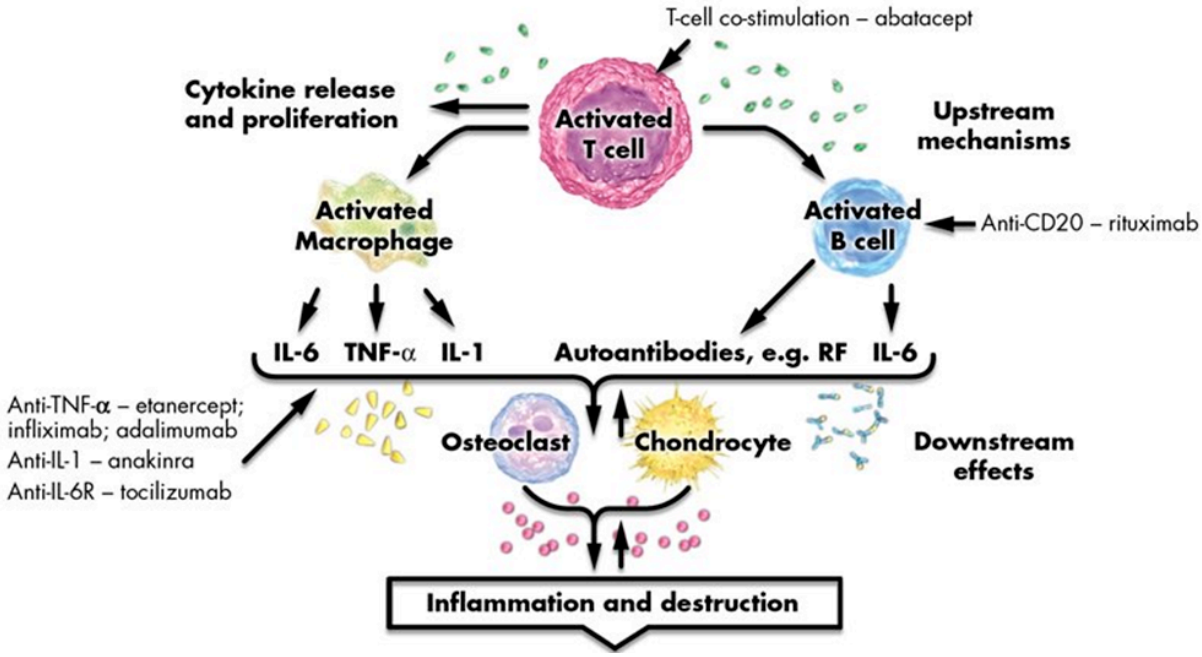

Figure 6: This picture is a summary of the different types of cells (hematopoietic as well as nonhematopoietic) involved in RA, and their interactions. These interactions are possible due to the depicted cytokines, which are released from the activated cells. These cytokines, in their turn can induce the production of other additional proinflammatory cytokines. Together these cytokines contribute to the pathogenesis of RA that ends up with joint destruction. Those cytokines with pathogenic potential have been identified and biological therapies are developed to block their action. This figure identifies those therapeutic modalities (http://www.ispub.com/ispub/ijrh/volume_3_number_1_51/abatacept_in_focus/abatacept-fig1.jpg).

There is still a need for new drugs. For example, because of the fact that, there are still patients that don't respond to one of the current therapies. One example of a recently developed biological for the treatment of RA is Tocilizumab. Tocilizumab is a IL-6 blocking agent that promises to have a great future and therefore will be discussed further in this thesis.

2. Tocilizumab and IL-6

2.1 Interleukin-6

Il-6 is a cytokine that consists of 4 α-helices and has a molecular mass of 20.8 kDa. (figure 7). One of the typical features of cytokines is that they show a wide variety of biological functions *on various tissues and cells.*

