Clinical studies and tissue analyses in the earliest phases of rheumatoid arthritis: In search of the transition from being at risk to having clinically apparent disease

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GENERAL DISCUSSION
In this thesis clinical aspects (part I) and analyses of the synovium (part II) and the immune response in different compartments (part III) were performed in the earliest stages of rheumatoid arthritis (RA). With these studies we aimed to get more insight into the mechanisms behind the transition from a phase during which individuals are at risk for developing RA, characterized by systemic autoimmunity associated with RA, to having clinically apparent disease.

I. CLINICAL ASPECTS DURING THE EARLIEST STAGES OF RA

Application of the 2010 ACR/EULAR criteria results in earlier fulfillment of classification criteria but increased heterogeneity of RA

Since a diagnostic test is not available for RA, patients are classified for research purposes based on mainly clinical features according to the 1987 American College of Rheumatology (ACR) criteria\(^1\). However, these criteria lead to classification of RA during a late stage of the disease. Moreover, a subset of the early arthritis patients will never fulfill classification criteria and are diagnosed as unclassified arthritis (UA). Accordingly, in this group of patients proper treatment may not always be initiated. To be able to classify RA patients in an earlier phase, and thereby to start early appropriate intervention, new classification criteria were developed, the 2010 ACR/European League Against Rheumatism (EULAR) criteria\(^2,3\). In chapter 2 we studied the implication of introduction of the 2010 ACR/EULAR criteria for RA by comparing application of these new criteria with that of the 1987 ACR criteria in a cohort of early arthritis patients (arthritis duration < 1 year; disease-modifying antirheumatic drug (DMARD) naive). We found that, using 2010 ACR/EULAR criteria, significantly more early arthritis patients were classified as RA in an earlier phase than when using the 1987 ACR criteria. At baseline 36% of the patients previously classified as UA were classified as RA according to the new criteria. Of the patients who were previously classified as UA at baseline, but who did fulfil RA 1987 ACR criteria after 2 years follow up, 85% could already be classified as RA at baseline when applying the new criteria. These are important observations, showing that the main goal of the new criteria, earlier classification of RA patients, was actually reached. In the mean time a large number of publications on the 2010 ACR/EULAR criteria have rapidly appeared and support an earlier diagnosis of RA by application of the new criteria, not only in early arthritis cohorts in Europe\(^4,6\), but also in Canada\(^7\) and New Zealand\(^8\). Although the new criteria result in an earlier classification of RA, we observed two important aspects in association with introduction of these criteria which need further consideration. First, we observed an increased proportion of RA patients with self-limiting disease after follow up when applying the new criteria, similar to results obtained in the Birmingham early arthritis cohort\(^4\). This implies that overdiagnosing could be a result of application of the new criteria, which may lead to unnecessary start of aggressive treatment. Moreover, using the new criteria, we and others have shown that a subset of UA patients has persistent disease\(^9,10\) or will develop erosions\(^5,11\) over time, as is the case when using the 1987 ACR criteria. This suggests that, to facilitate decision making in starting early treatment, there is a sustained need for prediction models for the development of persistent and erosive disease in early arthritis patients, which may be different from fulfillment of classification criteria for RA.
Second, we observed that the clinical characteristics of patients with RA according to the new criteria differ on some important aspects from RA patients fulfilling the 1987 ACR criteria, which was later confirmed in another cohort. We showed that the disease is generally less severe, more often mono- or oligoarticular and less frequent autoantibody positive. This raised the suggestion that RA as classified according to the 2010 ACR/EULAR criteria could be in part a different disease entity than RA as classified according to the 1987 ACR criteria. To investigate this in more detail, our group analysed the synovial tissue infiltrate in a subgroup of these early RA patients, but did not find differences between RA patients who fulfilled 2010 ACR/EULAR criteria only and those who fulfilled 1987 ACR criteria (all also fulfilling 2010 ACR/EULAR criteria) after 2 years of follow up. In addition, a more recent study showed that the incidence rates of RA according to the two different classification criteria converged in the Norfolk Arthritis Register, consisting of 197 early arthritis patients, at around 3.5 years after onset of symptoms. The authors suggested that the new criteria may therefore describe RA in the acute phase whereas the old criteria may describe RA in the established phase when the disease has become chronic. Overall, it is important to recognize that, with respect to clinical characteristics and prognostic outcome, RA disease heterogeneity has increased with the introduction of the 2010 ACR/EULAR criteria. How this will affect clinical trials and whether more strict inclusion or exclusion criteria should be used or stratification by individual criteria is required needs to be addressed in future studies.

