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

de Hair, M.J.H.

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GENERAL INTRODUCTION

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Maria J.H. de Hair*, Leonard C. Harty*, Danielle M. Gerlag, Constantino Pitzalis, Douglas J. Veale, and Paul P. Tak
*Both authors contributed equally

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RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by pain, swelling and stiffness of the joints due to synovial inflammation. Inflammation of the synovium is the hallmark of the disease. In socioeconomic terms, RA is the most common and most important of the inflammatory arthritides. The inflamed synovium expands into and destroys the underlying cartilage and bone resulting in irreversible erosion of the bone and eventually in loss of normal joint architecture and disability. It is a debilitating chronic erosive disease that affects 1-2% of the population worldwide and affects females three times more than males. It is associated with an increased incidence of cardiovascular morbidity and mortality.

The management of RA includes both drug therapy and non-pharmacological measures, such as physiotherapy, occupational therapy and, in case of destructive disease, joint surgery. Drug therapy consists of (a combination of) non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and disease-modifying antirheumatic drugs (DMARDs). During the last few decades, treatment options for RA have largely improved, not only by an increase in the availability of DMARDs, but also by increased availability of biological targeted therapies, such as tumor necrosis factor (TNF) inhibitors, B-cell depleting therapy, CTLA4-Ig treatment, and interleukin-6 (IL-6) receptor antibody treatment. Patient-specific treatment should be initiated early after disease onset, as there is a therapeutic window of opportunity during which antirheumatic treatments are more effective and joint destruction can be reduced or halted.

To allow initiation of appropriate treatment, RA patients should be diagnosed in an early stage of the disease. However, a subset of the early arthritis patients cannot be diagnosed during early disease due to the heterogeneity of the disease and the lack of definitive diagnostic markers, and are diagnosed as undifferentiated arthritis patients. Accordingly, in this group of patients proper treatment may not always be initiated.

DISEASE HETEROGENEITY AND DIAGNOSIS OF RA

Although RA is most typically characterized by symmetrical pain and swelling in the small joints of hands and feet, it is a heterogeneous disease, both on a clinical and a molecular level. Clinically this is characterized for example by variability in joint involvement, which can be symmetric or asymmetric and can include small joints only, large joints only, or a combination. Patients can present with or without extra-articular manifestations, such as rheumatoid nodules, pleuritis and vasculitis. Moreover, there is large variation with respect to response to treatment regimens. In addition, the prognostic outcome can be variable, ranging from self-limiting disease to persistent disease with or without joint destruction. On a molecular level, the heterogeneity of the disease is characterized for example by the presence or absence of RA-specific autoantibodies like IgM rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), which are present in up to 80% of the RA patients. When we look into the synovial tissue, there is heterogeneity in patterns of cell infiltration and gene expression signatures. Together, this suggests that the phenotype described as RA may be the result of different pathogenetic pathways.
Due to heterogeneity of the disease it is difficult to diagnose patients based on a single diagnostic test. For research purposes, RA patients have been classified for many years based on mostly clinical features, according to the 1987 American College of Rheumatology (ACR) criteria for RA\textsuperscript{10} (Table 1).

Of importance, these criteria were developed to differentiate RA patients from arthritis patients having other established rheumatologic disorders. One of the criteria, for example, is ‘radiographic changes typical of RA’, which is generally a feature of relatively late disease. The 1987 ACR criteria for RA were developed to classify RA patients during a late stage of the disease. More recently, new classification criteria focusing on early RA were developed by a joint venture of the ACR and the European League Against Rheumatism (EULAR), resulting in the 2010 ACR/EULAR criteria for RA\textsuperscript{11,12} (Table 2).

An important difference with the 1987 ACR criteria is the omission of ‘radiographic changes’ from the criteria. Secondly, the more recent discovery of ACPA being highly specific for RA\textsuperscript{7} led to the inclusion of ACPA in the criteria.

The advent of the new criteria leads to new questions. Could the 2010 ACR/EULAR criteria result in false positive classification of patients with self-limiting disease as having RA? What would be the effect on the disease heterogeneity when these classification criteria are applied?