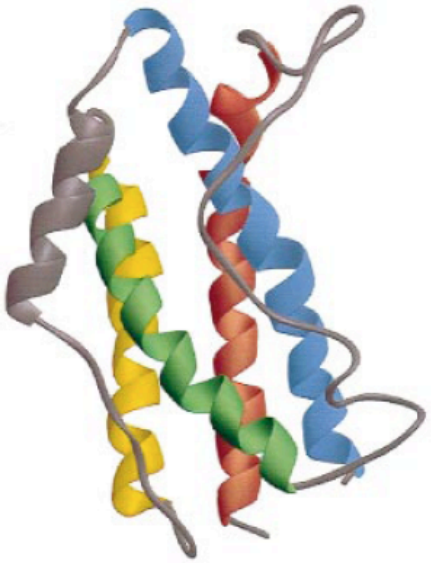

Figure 7: The structure of IL-6

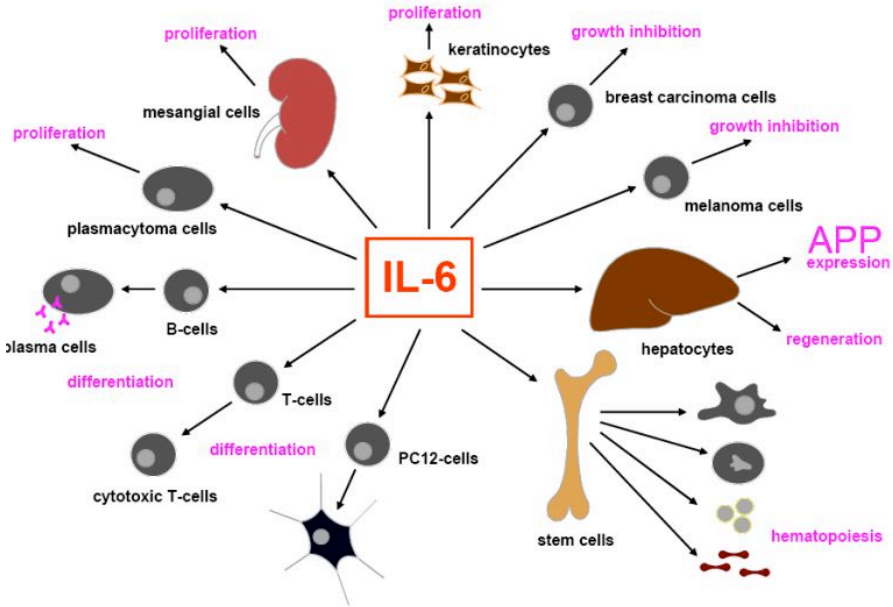

Figure 8: The biological activities of IL-6 (http://www.biochemie.uni-freiburg.de/heinrich/research.html)

Furthermore, have several different cytokines overlapping or even similar functions on a cell (Kishimoto et al., 1995). IL-6 is a good example of such a pleiotropic cytokine, as is depicted in figure 8. It acts, among others, as a stimulator of B and T cell functions and it promotes proliferation of plasmablasts into immunoglobulin producing plasma cells (van Snick. 1990).
IL-6 is involved in a couple of processes that play a role in the local synovial as well as the systemic inflammation in RA. For example:

- The induction of the acute phase response (Streetz et al., 2001).
- The recruitment of leukocytes towards inflammatory sites (Kishimoto, 2005).
- The stimulation of synoviocyte proliferation (Kishimoto, 2005).
- The pannus formation and bone resorption in inflamed joints (Tamura et al., 1993).

To produce these effects, IL-6 signals through a receptor composed of two different subunits: the IL-6 binding receptor and gp130, a receptor subunit shared in common with other cytokines in the IL-6 family, such as IL-11 and LIF. There are two forms of the IL-6 binding receptor, namely the membrane bound IL-6 receptor and the soluble IL-6 receptor which circulates in blood and synovial fluid (Heinrich et al., 2003).

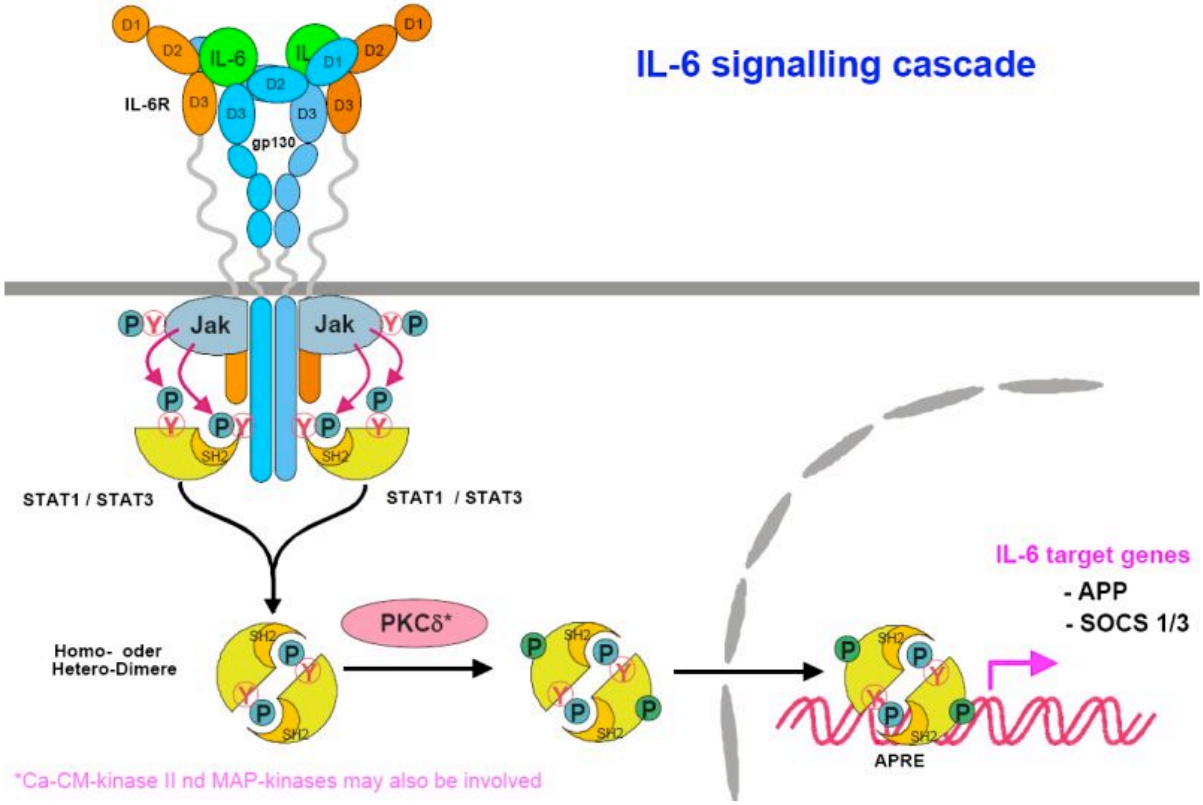

Figure 9: The IL-6 signal transduction. This signal transduction occurs via the JAK/ STAT pathway, i.e. tyrosine kinases of the Janus family activate signal transducers and activators of transcription (http://www.biochemie.uni-freiburg.de/heinrich/research.html).

