Systemic immune markers characterizing early stages of rheumatoid arthritis
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Chapter 1

Introduction and outline of the thesis
The essence of immunity is to defend against foreign matter (i.e. pathogens) while maintaining tolerance to self. This state of immune tolerance is the cornerstone of protection against autoimmune diseases. The balance between an effective immune response and its ability to timely auto-suppress such response, ensures healthy living. Among numerous factors (internal and external) that disrupt this balance, aging is considered to be important. The human body's ability to defend itself against infectious diseases, cancer and autoimmune diseases has been demonstrated to decline with age. Accordingly, development of rheumatoid arthritis (RA), one of the most common autoimmune diseases, has been associated with the aging of the immune system (1).

Numerous alterations of the immune response have been described in RA patients. These include increased expression levels of various cytokines, such as IL-1β, IL-2, IL-6, TNF-α in the peripheral blood (PB), synovial fluid (SF) and synovial tissue from RA joints (2-5). Further immune alterations attributed to RA pathology are: increased numbers of T-cells with a senescent or exhausted (terminally differentiated) phenotype (6-8); decreased numbers of circulating NK-cells (9, 10); disturbed regulatory T-cell function (11); telomere erosion of hematopoietic stem cells (12-14) and subsequent premature telomere shortening of circulating leukocytes (15, 16); expansion of monocytes (17, 18), T-cells (5, 19) and B-cells (3, 20, 21) with pro-inflammatory effector functions in PB and SF. Furthermore, RA is characterized by the presence of autoantibodies. Approximately 70% of RA patients are seropositive for autoantibodies, such as anti-cyclic citrullinated peptide antibodies (ACPA) and ~80% are seropositive for rheumatoid factor (RF) (22, 23). Seropositivity for autoantibodies (e.g. ACPA) is not only one of the diagnostic criteria for RA (24), but its presence in otherwise healthy people indicates a high risk of future RA development (25-29).

Autoantibodies are not just disease markers but may have an active, functional role in RA pathogenesis. Autoantibodies can induce pro-inflammatory cytokine production by FcγR-dependent triggering of macrophages in vitro (30-32). In the pre-clinical stage of RA, emergence of ACPA and RF as well as a broadening of the ACPA repertoire (epitope spreading) preceded the elevation of serum cytokine levels (27, 29).

Aggressive treatment early in the disease course has been shown to lead to long-term improvement and reduction of the risk of radiographic progression (33-36). A step forward in RA research, based on the concept of the therapeutic “window of opportunity”, involves postponing/preventing the disease development by therapeutic intervention before the onset of all clinical symptoms of RA (37-39). Recent studies suggest that subjects at high risk of RA development, thus most eligible for a preventive intervention, include first-degree relatives of RA patients and seropositive arthralgia patients (SAP) (40, 41).

Inclusion of well-defined cohorts, such as SAP, in basic translational research and in clinical studies may thus likely improve our understanding of the immunopathogenesis of RA. Similarly important is the inclusion of RA patients who have only recently been diagnosed with RA and who are not yet treated
with disease modifying anti-rheumatic drugs (DMARDs, second-line treatment), corticosteroids or biologics (third-line treatment). This approach may allow to identify immune defects primarily involved in the disease, and to avoid focusing attention on changes only secondary to a long-term inflammatory process. It also excludes the possibility that the observed immune alterations (or the lack thereof) are a consequence of the drug’s effects (or the drug-induced normalization of the primary immune defects).

Our overall goal was to identify systemic immune markers characterizing early stages of RA, as these may provide clues as to the primary factors involved in the development of a pathologic autoimmune response. Therefore, in all our studies, patients at the very early stage of the disease - newly diagnosed with RA, DMARD/glucocorticoids/biologics-free as well as seropositive arthralgia patients (SAP) were asked to participate. SAP are at high risk of developing RA (41). According to the European League Against Rheumatism (EULAR) recommendations, this risk is based on the presence of systemic autoimmunity associated with RA and symptoms without clinical arthritis (42) (Fig. 1). Similar to newly diagnosed RA, immune alterations occurring in SAP may represent primary defects involved in the pathogenesis of RA, rather than secondary effects of chronic immune stimulation as seen in later phases of RA.