\textbf{Table 1. 1987 ACR criteria for RA}\textsuperscript{10}

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td><strong>1. Morning stiffness</strong></td>
<td>Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement</td>
</tr>
<tr>
<td><strong>2. Arthritis of 3 or more joint areas</strong></td>
<td>At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints</td>
</tr>
<tr>
<td><strong>3. Arthritis of hand joints</strong></td>
<td>At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint</td>
</tr>
<tr>
<td><strong>4. Symmetric arthritis</strong></td>
<td>Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)</td>
</tr>
<tr>
<td><strong>5. Rheumatoid nodules</strong></td>
<td>Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician</td>
</tr>
<tr>
<td><strong>6. Serum rheumatoid factor</strong></td>
<td>Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in &lt;5% of normal control subjects</td>
</tr>
<tr>
<td><strong>7. Radiographic changes</strong></td>
<td>Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)</td>
</tr>
</tbody>
</table>

For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded.
Table 2. 2010 ACR/EULAR criteria for RA\textsuperscript{11, 12}

<table>
<thead>
<tr>
<th>Target population (Who should be tested?): Patients who</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>1. have at least 1 joint with definite clinical synovitis (swelling)*</td>
<td></td>
</tr>
<tr>
<td>2. with the synovitis not better explained by another disease†</td>
<td></td>
</tr>
</tbody>
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Classification criteria for RA (score-based algorithm: add score of categories A–D; a score of ≥6/10 is needed for classification of a patient as having definite RA)‡

A. Joint involvement §
- 1 large joint¶ 0
- 2-10 large joints 1
- 1-3 small joints (with or without involvement of large joints) 2
- 4-10 small joints (with or without involvement of large joints) 3
- >10 joints (at least 1 small joint)** 5

B. Serology (at least 1 test result is needed for classification)††
- Negative RF and negative ACPA 0
- Low-positive RF or low-positive ACPA 2
- High-positive RF or high-positive ACPA 3

C. Acute-phase reactants (at least 1 test result is needed for classification)‡‡
- Normal CRP and normal ESR 0
- Abnormal CRP or abnormal ESR 1

D. Duration of symptoms§§
- <6 weeks 0
- ≥6 weeks 1

* The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of rheumatoid arthritis (RA) with a history compatible with prior fulfilment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.
† Differential diagnoses vary among patients with different presentations, but may include conditions such as systemic lupus erythematosus, psoriatic arthritis, and gout. If it is unclear about the relevant differential diagnoses to consider, an expert rheumatologist should be consulted.
‡ Although patients with a score of <6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.
§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment. Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.
¶ “Large joints” refers to shoulders, elbows, hips, knees, and ankles.
# “Small joints” refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.
** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).
†† Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low-positive for RF. ACPA = anti-citrullinated protein antibody.
‡‡ Normal/abnormal is determined by local laboratory standards. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.
§§ Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.
PATHOGENESIS OF RA

The etiology of RA is largely unknown, but several risk factors associated with RA have been recognized. Genetic risk factors as well as environmental risk factors and the interaction between these play a key role. However, the factors leading to the onset of synovial inflammation are currently unknown.

Risk factors

Besides the presence of RA-specific autoantibodies, several other risk factors have been associated with RA. HLA-DRB1 and PTPN22 (the latter in Caucasian populations) have the largest genetic contribution to RA susceptibility, but many other genes have been associated with RA as well, such as CTLA4, CD40, PADI4, CD2/CD58, and CD2/CD58, although with much smaller contributory effect. The genetic loci which have currently been associated with RA have mainly been associated with ACPA-positive RA. In addition, these genetic factors probably contribute for not more than 50% to the genetic susceptibility, and overall explain only a part of the susceptibility to RA. Environmental factors appear to contribute to the pathogenesis of RA as well. Examples are smoking, obesity, low vitamin D levels, viral infections and a history of periodontitis. The exact mechanisms of how these factors contribute to the development of RA are unknown, although an important interaction between autoimmune, genetic and environmental factors has recently been shown. Collectively, an accumulation of several risk factors and their interaction may lead to a breach of immune tolerance.

Inflammation of the synovium

The synovium is a soft tissue layer lining non-cartilagenous joint surfaces. Its physiologic function is to secrete synovial fluid which lubricates the joint and nourishes the avascular cartilage. In the healthy state the synovial tissue is composed of one to three layers of specialized columnar cells called fibroblast-like synoviocytes (FLS) with interspersed macrophages. Lymphatic and blood vessels as well as nerve fibres are present in the healthy synovium and it can contain adipocytes. It is divided into an intimal lining layer without an underlying basement membrane, and a synovial sublining layer which is continuous with the joint capsule.