Smoking and overweight determine the likelihood of developing RA

Although a diagnostic test is lacking for RA, IgM rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) are present in up to 80% of RA patients and can be present up to 14 years before onset of clinical symptoms. Only a subset of these autoantibody-positive individuals will develop RA over time, but, currently it is unknown which subset will and which subset will not, and why. Importantly, detection of RF and/or ACPA has enabled us to identify individuals who are at increased risk of developing RA and, consequently, to study the mechanisms behind the transition from a phase during which individuals are at risk for developing RA to having clinically apparent disease. Besides the presence of RF and/or ACPA several other risk factors have been associated with RA. Among these are genetic factors, such as HLA-DRB1 alleles and a PTPN22 single nucleotide polymorphism, and environmental factors, such as smoking. So far, most of the risk factors have been studied in cross sectional studies of established RA patients. In chapter 3 we investigated the contributory role of the modifiable factors smoking and overweight to the development of RA in a prospective observational study of autoantibody-positive individuals. We observed an association for smoking and overweight with the development of arthritis. In this cohort the overall arthritis risk of 28% after a median of 27 months follow up increased to 60% in individuals with a smoking history combined with overweight.

Smoking has been associated with both ACPA positive and RF-positive RA, although most studies investigating the mechanism behind the association of smoking and RA have focused on ACPA-positive RA. Whether smoking is associated with merely the presence of ACPA, the height of ACPA levels or with the presence of specific citrullinated peptides...
is as yet unclear. Increased expression of citrullinated peptides\(^{21,26}\) and peptidylarginine deiminase (PAD) enzymes\(^{26}\), which catalyze the citrullination of peptides, has been observed in healthy lungs of smokers compared to non-smokers. However, these citrullinated peptides and PAD enzymes are also present in a subset of non-smokers. In addition, in the periodontium of smokers higher levels of the bacterium Porphyromonas gingivalis are present compared to non-smokers\(^ {27}\). *P. gingivalis* is the only bacterium expressing a PAD enzyme and has the ability to citrullinate proteins\(^ {28}\). Besides the observation that smoking may lead to local citrullination of peptides, smoking may additionally trigger an immune reaction to citrullinated peptides, suggesting that the lung and periodontium may be an early site of RA-related autoimmunity. This is supported on a more clinical level by a study of autoantibody-positive individuals at risk for the development of RA, in which airway abnormalities were comparable to those found in RA patients, and significantly more frequent than in autoantibody-negative controls\(^ {29}\).

Thus far, the results of association studies of obesity and the risk of RA have been variable\(^ {30-36}\), with different results for ACPA positive and negative RA\(^ {33,36}\) and males and females\(^ {34,36}\). Of importance, our study is the first prospective study addressing the possible contributory role of overweight to the development of RA. Mechanistically, not much is known about the contribution of overweight to RA. In established RA BMI seems to be related to disease activity\(^ {37}\) and high BMI has been shown to be associated with a poor response to anti-TNF therapy\(^ {38}\), but BMI is inversely related to joint damage in early RA patients\(^ {37}\). This suggests that body weight might have differential effects on inflammation and joint damage. It has been suggested that the protective effect on joint destruction could be the result of the positive effect of estrogen, produced by the increased proportion of adipose tissue, on bone turnover\(^ {39}\). On the other hand, obese adipose tissue is characterized by adipocyte hypertrophy, leading to activation of inflammatory pathways and recruitment of inflammatory cells, such as macrophages and CD8 positive T-cells, into the adipose tissue\(^ {40}\). It is unknown whether adipose tissue inflammation could lead to citrullination of proteins as well. It is possible that pro-inflammatory activity of adipose tissue in the synovial sublining of the joint contributes to synovial inflammation. However, at present it is unknown whether overweight is associated with increased adipose tissue in the joints or with an increased pro-inflammatory profile of joint adipose tissue. Previous work has shown that articular adipose tissue obtained from RA patients produced both pro- and anti-inflammatory (adipo-) cytokines upon activation, and stimulated production of interleukin (IL-) 6 and IL-8, for example, by fibroblast-like synoviocytes\(^ {41}\). To get more insight into the involvement of adipose tissue in the joint in the development of RA, it would be important to investigate whether overweight is related to local adipocyte hypertrophy in the joint and to investigate the presence and function of adipose tissue derived factors, such as lipid droplets and (adipo)cytokines.

