Systemic immune markers characterizing early stages of rheumatoid arthritis
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Chapter 9

Discussion and Summary
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The main aim of the studies described in this thesis was to identify immune alterations characterizing the early stages of rheumatoid arthritis development. Integration of the data obtained by us with data from studies by others contribute to an improved understanding of the causes of RA from an immune perspective. The picture of the events leading to RA development, however, seems to be more clear for seropositive RA than for seronegative RA. RA patients seronegative for ACPA and RF represent a significantly smaller group. As autoantibodies play a major role in the immune alterations contributing to development of seropositive RA, the mechanistic outline pertains to seropositive RA only (Fig.1).

Development of seropositive RA

It has been suggested that the initial step in the development of seropositive RA is the emergence of systemic autoimmunity associated with RA, which refers to the emergence of ACPA and/or RF in the peripheral blood. This phenomenon has been attributed to the ill-defined interaction between the genetic (shared epitope-containing HLA-DR alleles) and environmental (smoking) factors; leading to the formation of neoepitopes by citrullination of proteins (1, 2). The presence of autoantibodies has been associated with the upregulation of various inflammatory markers in the peripheral blood. In line with other studies (3-5), we propose a functional role of autoantibodies in the induction of pro-inflammatory cytokine expression. Until now, in vitro studies demonstrated autoantibody-mediated induction of TNF-α and IFN-γ by monocytes and macrophages (3-5). IgG-containing autoantibodies, in the form of immune complexes, bind to immune cells expressing Fc receptors for IgG (FcγR). These cells include monocytes/macrophages (expressing FcγRI, FcγRIIA, FcγRIIC, FcγRIIIA) and NK-cells (expressing FcγRIIC, FcγRIIIA) (6). While binding of autoantibody-immune complexes to FcγR was shown to stimulate cytokine expression by both monocytes/macrophages and NK cells (4, 5, 7-9), these immune complexes may also trigger the apoptosis of NK-cells (8, 10-12). The systemic consequences of the ACPA/RF presence are the upregulation of pro-inflammatory cytokines and a drop in NK-cell numbers (Chapter 3 and 4, this thesis). These immune alterations may affect the process of RA development in several ways. The systemic increase of TNF-α enhances apoptosis and inhibits the proliferation of hematopoietic stem cells (HSC) (13), representing the progenitors of all immune cell types. This results in a decrease of HSC in the bone marrow and the periphery, a decrease of recent thymic emigrants (T-cells) and eventually in reduced numbers of circulating naive T-cells (14-17). In contrast, differentiated effector T-cells, i.e. CD161-expressing CD4+ T-cells, were found increased in seropositive arthralgia patients (Chapter 5, this thesis), regarded as an early stage of RA development. TNF-α, a key cytokine in RA pathogenesis, stimulates expression of various pro-inflammatory cytokines (i.e. IL-1β, IL-6) and chemokines (i.e. MCP-1), through the activation of NF-κβ (18-20). Augmentation of the pro-inflammatory phenotype, significant for the process of RA development, may be dependent on the concomitant presence of IL-17. Combination of TNF-α and IL-17 had profoundly greater effect on the
expression of cytokines, pro-thrombotic and pro-coagulant factors than either of these cytokines alone (20). IL-17 and IL-17-producing Th cells are implicated in the early stages of RA development (21, 22). Decrease of NK-cells, particularly CD56dim NK-cells, may allow for an uncontrolled expansion of pro-inflammatory T-cells, as NK-cells play an immunosurveillance role against cells with tissue-destructive properties (23, 24). Furthermore, a direct role in killing autoreactive T-cells and B-cells by NK-cells has been demonstrated (25). At this initial stage of RA development (SAP) immune alterations are present at the level of peripheral blood, while the joints are not (yet) affected. This notion is supported by the data showing lack of signs of inflammation or increased immune cell infiltration within the synovium of SAP (26, 27).

Figure 1. Schematic depiction of the immune alterations induced by autoantibodies (ACPA or RF immune complexes) in seropositive RA patients.

Development of seronegative RA

Events preceding the development of seronegative RA are ill-defined and there is no consensus on systemic or synovial markers discriminating between SP and SN RA. Results presented in this thesis revealed clear immune alterations that may be specific for seronegative RA. These include increased numbers of CD56bright NK-cells (Chapter 4, this thesis), increased systemic levels of IL-10 and decreased systemic levels of Eotaxin (Chapter 3, this thesis). Due to the scarcity of available data, the role of these alterations in seronegative RA development remains to be investigated.