Binding of IL-6 to one of its receptors leads to dimerization of the two subunits. As depicted in figure 9 does this dimerization initiates cellular events including activation of JAK kinases. Activated JAK kinases, phosphorylate and activate STAT transcription factors that move into the nucleus to activate transcription of genes. (Kishimoto, 2005) The ras-mediated pathway, via Map kinases, activates transcription factors such as ELK-1 and NF-IL-6 (C/EBP-beta) that can act through their own cognate response elements in the genome (Heinrich et al., 2003). These factors together regulate a variety of complex promoters and enhancers that respond to IL-6 and other signalling.

2.2 Tocilizumab

Tocilizumab is a humanized antihuman IL-6 receptor monoclonal antibody, as shown in figure 10. (Sato et al., 1993). At first, tocilizumab was called MRA, which stands for myeloma receptor antibody, because it has shown potential (It was able to inhibit multiple myeloma cell growth) therapeutic benefit in multiple myeloma (Sato et al., 1993). The humanization of tocilizumab resulted in decreased antigenicity in humans. Additionally, this lead to a prolonged half-life of tocilizumab and low production of neutralizing antibodies (Sato et al., 1993).

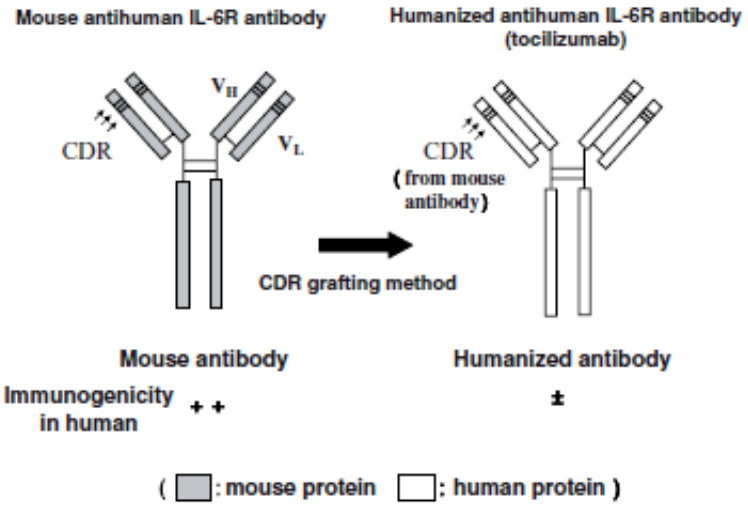

Figure 10: Humanized antihuman IL-6 receptor (tocilizumab). The humanizing of the mouse antihuman IL-6 receptor lowers the antigenicity in humans (Nishimoto et al., 2008).

Tocilizumab specifically binds to both forms of the IL-6 receptor (membrane-bound and soluble IL6-R). By binding the receptor, native IL6 can no longer bind. Due to this blocking, dimerization of the two subunits of the receptor doesn't occur. Therefore, the cellular signal transduction, as shown in figure 11, is inhibited (Mihara et al., 2005).

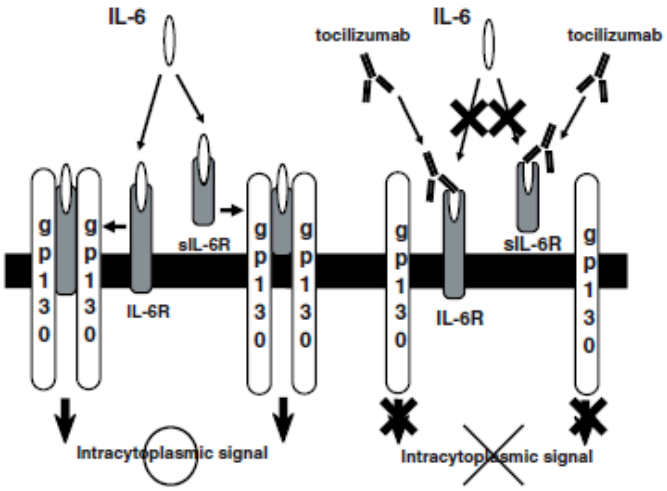

Figure 11: The mechanism for tocilizumab to inhibit the IL-6 signaling (Nishimoto et al., 2008).

2.3 The clinical value of tocilizumab in RA

Since 1999, a number of, multicentre clinical trials conducted all over the world, have investigated the effect of treatment with tocilizumab in adult RA patients.
The first phase I/II studies, one Japanese and one conducted in the UK, showed that the best drug protocol, which significantly lowers the disease activity, consists of the intravenous administration of tocilizumab every four weeks at a concentration of 8 mg per kg bodyweight (Nishimoto et al., 2004 + Maini et al., 2006). These Phase II studies where allowed because of the fact that the results of the phase I study were very promising, as depicted in figure 12. and included improvement in number of swollen and painful joints, as well as a normalization of the acute phase proteins in the blood of RA patients with measurable blood-level tocilizumab. This measurable blood-level tocilizumab was seen in patients that were treaded with 4 or 8 mg tocilizumab per kg bodyweight (Nishimoto et al., 2003).