Besides having local pro-inflammatory effects, both smoking and overweight are associated with systemic inflammation. Smoking\(^ {42}\) and overweight\(^ {43}\) lead to an increase in reactive oxygen species, which may result in oxidative stress and subsequent oxidation and damage of lipids and other cell membrane constituents, proteins and DNA, and stimulation of pro-inflammatory signaling cascades. Smoking\(^ {42}\) and overweight\(^ {43}\) may
thus lead to increased apoptosis and thereby increased presentation of intracellular self-antigens. Recently, an interaction between smoking and oxidative stress, assessed by measuring levels of oxidative damage and antioxidant markers in blood samples of RA patients and healthy controls, has been associated with an increased risk of RA. In addition, smoking is associated with increased C-reactive protein (CRP) levels and white cell count as well as increased production of pro-inflammatory cytokines, lipopolysaccharides (LPS) and circulating T cells. Overweight is characterized by increased CRP levels and pro-inflammatory cytokines such as IL-6 and tumor necrosis factor (TNF). An association between BMI and systemic inflammation, assessed by CRP levels and erythrocyte sedimentation rate (ESR), was also observed in our cohort of autoantibody-positive individuals (data not shown). Moreover, overweight is associated with decreased levels of vitamin D and low vitamin D levels have been found in RA patients; of note, vitamin D has a regulatory role in both innate and adaptive immune responses.

Since smoking and overweight are modifiable factors, our observations may have important consequences in clinical practice. However, these results need to be confirmed in larger independent cohorts and it needs to be proven whether smoking cessation and weight reduction will decrease the risk of developing RA in autoantibody-positive individuals. Importantly, an intensive prevention program in Finland aiming at dietary changes and smoking cessation has resulted in long-term prevention of cardiovascular diseases, which was accompanied by a similar decline in the incidence of RA. Such programs might contribute to the prevention of RA and related co-morbidities.

Overall, there is increasing interest in these modifiable factors and their association with RA and knowledge on their mechanistic contribution to RA is steadily growing. However, it is unclear if smoking and overweight specifically contribute to the actual development of synovial inflammation or whether these factors, in this phase of the disease, merely induce and/or increase a systemic autoimmune reaction and a systemic inflammatory state and thereby aid to pass the threshold towards development of arthritis.

II. THE SYNOVIOUM DURING THE EARLIEST STAGES OF RA

Mini-arthroscopy is well tolerated, which supports the use in a research setting from a patient perspective

Studying the target tissue of RA, the synovium, is highly important for several reasons. First, synovial tissue analysis could give insight into disease mechanisms associated with the development and chronicity of the disease and aid to identify therapeutic drug targets. Second, it could be used for studying the mechanism of action of newly developed therapeutic compounds. Third, it could be used to provide proof of mechanism during early clinical development. Synovial biopsies can be obtained by mini-arthroscopic biopsy sampling, performed under local anaesthetics at the outpatient clinic. It is currently performed in a number of research centers and is generally well tolerated with low complication rates. Still, mini-arthroscopy is generally seen as a procedure that is quite invasive and therefore not easily implemented in clinical trials. This is partly due to a lack of experience by researchers and by members of institutional review boards, and
by insufficient information about the patient’s perspective. In chapter 4 we therefore investigated expectations and experience of patients undergoing mini-arthroscopic synovial biopsy sampling in comparison to dynamic contrast-enhanced (DCE) MRI for research purposes. We observed that mini-arthroscopy is well tolerated compared to DCE-MRI for studying synovial inflammation, both in early arthritis patients and in autoantibody-positive individuals without arthritis who are at risk for developing RA. Interestingly, there was a trend, although not statistically significant, towards more early arthritis patients preferring mini-arthroscopy compared to DCE-MRI. Although at baseline the group of autoantibody-positive individuals without arthritis was more reluctant to undergo mini-arthroscopy, after having experienced both procedures the percentage of individuals preferring mini-arthroscopy increased. Overall, these results refute the belief that mini-arthroscopy would be a procedure not well experienced by study subjects and support the use of mini-arthroscopy in a research setting from a patient perspective. Together with the low complication rates, these results support the implementation of mini-arthroscopy for research purposes. This may increase options for collaborations between multiple research centres.