As synovitis is the primary pathogenic event underlying signs and symptoms of arthritis in RA, we endeavour to better understand its pathology. Autoimmune activation, coupled with up-regulation of pro-inflammatory cytokines and mobilization of inflammatory cells to the synovium play considerable roles but the precise etiology of the disease is as yet unclear. Microscopic analysis of the synovium has given us some insight into the pathogenesis of RA. Rheumatoid synovial tissue is hypertrophic and edematous and is characterized by marked intimal lining hyperplasia and by accumulation of T lymphocytes, plasma cells, macrophages, B lymphocytes, neutrophils, mast cells, natural killer cells, and dendritic cells (DC) in the synovial sublining. Villous projections of synovial tissue protrude into the synovial cavity and erode into the underlying cartilage and bone. Neo-angiogenesis, the development of new blood vessels, within the inflamed synovium facilitates the migration of leukocytes and contributes to the perpetuation of this chronic disease.
As already mentioned, the factors leading to synovial inflammation are currently unknown. Presumably, antigens in close contact to the synovium are presented to antigen presenting cells (APCs) such as DCs and FLS, leading to T- and B-lymphocyte activation. Upon activation T lymphocytes produce pro-inflammatory cytokines and adhesion molecules, and B lymphocytes start to produce antibodies which may lead to deposition of immune complexes in the joint and subsequent cytokine production. In addition, activated B lymphocytes may play a role in co-stimulation of T-lymphocytes. Together, the immune activation may result in recruitment of other inflammatory cells into the synovium. The presence of immune cells in a pro-inflammatory environment may trigger an inflammatory cascade, resulting in severe synovial inflammation.

At current, RA-specific antigens have as yet not been detected, and it is believed that self-antigen, recognized by autoreactive T- and B lymphocytes, may play an important role. Candidate antigens are citrullinated peptides, to which ACPAs are directed, which have been demonstrated in synovial tissue. However, antigens initiating this immune response are not specific for RA and may not be joint-specific, since the presence of citrullinated proteins is also found in other forms of inflammation. It has been hypothesized that citrullination of peptides in the lungs, due to cigarette smoking, or in the gingiva, due to periodontitis, may be important for triggering the immune activation cascade, leading to systemic autoimmunity. What determines the transition from the phase characterized by circulating ACPAs to chronic synovitis? We have previously proposed that a ‘second hit’ to the synovium may be necessary to induce citrullination of peptides in the synovium. This might, for example, be a minor trauma or a viral infection. In the presence of pre-existing immunity against citrullinated antigens at sites other than the synovium, citrullination of peptides in the synovial tissue due to an inflammatory response to this second hit, might lead to epitope spreading and autonomous progression of synovitis. Clearly, this hypothesis still needs to be proven.

There has been an enormous upsurge of studies on the synovial tissue response to treatment during the last 20 years. Synovial cell infiltration, particularly by CD68 positive macrophages, and macrophage-derived cytokine expression are reduced after prednisolone therapy with a significant correlation with beneficial clinical effect. This effect is not restricted to corticosteroid treatment, as several experiments have consistently shown that the quantity of CD68 positive macrophages in the synovial sublining is reduced concurrent with a reduction in disease activity. It has also been shown that when therapy has failed and inflammation persists, CD68 positive macrophages do not decrease in number, further supporting its use as an accurate biomarker that can be used on the group level to distinguish effective from ineffective treatment. Accordingly, the expression of pro-inflammatory cytokines and inflammatory cells is reduced in the synovial membrane of treated RA patients with low disease activity. Synovial tissue analysis has also provided insight into biomarkers predictive of the response to treatment in individual patients. Pre-treatment synovial inflammation levels, TNF expression and the presence of lymphocyte aggregates all correlate with the therapeutic response to infliximab. The clinical response to rituximab treatment is related to the change in B cell derived plasma cells in the synovium. Together, these findings illustrate that
descriptive studies of the rheumatoid synovium help us to understand the events that take place in vivo and complement experimental animal studies as well as in vitro studies.

**PRECLINICAL RA**

RF and ACPA can be present 10-14 years before the development of RA. Besides the presence of RA-specific autoantibodies before the onset of arthritis, other signs of systemic immune activation have been observed during that phase, such as increased C-reactive protein and monocyte chemoattractant protein-1 levels. In addition, broadening of the ACPA repertoire due to epitope spreading towards the onset of arthritis in ACPA-positive RA patients has been reported. Together, these observations suggest the presence of an RA-specific adaptive immune response before clinical signs of arthritis are present. However, only a subset of the individuals who are positive for RF and/or ACPA will develop RA over time. At current, it is unknown which subset of these autoantibody-positive individuals will make the transition to the development of clinically apparent RA and which subset will not, and why.