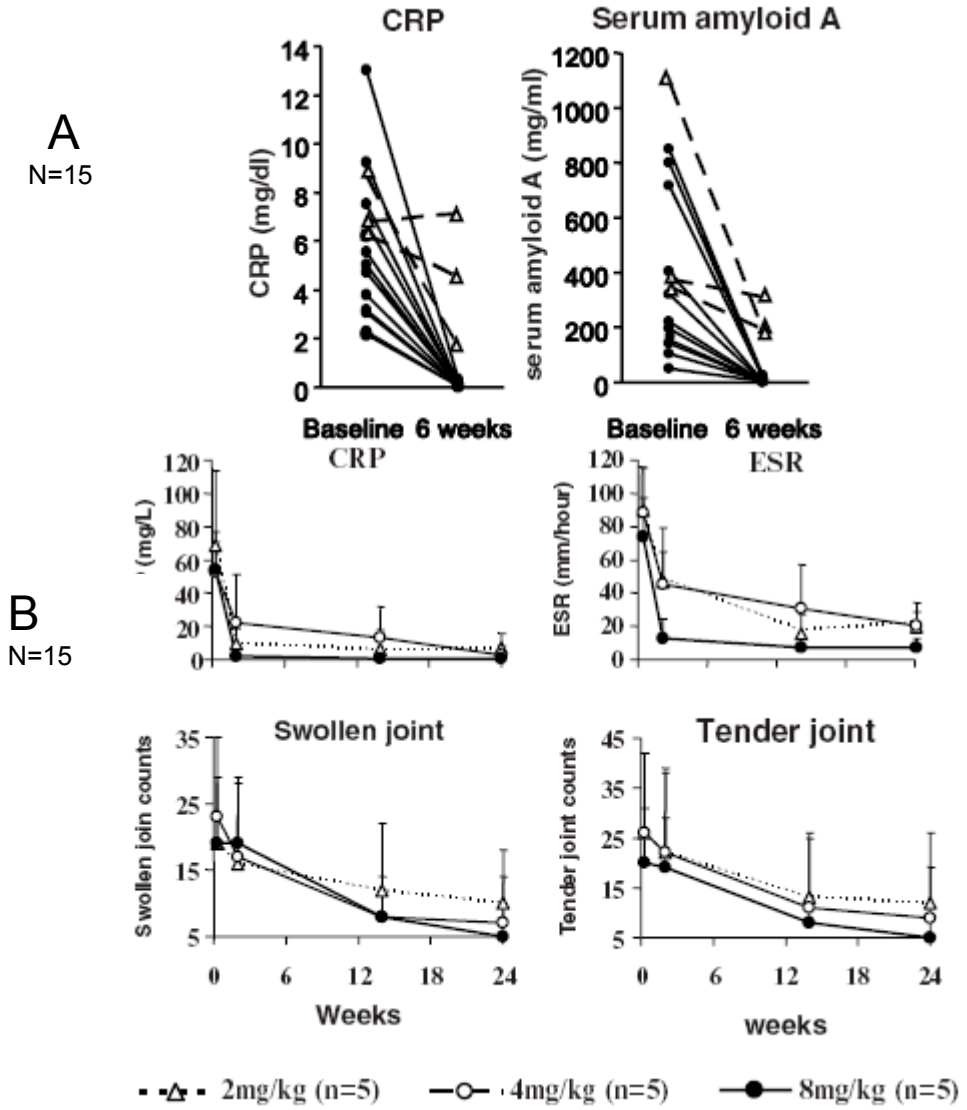

Figure 12: The clinical effects of tocilizumab. (A) Quantitative changes in acute-phase proteins in the blood: acute-phase proteins, CRP and serum amyloid A were normalized in patients with measurable blood-level tocilizumab (4/8 mg per kg; filled circle). This normalization was not shown in patients who didn't maintain measurable blood-level tocilizumab (2mg per kg; open triangle). (B) Treatment with tocilizumab quantitatively improved swollen and painful joints, as well as CRP values and the erythrocyte sedimentation rate (ESR) (Nishimoto et al., 2003).

The results of the Japanese phase II study are shown in figure 13. This figure clearly shows that tocilizumab lowers the RA disease activity. For example the ACR20 response rate of 8 mg per kg bodyweight was 78%, whereas that of placebo was only 11 %. ACR20 means that there is 20% or more improvement in tender and swollen joint counts and 20% improvement in 3 of the 5 remaining ACR core set measures: patient and physician global assessments, pain, disability, and an acute-phase reactant, such as CRP or ESR (Nishimoto et al., 2004).