Absence of evident synovial inflammation in the preclinical phase of RA

Previously, it has been shown that a period of subclinical synovial inflammation may precede the onset of clinically apparent arthritis, but the duration of this period in RA was not known. To examine this, a cohort study was initiated at the Academic Medical Center (AMC) in Amsterdam to analyze the synovial tissue of autoantibody-positive individuals who are at risk of developing RA. In a pilot study, no signs of inflammation were observed compared to healthy controls. In chapter 5 we extended these analyses and we examined the relationship between changes in the synovium and development of arthritis over time in a markedly larger, prospective study. Consistent with the previous observations, analyses of MRI-parameters associated with RA and synovial tissue biopsies by immunohistochemistry for the presence of a range of inflammatory cells did not show clear signs of synovial inflammation. We only observed a trend towards an association between subtle synovial infiltration by T cells and the development of RA.

Previous studies have shown that the characteristics of the synovial infiltrate of early RA patients are comparable to those of established RA patients. Our observations indicate that this is not the result of longstanding subclinical synovitis before the onset of RA, but that, when the threshold towards synovial infiltration is passed, the synovial tissue is rapidly infiltrated by immune cells. In addition, our results cannot explain the presence of erosions in patients early after arthritis onset. This might be explained by differences in definition of early disease, the early RA patients with erosions may have been in a chronic phase already, which would be consistent with the clinical experience that patients with a recent onset of clinical signs and symptoms do not exhibit erosive disease.

There was a trend towards an association between the presence of T cells in the synovium and the development of clinically manifest arthritis. When combining presence of synovial T cells with positive ACPA status, the risk of RA increased even more. The expression of citrullinated fibrinogen in the synovial tissue was not associated with
development of arthritis or ACPA status. The fact that we only investigated the expression of citrullinated fibrinogen is a limitation since several other citrullinated antigens could be involved. However, previous work has shown that the presence of citrullinated peptides is not specific for RA synovial tissue\textsuperscript{60, 61}. Initial ACPA formation may not necessarily be directed against joint specific peptides, but rather against citrullinated peptides in other compartments of the body such as the gingiva or lung, as discussed previously. We have previously proposed that a second hit, such as a trauma or viral infection, may lead to citrullination of specific peptides in the synovium, followed by epitope spreading and autonomous disease progression\textsuperscript{53}. This remains speculative, however, and needs to be investigated in more detail.

Overall, these results do not support the hypothesis of a long-lasting period of subclinical synovial inflammation before the onset of arthritis, but suggest that synovial tissue infiltration is a process relatively late in RA pathogenesis and happens (sub)acutely, leading to clinically apparent arthritis. Although clinical symptoms of RA start in the joint, the synovium would thus be a tissue involved in a relatively late phase of the disease, following (long term) systemic autoimmunity. It should be noted, however, that we have selected subjects based on being autoantibody positive. We cannot exclude that other mechanisms may be operative in other subpopulations. It is for instance conceivable that some patients will present with clinically manifest arthritis and subsequently become autoantibody positive. This mechanism would clearly not be captured by our approach. Together, our observations may have some important implications. First, we do not believe that analysis of synovial tissue in autoantibody-positive individuals will be very useful in clinical practice to define who will develop RA. Second, our results may perhaps suggest a role for synovial T cells in the earliest phase of synovial inflammation, which needs to be further explored. Third, the fact that we did not observe clear signs of synovitis in the preclinical, autoantibody-positive, stage makes this a likely phase for effective intervention to prevent the transition to clinically apparent RA. The aim of such preventive regimens would be to prevent infiltration of immune cells into the synovium and hereby to prevent the evolution to a tissue showing autonomous disease progression.

**Arthralgia in the earliest phase of RA cannot be clearly explained by synovial expression of PGE\textsubscript{2} pathway enzymes**