Figure 1. Depiction of the cohorts, as well as body compartments: peripheral blood (indicated with upper circles) and joints (indicated with lower circles) studied.

<table>
<thead>
<tr>
<th>HC</th>
<th>SAP</th>
<th>RA</th>
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<tbody>
<tr>
<td>- Systemic autoimmunity associated with RA</td>
<td></td>
<td></td>
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<tr>
<td>- Symptoms without clinical arthritis</td>
<td></td>
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<tr>
<td>(Gerlag et al, 2012)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Joint involvement (synovitis)</td>
<td></td>
<td></td>
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<tr>
<td>- Symptom duration ≥ 6 weeks</td>
<td></td>
<td></td>
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<tr>
<td>- RF and/or ACPA positive</td>
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<td></td>
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<tr>
<td>- CRP and/or ESR positive</td>
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<td>(2010 ACR/EULAR classification criteria for RA)</td>
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</table>
Some of the immune markers investigated in our cohorts, were previously described in long-standing, treated RA only.

In order to elucidate a role of autoantibodies in inflammatory processes, early RA cohorts were stratified according to ACPA/RF status, into seropositive (ACPA+ and/or RF+) and seronegative (ACPA- and RF-).

In the studies reported in this thesis, we focused primarily on the periphery, while the local inflammatory sites (synovial fluid and tissue) were studied only in late-stage RA (Fig. 1). This approach stems from the notion that RA does not begin at the level of the joint but rather that systemic inflammation precedes synovitis (29). In line with this notion, recent studies showed no evidence for the presence of subclinical synovitis in SAP (43, 44).

The main objective of our studies was to identify immune alterations that may play a role in RA onset:

In Chapter 2, in a comprehensive review, we have summarized and evaluated published data on the role of immunosenescence in rheumatoid arthritis. We addressed the still open question whether premature immunosenescence in RA is the primary, genetically determined factor underlying the disease or whether it is a secondary consequence of the already ongoing RA-associated inflammation. It could be concluded that convincing evidence necessary to answer this question, is still lacking. Therefore, suggestions for future (longitudinal) studies in well defined patient’s cohorts are provided.

In Chapter 3 the expression of various serum immune markers (cytokines, chemokines, cytokine receptors) in samples obtained from SAP, seropositive RA (SP RA) and seronegative RA (SN RA) compared to healthy controls, was analyzed. The aims of the study were to identify markers discriminating between SP and SN RA and markers identifying SAP at high risk of RA development.

Although NK cells have various immunomodulatory capacities, the role of NK-cells in the immunopathogenesis of RA is unclear. Therefore, in Chapter 4, we investigated causes and consequences of alterations of NK-cell subsets in the early stages of RA development.

In Chapter 5 the pathogenic role of CD4+ T-cells expressing CD161; recently identified as a common marker of Th17 progeny; in SAP, early RA and late RA patients was assessed. Our findings of a pro-inflammatory role of CD4+CD161+ T-cells primarily in the inflamed synovium, prompted the study described in Chapter 6. In this study, expression of lectin-like transcript 1 (LLT1), representing the endogenous ligand for CD161, in RA joints and in the periphery was investigated.
Previously, we reported that CD70-expressing CD4+ T cells are enriched in RA and promote autoimmunity via IFN-γ and IL-17 expression (45). In **Chapter 7** we studied if CD70-expressing T-cells are modulated in the different phases of RA development. Also, we studied the dynamics of CD70 expression regulation in vitro.

**Chapter 8** discusses the role of immunosenescence in the progression to chronic inflammation, which is characteristic for autoimmune-mediated inflammatory diseases (AIMIDs), such as RA.

Finally, in **Chapter 9** we present an overview of our findings on immune marks in the different stages of disease. We discuss the implications of our findings for the current understanding of the RA disease process and for early recognition of the at-risk subjects. Also, directions for future research are proposed.

**References**


Chapter 1


Introduction and outline of the thesis