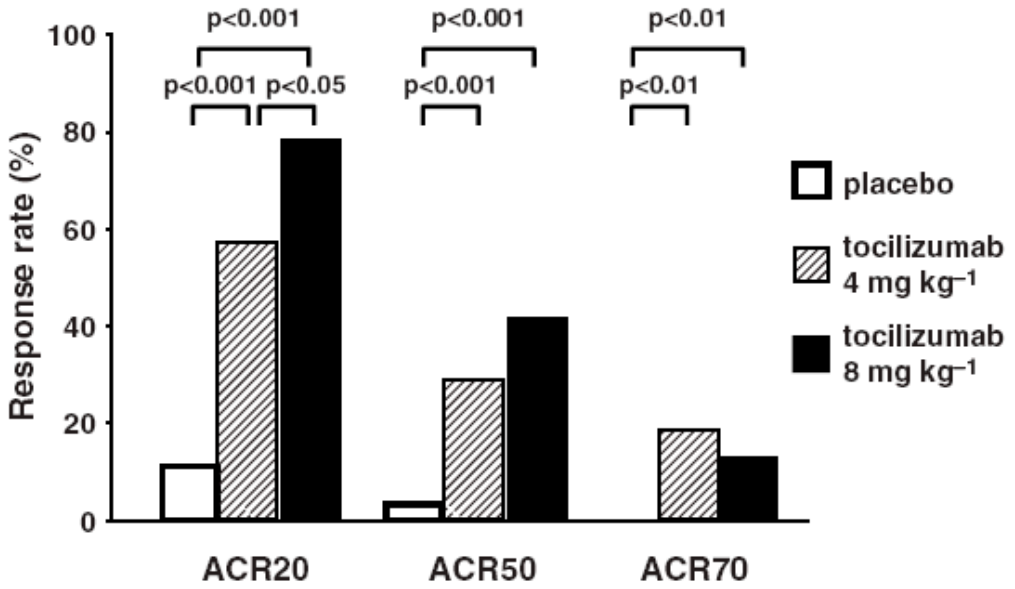

Figure 13: Treatment with tocilizumab significantly lowers RA disease activity in comparison with placebo treatment (Nishimoto et al., 2004).

These promising results of the phase II trails led to the start of 7 global, multicenter phase III studies. These studies all have an acronym and in this thesis they will be referred to with this acronym. The clinical results of these 7 studies are shown in table 2. Without any exception tocilizumab has been shown to have a great potential as a treatment for RA (as shown in table 2). These results led to approval of tocilizumab, in 2008 for Japan and since the beginning of 2009 also in the rest of the world, as a medicine for RA (Oldfield et al., 2009).
The differences between the studies and the main results of these studies will be discussed further in this thesis.

	ACR20, % (*P*)	ACR50, % (*P*)	ACR70, % (*P*)	DAS28 remission, % (*P*)
SAMURAI trial				
Tocilizumab (8 mg/kg)	78 (<0.001)	64 (<0.001)	44 (<0.001)	59 (<0.001)
DMARDs	34	13	6	3
SATORI trial				
Tocilizumab (8 mg/kg)	80 (<0.001)	49	40	43 (<0.001)
MTX	25	11	6	2
OPTION study				
Tocilizumab (8 mg/kg)	59 (<0.0001)	44 (<0.0001)	22 (<0.0001)	27 (<0.0001)
Tocilizumab (4 mg/kg)	48 (<0.0001)	31 (<0.0001)	12 (<0.0001)	13 (=0.0002)
Control	26	11	2	1
TOWARD study				
Tocilizumab (8 mg/kg)	61 (<0.0001)	38 (<0.0001)	21 (<0.0001)	30 (<0.0001)
Control	25	9	3	3
RADIATE study				
Tocilizumab (8 mg/kg)	50 (<0.001)	29 (<0.001)	12 (0.001)	30 (0.001)
Tocilizumab (4 mg/kg)	30 (<0.001)	17 (<0.001)	5 (0.1)	8 (0.053)
Control	10	4	1	2
AMBITION				
Tocilizumab (8 mg/kg)	70 (<0.0001)	44 (0.0023)	28 (0.0002)	34
MTX	53	34	15	12
LITHE				
Tocilizumab (8 mg/kg)	56 (<0.0001)	36 (<0.0001)	20 (<0.0001)	47 (<0.0001)
Tocilizumab (4 mg/kg)	47	29	16	30
Control	25	10	4	8

Table 2: The efficacy of tocilizumab therapy in the 7 global phase III trials (obtained from Mima et al., 2009).

SATORI: *Study of Active-controlled TOcilizumab monotherapy for Rheumatoid arthritis patients with Inadequate response to methotrexate. (Japanese study, n=127).*

The SATORI trial was a Japanese trial which investigated if tocilizumab monotherapy was able to improve the clinical features of RA in patients who had an inadequate response to low dose MTX (Nishimoto et al., 2008). It has been shown in the SATORI trial that monotherapy with tocilizumab was effective for RA patients who had an insufficient response to a low dose of MTX (Table 2). The impairment of physical function was improved. Additionally, the trial showed that the switch from MTX to tocilizumab didn't bring problems for the patients (Nishimoto et al., 2008). All the results were remarkable and noteworthy but one finding stood out. The response to tocilizumab is probably due to decreasing levels of Vascular endothelial growth factor (VEGF) in the serum (Nishimoto et al., 2008). This is depicted in figure 14. VEGF is known to play an important role in the angiogenesis, which is a characteristic histological feature of RA. VEGF is necessary to get oxygen to the synovial tissue in RA (Nishimoto et al., 2008). Furthermore is it also a mediator of inflammation (Mima et al., 2009).