The fact that we did not observe clear signs of synovial infiltration in autoantibody-positive individuals was somewhat surprising, since, in this phase of the disease, arthralgia is not uncommon. This suggests that arthralgia is not necessarily related to the presence of inflammatory cells, at least in the preclinical phase of the disease, but would be caused by the presence of specific pain mediators. One of the major candidate pathways involved in arthralgia is the prostaglandin (PG) E\textsubscript{2} pathway, as is illustrated by the beneficial effects of non steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase (COX) inhibitors on arthritic pain. In addition to its importance in pain sensation, PGE\textsubscript{2} has pro-inflammatory characteristics\textsuperscript{62} and enzymes of the PGE\textsubscript{2} pathway are clearly expressed in RA synovial tissue\textsuperscript{63}. In chapter 6 we therefore investigated whether synovial expression of mediators of the PGE\textsubscript{2} pathway was positively related to arthralgia and contributed
to the development of arthritis in autoantibody-positive individuals at risk of developing RA. In addition, we studied the expression of this pathway in early arthritis patients to determine if it was positively related to arthralgia and associated with disease activity and specificity, or prognostic outcome after follow-up. Since PGE$_2$ is an unstable molecule, we measured its upstream (COX-1, COX-2, microsomal PGE synthase 1 (mPGES-1)) and downstream (15-hydroxy prostaglandin dehydrogenase (15-PGDH)) regulating enzymes. See figure 1 for a schematic overview of the PGE$_2$ pathway.

In both study groups expression of enzymes of the PGE$_2$ pathway was clearly present, both in the intimal lining layer and in the synovial sublining. In both autoantibody-positive individuals and early arthritis patients we did not find clear evidence that arthralgia can be explained by the expression of these enzymes in the synovial tissue. Our observation that synovial PGE$_2$ would not play a major role in pain sensation was supported by the fact that we did not observe lower expression of PGE$_2$ enzymes in the synovial tissue of patients who used NSAIDs/COX inhibitors compared to patients who did not use NSAIDs/COX inhibitors. Since PGE$_2$ can mediate hyperalgesia both locally and centrally, by spinal release, we hypothesize that PGE$_2$ would contribute to pain sensation centrally rather than in the synovium. Moreover, PGI$_2$ (prostacyclin), which has been shown to mediate pain hypersensitivity, could play a role in arthralgia as well. In addition, we cannot exclude a role for other pain mediators which could exert their effects in the synovium, such as bradykinin, histamine, adenosine triphosphate (ATP) and acetylcholine. Moreover, the pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin-6 (IL-6) may be involved as well.

With respect to PGE$_2$ as a possible stimulus of inflammation we did not find evidence for a major role in the development of arthritis in autoantibody-positive individuals. In

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**Figure 1.** Schematic overview of the PGE2 pathway (by P. Leclerc). A.A.: arachidonic acid, COX: cyclooxygenase, PG: prostaglandin, mPGES-1: microsomal PGE synthase 1, 15-PGDH: 15-hydroxy prostaglandin dehydrogenase, PGI2: prostacyclin, Tx: thromboxane, NSAIDS: non-steroidal anti-inflammatory drugs, Coxibs: COX inhibitors. To produce PGE2 arachidonic acid is metabolized into prostaglandin H2 by the enzymes COX-1 or COX-2. PGH2 yield from cyclooxygenase activity is subsequently transformed into PGE2 by one of three prostaglandin E synthases. Under inflammatory conditions mPGES-1 plays, together with COX-2, a predominant role in the synthesis of PGE2. PGE2 levels are further regulated at the catabolic end by the enzyme 15-PGDH.
early arthritis patients, expression of these enzymes correlated with infiltration by T cells and macrophages, but was not related to prognostic outcome. The role of the PGE_2 pathway is complex, however, since PGE_2 can also negatively regulate inflammation by stimulating the production of anti-inflammatory cytokines such as IL-10. In the group of early arthritis patients we observed increased synovial expression of mPGES-1 and COX-1 in spondyloarthritis (SpA) patients compared to RA and UA patients and a similar trend for COX-2. This supports a previous study in which synovial expression of COX-2 was higher in SpA patients compared to RA patients. Moreover, a role for the PGE_2 pathway in the pathogenesis of SpA has been shown by the positive effects of the use of NSAIDs, which are the first-line drug treatment for complaints of pain and stiffness in SpA patients. In addition, NSAIDs have a beneficial effect on the ossification of the spine in SpA patients. This is most probably the result of interference with the stimulatory effects of PGE_2 on osteoblastogenesis by increasing the replication and differentiation of osteoblasts. Our results suggest that this might be linked to the presence of PGE_2 produced in the synovial tissue. Further research should elucidate the role of the PGE_2 pathway in the pathogenesis of SpA in more detail.