Detection of RF and ACPA has enabled us to identify individuals having systemic autoimmunity associated with RA who are at risk of the development of the disease. This has greatly facilitated research on the earliest stages of the disease.

**The synovium during preclinical RA**

Despite growing insight into the characteristics of the synovial inflammation in RA, which has led to better directed therapies targeting specific molecules and pathways, not much is known about the local processes at play before the development of RA. Studies comparing characteristics of the inflamed synovial tissue of early arthritis patients with those of patients with established disease have shown that there are no major differences with respect to the cellular infiltrate, cytokines or adhesion molecules, suggesting that early arthritis already reflects chronic disease and that, before arthritis becomes clinically apparent, a period of subclinical synovial inflammation might exist. Firstly, this is supported by the observation that a subset of early onset arthritis patients presents with erosions. Secondly, several studies analyzing unaffected joints of RA patients have revealed comparable infiltration of the synovium by the major inflammatory cell types and expression of cytokines to affected joints. Thirdly, using ultrasound imaging in patients with early oligoarthritis and in autoantibody-positive individuals who are at risk of developing RA signs of synovitis were observed in joints without clinically apparent arthritis. Lastly, using a collagen induced arthritis model in rhesus monkeys, increased infiltration of the synovial tissue by T cells and macrophages was observed after immunization with type II collagen before the onset of clinical arthritis. In contrast, the first analyses of the synovial tissue in a small group of autoantibody-positive individuals at risk of developing RA have not revealed clear inflammation when compared to the synovium of healthy controls. To be more conclusive about possible subclinical synovial inflammation preceding the development of arthritis in autoantibody-positive individuals, a prospective follow-up study is needed in a larger group of individuals.
AIM AND OUTLINE OF THIS THESIS

In this thesis we studied the earliest stages of RA to get more insight into the mechanisms behind the transition from a phase in which individuals are at risk for developing RA, characterized by systemic autoimmunity associated with RA, to having clinically apparent disease. Part I of this thesis focuses on clinical aspects, part II focuses on the main target tissue of RA, the synovium, and part III focuses on the immune response in different compartments during the earliest stages of RA.

PART I: CLINICAL ASPECTS DURING THE EARLIEST STAGES OF RA

In chapter 2 we investigate the impact of application of the 2010 ACR/EULAR criteria instead of the 1987 ACR criteria for RA on the number and the time frame of classification of early arthritis patients as RA as well as on the clinical characteristics of patients classified as RA.

In chapter 3 we address the contributory role of smoking and high body mass index (BMI) to the development of RA in a prospective cohort of autoantibody-positive individuals with an increased risk of developing the disease.

PART II: THE SYNOVIIUM DURING THE EARLIEST STAGES OF RA

In chapter 4 we investigate the experience of patients undergoing mini-arthroscopy in comparison to MRI in a research setting.

In chapter 5 we perform synovial tissue analysis in autoantibody-positive individuals with an increased risk of developing RA in relation to the development of the disease, to get more insight into the pathogenic events in the synovial tissue which may lead to clinically apparent disease.

In addition, since these autoantibody-positive individuals may suffer from arthralgia, we investigate the prostaglandin (PG) E\textsubscript{2} pathway, a major pathway in pain sensation, in the synovial tissue of these individuals in chapter 6. In parallel, since PGE\textsubscript{2} is also a pro-inflammatory mediator, we investigate this pathway in the synovial tissue of early arthritis patients in relation to several disease characteristics.

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

Besides analysing the synovial tissue in studying the earliest phases of RA, it might be important to investigate other compartments of the immune system which may be involved in early immune activation. In chapter 7 we describe the goals and the procedure of inguinal lymph node biopsy sampling for studies investigating the earliest phases of RA pathogenesis and in chapter 8 we describe the cellular composition of these lymph node biopsies.

In chapter 9 we investigate the T-cell receptor repertoire in peripheral blood and synovial tissue of early and established RA patients, to evaluate the possible role of expanded T-cell clones in the pathogenesis of RA.
REFERENCE LIST