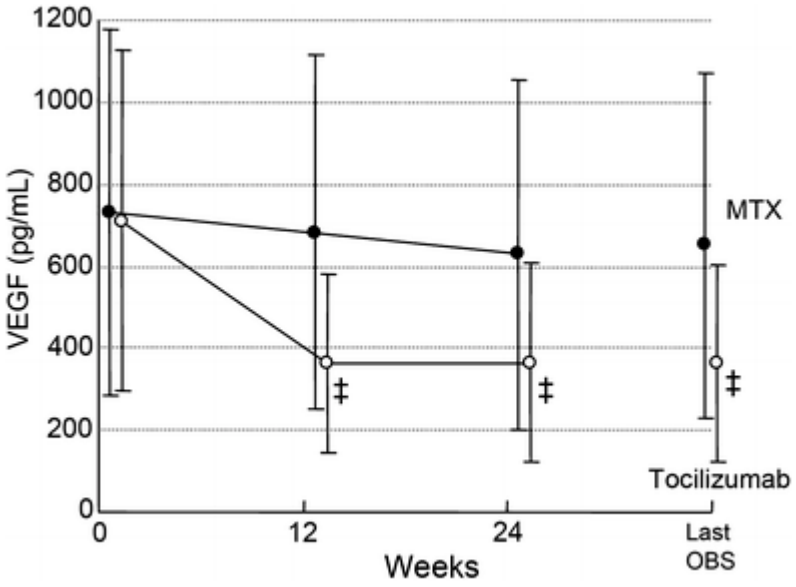

Figure 14: Change from baseline in serum levels of VEGF. The mean serum VEGF levels showed a marked decrease in the tocilizumab group. Mean changes from baseline were −74.0 pg/ml in the control group and −346.9 pg/ml in the tocilizumab group at week 24 ($P < 0.001$) (Nishimoto et al., 2008).

AMBITION: *Actrema versus Methotrexate double-Blind Investigative Trial in mONotherapy (Western study, n=673).*

In the AMBITION trial they also investigated the efficacy of Tocilizumab as a monotherapeutic drug. The difference with the SATORI study is that the RA patients in the AMBITION study did show a clinical response to MTX (Jones et al., 2008). This study showed, as is seen in table 2, that tocilizumab is the first biological that is superior to MTX in monotherapy for lowering symptoms in patients who haven't failed any prior therapy (Jones et al., 2008).

SAMURAI: *Study of Active controlled Monotherapy Used for Rheumatoid Arthritis, an IL-6 Inhibitor trial (Japanese study, n=306).*

There are two studies in which the progression of the structural joint damage, which is seen in RA patients was measured as a credit to measure the efficacy of tocilizumab. The first study to investigate this was the SAMURAI trail. Patients in this study had early RA, with an average duration of less than 2 years (Nishimoto et al., 2007). Figure 15 shows that in this study it was a proven that tocilizumab, even in monotherapy, is capable to prevent structural joint damage to occur in RA patients (Nishimoto et al., 2007).

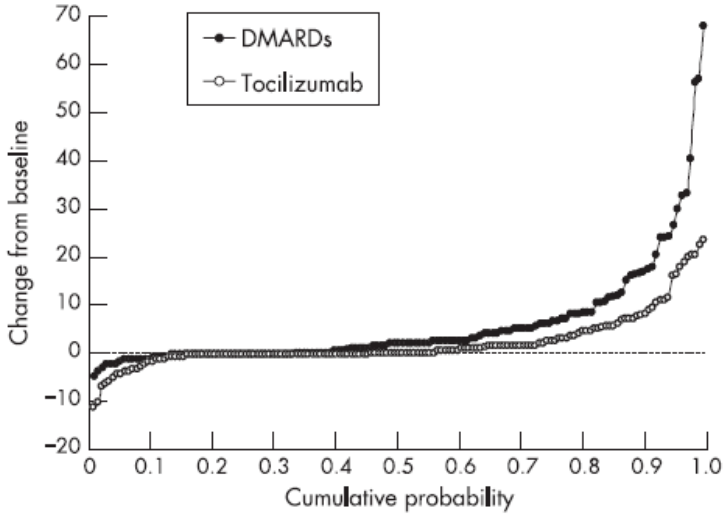

Figure 15: Cumulative probability distribution of radiographic changes in total Sharp/van der Heijde scores from baseline to week 52 for patients treated with tocilizumab or with conventional DMARD'S. The space between the curves indicates the different treatment effects with a considerable difference in favour of the tocilizumab group (Nishimoto et al., 2008).

LITHE: *tociLIzumab safety and THE prevention of structural joint damage (Western study, n= 1196).*

The second study, which looked at whether tocilizumab could prevent joint damage in RA patients was the LITHE study. During this trial the RA patients received tocilizumab on top of their normal MTX doses. This LITHE study proved that the addition of tocilizumab to MTX, significantly inhibited the progression of structural joint damage more than MTX alone (Kremer et al., 2008).

OPTION: *tOcilizumab Pivotal Trial in methotrexate Inadequate responders (Western study, n=623).*

Addition of tocilizumab to MTX has also been investigated in the OPTION trial (Smolen et al., 2008). The patients in this study had moderate to severe active rheumatoid arthritis and had an insufficient response to MTX. The study showed that the addition of tocilizumab to MTX also improves the quality of life in RA patients who had an insufficient response to MTX (Smolen et al., 2008).

TOWARD: *Tocilizumab in cOmbination With traditional DMARD therapy (Western study, n=1220).*

Not only that tocilizumab has an additive effect on MTX, but the TOWARD trial proved that RA patients, who had an insufficient response to a traditional DMARD therapy (methotrexate, chloroquine, hydroxychloroquine, parenteral gold, sulfasalazine, azathioprine, and leflunomide), had a superior improvement in RA symptoms when they received tocilizumab in combination with the doses of the traditional DMARDs that they received before the study (Genovese et al., 2008).