Overall, we did not find clear evidence that arthralgia in subjects at risk of developing RA or in early arthritis patients can be explained by altered synovial expression of enzymes of the PGE_2 pathway. Pain in these patients may therefore be regulated by other pathways or originate at sites other than the synovium, which needs to be addressed in future studies. In addition, we observed increased synovial expression of enzymes of the PGE_2 pathway in SpA patients compared to RA and UA patients, which is in line with previous studies suggesting involvement of the PGE_2 pathway in the pathogenesis of SpA and with the beneficial effect of NSAID treatment in these patients.

III. THE IMMUNE RESPONSE IN DIFFERENT COMPARTMENTS DURING THE EARLIEST STAGES OF RA

Inguinal lymph node biopsy sampling and analysis is a feasible research tool and shows increased immune cell activation during the earliest phases of RA

In chapter 5 we showed that systemic autoimmunity precedes synovial inflammation in RA. Since even in individuals who developed arthritis 1 month after synovial biopsy sampling no clear signs of subclinical synovitis were observed, we propose that synovial inflammation develops in a (sub)acute way. This hampers studies of the synovium during the transition phase. To gain new insights into the earliest phases of RA pathogenesis and in the initiation of synovial infiltration it will be important to study compartments other than the synovium. As a general principle, the recruitment of activated immune cells to the site of inflammation is initiated after informing a nearby lymph node of a danger signal. Thus, the immune reaction in lymph nodes generally precedes the influx of effector cells into the target tissue. For this reason, we initiated lymph node biopsy sampling in different phases of RA. In chapter 7 we described the technique of core-needle biopsy sampling of inguinal lymph nodes and we presented its purpose as a new research tool in RA, which may facilitate research in other centers. Moreover, we showed that the procedure was generally well tolerated and that, other than a small hematoma requiring no therapy in most of the
cases, no major complications were reported. In chapter 8 we investigated the cellular composition of lymph node biopsies of autoantibody-positive individuals in comparison to healthy controls and early arthritis patients. First, we observed an increase in activated CD8 positive CD69 positive T cells in early arthritis patients compared to healthy controls. This may be in line with animal models of arthritis showing a decreased CD4/CD8 ratio in lymph nodes before the onset of arthritis\textsuperscript{71,72}. However, since none of the autoantibody-positive individuals in this cohort had as yet developed arthritis, due to a short follow up period, future studies are needed to confirm this. Nevertheless, our observations do seem to support a role for T cells in the earliest phase of RA\textsuperscript{73-75}. Future research should focus on the identification of T-cell subsets and antigen-specificity in association with the development of RA. Second, we observed an increase in CD19 positive B cells in early arthritis patients compared to healthy controls and a similar trend for the autoantibody-positive individuals compared to healthy controls. The latter would be in line with findings in animal models of arthritis\textsuperscript{71,76}. Previously, gene expression profiling in autoantibody-positive individuals revealed a low peripheral blood B-cell signature compared to RA patients, especially in those individuals who developed arthritis after follow up\textsuperscript{77}. In chapter 5 we showed that B cells were clearly absent in the synovium of all but one of the autoantibody-positive individuals studied. Together with the observed increase in B cells in the lymph nodes of autoantibody-positive individuals, this could imply that B cells are retained in the lymph nodes to ensure maturation and differentiation during the immune response after which they may infiltrate the synovium. To investigate this in more detail, it would be important to analyze serial and paired samples of all three compartments in autoantibody-positive individuals towards the onset of arthritis.

Increased expansion of synovial T cells especially during the early phase of RA

Interestingly, our observations in both chapter 5 and chapter 7 suggest involvement of T cells in the earliest phases of RA. The exact role of T cells in the pathogenesis of RA is not completely clear\textsuperscript{73,75}. However, the abundant presence of T cells in the synovium of RA patients, already in the early phase of the disease\textsuperscript{55}, and the association of RA with T-cell related HLA-DRB1 alleles and a PTPN22 single nucleotide polymorphism\textsuperscript{18,19} suggest an important role for T cells.