Characteristics of late-stage RA

Additionally, data presented in this thesis may serve to increase our understanding not only of the events in early stages of RA development, but also of alterations involved in maintaining local inflammatory processes in long-standing RA. When compared with healthy subjects, the peripheral blood of long-standing RA patients showed increased numbers of effector T-cells with pro-inflammatory functions. Several different populations of T-cells have been identified. Despite the significant likelihood that these
populations show a phenotypical and functional overlap, and may originate from a common precursor population, this has not been assessed. Such T-cell populations, enriched in RA, include CD4+CD70+ T-cells, showing potent IFN-γ and IL-17 expression (28), Th17 cells (29), Th1 cells or cytotoxic and IFN-γ-producing CD28- T-cells (17). As to the question of a common precursor, we here demonstrated (Chapter 5, this thesis) a pathogenic role of precursor Th17 cells, defined as CD4+CD161+ T-cells, switching to non-classical Th1 cells at the level of the joint (likely by local IL-12 skewing (30)). Furthermore, our data suggest that the ligand for CD161, namely lectin-like transcript 1 (LLT1), expressed by macrophages in the RA synovium, putatively providing a co-stimulatory signal contributing to the Th1 phenotype, may be involved in the modulation of the pathogenic T-cell response (Chapter 6, this thesis).

SAP progression to RA
Events responsible for the expansion of the pathogenic immune response from the periphery to the joints, which underlie the progression to classifiable RA, are still subject of speculations. Increase of ACPA levels, broader ACPA specificity (epitope spreading) (3) and concomitant increase of multiple inflammatory markers in the peripheral blood (3, 31) have been associated with the switch from the pre-RA stage to full-blown RA. By comparing various immune markers in the periphery of at-risk patients (SAP) who progressed to RA during the follow-up and SAP who do not progress, we have identified cytokines that may be involved in the switch to RA. These include increased systemic levels of IL-5, MIP-1β, IL-1RA and IL-12; among which the best ability to predict RA development was found for IL-5 (Chapter 3, this thesis). Furthermore, we observed modulation of circulating CD4+CD161+ T-cells, characterized by a potent IFN-γ and IL-17 producing capacity, in SAP and newly diagnosed RA patients. The data obtained showed elevated frequencies of these cells in SAP but decreased frequencies in early RA, thereby suggesting the migration of pro-inflammatory CD4+CD161+ T-cells to the joints which may contribute to RA development (Chapter 5, this thesis). Our data also revealed a delay in the downregulation of CD70 expression in peripheral T cells from both SAP and early RA patients (Chapter 7, this thesis). Prolonged expression of an important co-stimulatory molecule on pro-inflammatory T-cells may contribute to increased autoreactivity.

In summary (Fig.2), we have:

- identified immune alterations involved in the development of seropositive RA (decline of NK-cells; increased systemic levels of pro-inflammatory cytokines, i.e. IL-1β, IL-1RA, IL-2, IL-2R, IL-15, IL-17);
- revealed novel systemic immune alterations characterizing seronegative RA (increase of CD56bright NK-cells, increase of IL-10, decrease of Eotaxin levels)
• added novel data about immune alterations that contribute to local inflammation and joint destruction at later stages of RA (expression of the ligand for CD161, LLT1, by synovial macrophages; accumulation of CD4+CD70+ T-cells and CD4+CD161+ T-cells in the synovial fluid and synovial tissue).
• identified immune markers in SAP that may help to identify high-risk (for progression to RA) patients (i.e. IL-5, MIP-1β, IL-1RA, IL-12)

Figure 2. Schematic summary of results described in this thesis, including the immune alterations and their localization in the studied cohorts.

Limitations and future perspectives
In this thesis we investigated the immune profiles in different phases of RA in a cross-sectional design. Although this can help in characterizing the different phases of RA, future studies should preferably be done in a longitudinal design. That would allow to more directly study cause-effect relationships within the context of the same individual. Clearly, this can only be done if basic research is combined with strict clinical monitoring.

Some of the markers in our studies were validated in independent cohorts. For the majority of the studied immune markers, however, we would recommend further validation in independent patient cohorts.

Our studies on differences in SP vs SN RA underline the importance of stratifying RA patients according to the autoantibody status in studies investigating pathological pathways involved in RA and also in the designs of clinical trials.
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References