RADIATE: *RheumAtoiD arthritis study in Anti-TNF-failurEs (Western study, n= 499).*

One of the phase III trials, namely the RADIATE trial, investigated whether tocilizumab was capable of improving the RA symptoms in patients that had an insufficient response to a TNF Inhibitor (Emery et al., 2008). The best known biologicals used in RA patients are TNF-blocking agents, namely infliximab (chimeric anti-TNF), etanercept (soluble TNF receptor) and adalimumab (humanised anti-TNF). All three of these drugs act by partly neutralising circulating and synovial TNF (Klareskog et al.,2009). In the RADIATE study it has been shown that tocilizumab in combination with MTX is effective and safe in RA patients who don't respond to TNF inhibitors. It was even so that RA patient responded to this dual therapy regardless of the number of failed TNF inhibitors. Normally this switching of one TNF inhibitor, towards another increases the chance of a non-satisfactory response (Emery et al., 2008).

STREAM: *long-term Safety and efficacy of Tocilizumab, an anti-interleukin-6 Receptor Monoclonal antibody, in patients with RA (Japanese study, n=164).*

All of these results are very promising, but up to now there has only been one study that investigated the long-term effects of tocilizumab, namely the STREAM study,. This open-label extension trial of tocilizumab demonstrated a sustained good efficacy (figure 16) and a generally good safety profile over 5 years (table 3). The high retention rate at 5 years indeed indicates the favourable efficacy and safety profile. In particular, only one of 143 patients withdrew due to unsatisfactory response, indicating that no general loss of response occurred during long-term treatment (Nishimoto et al., 2008). Moreover, does it seem that the response even gets higher when the years pass.

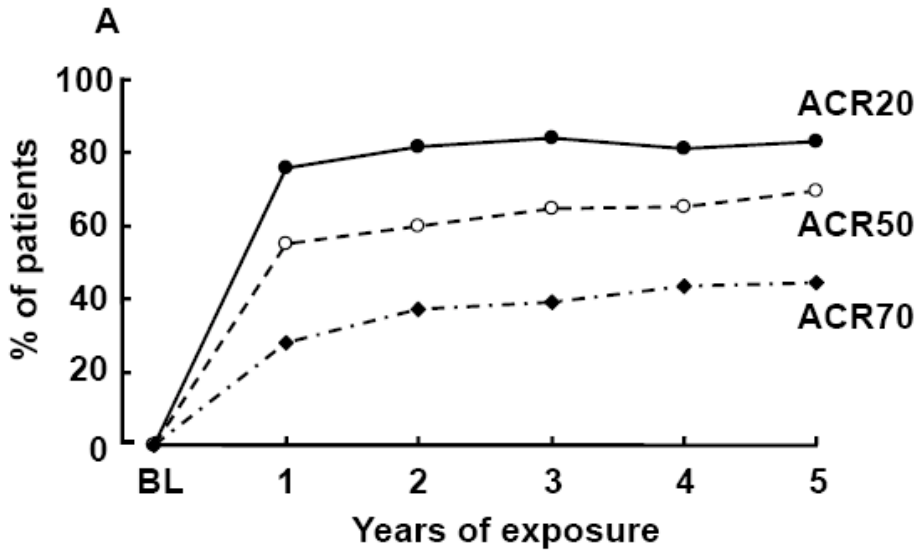

Figure 16: The efficacy of Tocilizumab therapy during 5 years of ongoing medication (Nishimoto et al., 2008).

		%
Any SAE	77	53.8
Joint surgery	20	(14.0)
Pneumonia	9	(6.3)
Herpes zoster	7	(4.9)
Tendon rupture	5	(3.5)
Humerus fracture	4	(2.8)
Spinal osteoarthritis	3	(2.1)
Femoral neck fracture	3	(2.1)
Joint dislocation	2	(1.4)
Back pain	2	(1.4)
Lumbar spinal stenosis	2	(1.4)
Bronchitis acute	2	(1.4)
Pyelonephritis	2	(1.4)
Brain stem infarction	2	(1.4)
Cataract	2	(1.4)
Pneumothorax	2	(1.4)
Liver function abnormality	2	(1.4)

Table 3: Serious adverse events (SAE) observed in RA patients during 5 years of ongoing tocilizumab therapy. 32 patients withdrew from the study due to SAE (Nishimoto et al., 2008).

All these 7 phase III trials proved the efficacy and safety of Tocilizumab for the treatment of RA. The drug has been proven highly effective in monotherapy as well as in combination with conventional DMARD'S. Furthermore tocilizumab is very efficient in patients with an insufficient response to a TNF inhibitor, which opens new doors for a lot of RA patients. All in all this promises a great future for tocilizumab as a medicine in the treatment of RA.

3 Discussion

The results in the previous chapter showed that tocilizumab is very efficient as a drug in the treatment of RA, and has a couple of advantages when compared with TNF inhibitors.
The first one is that TNF inhibitors aren't effective in every patient. Furthermore, the majority of the RA patients that do show a response, only have a partial clinical improvement in their disease (Mima et al., 2009).
Secondly, tocilizumab is the first biological that has a higher efficiency rate than MTX when used as a monotherapeutic drug in RA patients. Not only in RA patients with an inadequate response to MTX, but also in patients who haven't failed any prior therapy (Jones et al., 2008).
Thirdly, in the RADIATE study it has been shown that tocilizumab in combination with MTX is effective and safe in RA patients who do not respond to TNF inhibitors. Moreover was it even so that RA patients responded to this dual therapy regardless of the number of failed TNF inhibitors. Whereas, normally this switching of one TNF inhibitor toward an other, increases the chance of a non-satisfactory response (Emery et al., 2008).
Fourthly, TNF inhibitors often lead to an increase of tuberculosis in treated RA patients (Mima et al., 2009). The STREAM study showed that the treatment of RA patients with tocilizumab doesn't lead to an increased number of tuberculosis (TB) infections. (Patients who had a history of TB infections were not excluded from this study) (Nishimoto et al., 2008).
Finally has it been shown that tocilumab is the first biological to lower the acute phase response in almost all treated RA patients. In fact, it is able to get treated RA patients, as long as they have measurable blood level tocilizumab, completely negative for CRP and serum amyloid A (Nishimoto et al., 2003). This is a good reason to believe that the acute phase response is mediated via IL-6. TNF inhibitors are also able to lower CRP levels, but only a number of patients become completely negative for it (Charles et al., 1999).
The lowered acute phase response that was seen in the tocilizumab treated RA patients, was also seen in IL-6 knockout mice (Kopf et al., 1994). This is another proof that the acute phase response is mediated via IL-6. Moreover, a different mouse model showed the role of IL-6 in the development of RA. SKG mice, normally develop arthritis due to mutations of the T cell signalling pathway (Hata et al., 2004). When the IL-6 gene in these SKG mice was deleted no arthritis developed (Hata et al., 2004).