Sequencing of the T-cell receptor (TCR) repertoire is a technique that allows for the detection of clonal expansion of T cells. This may help to determine the presence of expanded (autoreactive) T cells and, vice versa, to identify autoantigens. In chapter 9 we quantitatively profiled the TCR repertoire in paired synovium and peripheral blood of patients with early and established RA to identify expanded, potential autoreactive, T-cell clones. We found an increased percentage of highly expanded T-cell clones in the synovium compared to the peripheral blood of early RA patients, which was not the case in established RA patients. The total number of these highly expanded clones was limited (suggesting oligoclonal expansion) and there was hardly any overlap in specific TCR sequences between highly expanded clones in the synovium and peripheral blood within patients. Together, this suggests that in early RA autoreactive T cells are retained in the synovium, possibly in response to the presence of specific (auto)antigen(s) in the synovium.
This notion is supported by the detection of overlapping TCR sequences in the synovial compartment within patients. However, since there was no overlap in TCR sequences of highly expanded T-cell clones between patients it seems that specific antigenic epitopes do not overlap between patients. The fact that we did not observe oligoclonal expansion in the chronic phase of the disease could be the result of epitope spreading during the disease, which would lead to an increase in the number of (auto)antigens and expanded T-cell clones, decreasing the overall frequencies of these clones in the T-cell repertoire. Another hypothesis could be that the early disease phase is characterized by a T-cell reaction to specific antigen(s) and that in a later phase the T-cell reaction is broadened in response to for example antigens from apoptotic cells. To further extend these analyses it would be necessary to characterize the expanded T-cell clones in detail. Moreover, since we observed an increased percentage of activated CD8 positive T-cells in lymph nodes of early arthritis patients it would be interesting to investigate whether there is overlap in expanded T-cell clones between lymph node and synovial tissue samples in these patients. If so, this would indicate that joint specific antigens are presented in the lymph nodes, upon which specific T cells could proliferate and subsequently migrate to the synovium. In addition, together with the observation in chapter 5 that subtle T cell infiltration may precede the onset of clinically apparent arthritis in autoantibody-positive individuals, it would be interesting to investigate T-cell clonal expansions in the synovium in this earlier disease phase as well to determine whether these T cells have specifically expanded upon the presence of synovial (auto)antigens. Also in this case further characterization of the expanded T-cell clones is essential.

CONCLUDING REMARKS

Over the past few years research in the field of RA has focused on the earliest stages of the disease. In 2011 the EULAR established the Study Group for Risk Factors for Rheumatoid Arthritis to facilitate research in this area. As a start off this study group has reported on terminologies to be used in the disease phase during which individuals are at increased risk of developing RA up to the actual development of RA. Main research topics that were defined were to identify risk factors and biomarkers for the development of RA as well as for long term outcomes. Moreover, it was established that in the systemic autoimmunity phase not only the main target tissue, the synovium, should be investigated, but also other tissues which may be involved in the initiation of the immune response. Important tissues in this respect may include the lymph nodes, the lungs and periodontal tissue. How do the findings described in this thesis contribute to these research areas?

The advent of the newly developed 2010 ACR/EULAR classification criteria for RA has been a major step forward in establishing an early diagnosis for research purposes. However, these criteria were not developed to establish the prognosis in the individual patient, and there is still a need for better prediction models, which could facilitate early therapeutic decisions.

Our results help to better define a population at high risk of developing RA, based on the presence of RF and ACPA in combination with a history of smoking and overweight. The importance of these findings is underscored by the increased incidence of RA over the
last decades, which cannot be explained by genetic factors, but is most likely influenced by environmental and lifestyle factors\textsuperscript{31}. It will be important to validate these observations, to determine the biological mechanisms behind their contribution to RA and to evaluate whether measures to stop or prevent smoking and overweight will decrease the risk of RA. Recently, a clinical trial has been initiated evaluating the effects of B-cell depleting therapy during the preclinical phase; the results of this study remain to be awaited. Since smoking and overweight are modifiable factors, preventive strategies focusing on these factors should be explored as well to reduce the risk of developing RA.

By studying different body compartments, including the synovium and lymph nodes, in the earliest stages of RA we showed that synovial infiltration is preceded by systemic autoimmunity and possibly by immune activation in the lymph nodes. In addition, this thesis provides some support for an important role for T cells in the earliest stages of RA. Together, these studies contribute to the existing knowledge on the disease pathogenesis. Future studies should build on these and pave the way for curative and preventive interventions.
REFERENCE LIST


9. de Hair MJ, Lehmann KA, van de Sande MG, Majer KI, Gerlag DM, Tak PP. The clinical picture of rheumatoid arthritis according to the 2010 American College of Rheumatology/European League Against Rheumatism criteria: is this still the same disease? Arthritis Rheum 2012;64(2):389-393.


68. Braun J, van den Berg R, Baraliakos X et al. 2010 update of the ASAS/EULAR recommendations for the management...