Although in all the tocilizumab studies the safety profile of this drug is acceptable there are a few things that ask for attention.

At First, Tocilizumab lowers the acute phase response and the number of neutrophils. This may for example suppress the symptoms of an infection such as pneumonia (Fujiwara et al., 2008). Therefore, is it very important to rule out every possible infection, even if the symptoms are minor. Of course is this also the case for other biologicals.

Secondly, the treatment with tocilizumab often leads to an increase in serum cholesterol levels. Moreover in the non-Japanese studies even cases of hyperlipidemia are detected. Although the higher cholesterol levels have not yet

been associated with cardiovascular complications, it would be wise to lower high levels of cholesterol with lipid-lowering agents (Mima et al., 2009).
Tocilizumab has proven to be a very promising drug for the treatment of RA. There are a lot of reasons that you can think of why blocking IL6 is effective in treating RA, but there are two reasons that might play a big role in this and can help to explain the precise mechanism of the drug and the role of IL-6 in RA.

At first there is the role of IL-6 in the formation of Th17 cells. These Th17 cells play a very important role in the development of autoimmune diseases (Iwakura et al., 2006). The induction of the Th17 cells occurs by transforming growth factor beta (TGF-ß) in the presence of IL-6, whereas TGF-ß in the absence of IL-6, induces T regulatory cells (Bettelli et al., 2006). These T regulatory cells prevent autoimmune disease, whereas Th17 cells recruit neutrophils to the site and thus enhance the inflammation. It would be very interesting to investigate the number of Th17 and T regulatory cells in RA patients and to look if the numbers alter in tocilizumab treated RA patients. This could give us a better inside in the role of IL-6 in the pathogenesis of RA and a explanation for the effectiveness of tocilizumab.

Secondly, The SATORI trial showed that tocilizumab decreased the levels of VEGF in the blood (Nishimoto et al., 2008). VEGF is known to play an important role in the angiogenesis, which is required to maintain the chronic inflammatory stateby transporting inflammatory cells to the side of synovitis as well as by supplying nutrients and oxygen to the pannus (Nishimoto et al., 2008). In an asthma study, a disease with also a high level of IL-6, it has been shown that IL-6 via the soluble IL-6 receptor is able to upregulate VEGF, as is depicted in figure 17 (Ammit et al., 2007). It would be very interesting to find the precise pathway in which this upregulation works.

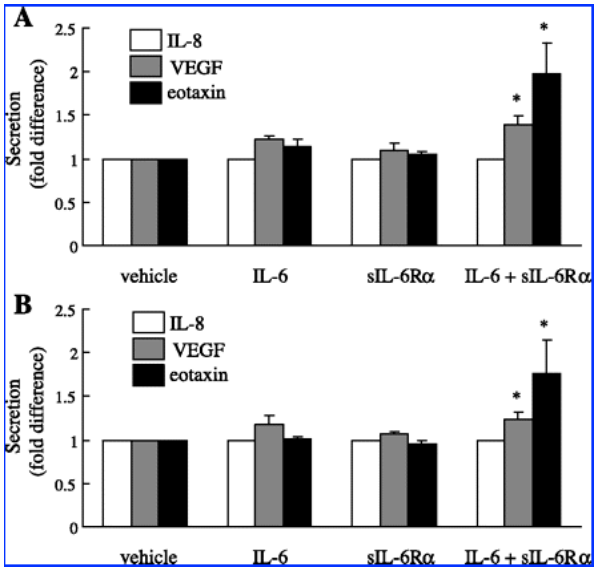

Figure 17: Il-6 induces secretion of proinflammatory cytokines.

Furthermore, a possible drawback of tocilizumab administration is that in all the tocilizumab studies mentioned in this thesis, tocilizumab increases the fatigue of the patients. It has been shown in IL-6 KO mice that IL-6 enhances the non-rapid eye movement (NREM) sleep (Olivadoti et al., 2008). Therefore it would be very interesting to measure the time spent in the NREM sleep in tocilizumab treated patients, because this could show a reason and possible solutions for the enhanced fatigue in tocilizumab treated RA patients.
Nevertheless, overall the results of the studies performed with tocilizumab are very promising. However, it still is very important to find a suiting method to

choose the right drug for each RA patient. If such a prediction becomes possible, patients will achieve clinical remission sooner and the treatment cost will go down. For now, tocilizumab seems to have great potential in treating RA.

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