Fc receptors and immunoglobulins in polyarthritis

A matter of function, supply and demand?

PETER MATT
Abstract

Fc receptors (FcR) and immunoglobulins (Ig) play important roles in the defence against pathogens. However, altered interactions of these may promote chronic inflammation in rheumatic diseases. An excess of Igs forming immune complexes (IC) could lead to continuous FcR activation and spreading of autoimmune inflammation to other tissues. This work focuses on the evaluation of the FcR status and function in the two most common polyarthritides - psoriatic arthritis (PsA) and rheumatoid arthritis (RA) – in relation to various Igs, joint and skin disease activity, and effect of anti-rheumatic treatment (determined by the EULAR response criteria for RA). Monocyte subpopulations (defined by surface CD14 and CD16 expressions) in patients and HC were also characterised, since different monocyte subsets may have opposing functions in inflammatory conditions. In addition, the effect and safety of long-term B-cell suppression in advanced RA was studied.

In PsA, elevated serum levels of IgG1, 2, and 3 were noted while the early naïve RA patients - besides being positive for autoantibodies like IgMRF, IgARF, IgGRF, and ACPA IgG - were distinguished by high levels of IgG1 and IgG3. Monocytes of PsA and RA patients were heavily loaded with IgG and expressed more CD64 (i.e. the high affinity FcγR) than HC. An increase in CD64 turnover was specific for early RA, while a higher CD16a (i.e. a low affinity FcγR) turnover was seen in both RA and PsA compared with HC. The FcγR function was impaired in both polyarthritides compared to HC, but the RA monocytes were more affected of this than the PsA monocytes. RA non-responders had a much lower capacity of IC binding compared with RA good responders. Alterations of the FcγR status and function reflected joint disease activity markers in both polyarthritides but not the skin disease activity in PsA. I therefore conclude that the observed FcγR statuses in both diseases were specific for joint inflammation. In addition, RA patients with high levels and the occurrence of several simultaneously appearing RF isotypes presented a minor FcγR function, while patients experiencing a good treatment effect were more likely to show low levels of Igs. This suggests that RFs/IC in excess could be important promotor of the ongoing inflammation in RA. However, for ACPA IgG no associations with the rheumatoid monocytic FcγR status/function were noted. CD16<sup>neg</sup>classical monocytes were elevated in early naïve RA, especially in the non-responders - while PsA patients showed an increase in CD16<sup>low</sup>expressing cells compared to HC. These observations indicate that different monocyte subpopulations could be important in the two polyarthritides. In a cohort of RA patients with advanced disease, long-term B-cell suppression resulted in conversion to RF negativity which indicated a good treatment response but not an increased risk of infection.

FcRs and Igs are important players that promote chronicity of inflammation in polyarthritis, especially in RA. An impaired FcγR function following an excess of Igs/IC reflects this state of the immune system. This work has identified an increased monocytic CD64 turnover as a RA specific feature. Future treatment options in RA might include supporting/normalizing the FcγR function. Today suppressing B-cell activity is an effective and relatively safe way to tackle the problem from the opposite side.

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To my family
List of papers


III Matt P, Rönnelid J, Kleinau S, Lindqvist U: Rheumatoid factor isotypes reflect the monocyte Fcγ receptor function and treatment outcome in early naïve rheumatoid arthritis. Submitted manuscript.

IV Matt P and Lindqvist U: B-cell depletion in rheumatoid arthritis; conversion to RF negativity indicates a good treatment response but not an increased risk of infection. Submitted manuscript.

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</tr>
<tr>
<td>ab</td>
<td>antibody</td>
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<tr>
<td>ACPA</td>
<td>anti–citrullinated protein antibody</td>
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<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
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<tr>
<td>ANA</td>
<td>antinuclear antibody</td>
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<td>APC</td>
<td>antigen presenting cell</td>
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<td>b/c DMARD</td>
<td>biologic/conventional disease-modifying anti-rheumatic drug</td>
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<tr>
<td>CD (as in CD64)</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DAS28</td>
<td>disease activity score (28 joint index)</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
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<td>EULAR</td>
<td>European league against rheumatism</td>
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<tr>
<td>FACS</td>
<td>fluorescence-activated cell sorting</td>
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<tr>
<td>FcR</td>
<td>Fragment crystallizable region Receptor</td>
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<tr>
<td>FcRγ chain</td>
<td>FcR gamma chain</td>
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<tr>
<td>FV/FU</td>
<td>first visit/follow up</td>
</tr>
<tr>
<td>HAQ</td>
<td>Health Assessment Questionnaire</td>
</tr>
<tr>
<td>HC</td>
<td>healthy control</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
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<tr>
<td>IC</td>
<td>immune complex</td>
</tr>
<tr>
<td>IFN (as in IFNγ)</td>
<td>interferon</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
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<tr>
<td>IL (as in IL-6)</td>
<td>interleukin</td>
</tr>
<tr>
<td>ITAM</td>
<td>immunoreceptor tyrosine-based activation motif</td>
</tr>
<tr>
<td>ITIM</td>
<td>immunoreceptor tyrosine-based inhibitory motif</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>MACS</td>
<td>magnetic-activated cell sorting</td>
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<tr>
<td>MDA</td>
<td>minimal disease activity</td>
</tr>
<tr>
<td>MFI</td>
<td>mean fluorescence intensity</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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</table>
MS morning stiffness
MTX methotrexate
NSAID non-steroidal anti-inflammatory drug
OCP osteoclast precursor
PASI psoriasis area and severity index
PBMC peripheral blood mononuclear cell
PG patient global assessment of health
PMA phorbol 12-myristate 13-acetate
PRED prednisolone
PsA psoriatic arthritis
RA rheumatoid arthritis
RBC red blood cell
RF rheumatoid factor
RTX rituximab
s (as in sCD64) soluble
SAA serum amyloid A
SASP salazopyrin (i.e. sulfasalazine)
SJC swollen joint count
TGFβ transforming growth factor beta
TJC tender joint count
TNFα tumor necrosis factor alpha
VAS visual analogue scale
Introduction

The pathogenesis of rheumatic diseases including the chronic polyarthritides is a major topic for research and with increased understanding of their complex immunological mechanisms new therapies have been developed. Thus, biologic drugs have markedly improved treatment outcome in arthritis in the last two decades. With these new drugs a tightening of treatment targets has been made possible, and today a disease state of remission is not only possible but also emphasized in many national recommendations. However, the road to tailored treatment for each patient is still long and winding and individual-based medicine is requested not only by patients but also by doctors. In future great emphasis will be put on predicting treatment outcome as approximately 1/3 of the arthritis patients still experience only minor benefit or adverse events from their therapy independently of the chosen drug. Consequently, the demand of objective markers for disease, disease activity and treatment effect is great.

In this work the focus was set on increasing the knowledge of the consequences of humoral and innate immune system interactions in the most common polyarthritides, namely rheumatoid arthritis and psoriatic arthritis.

Background

**Rheumatoid arthritis (RA)** is a chronic autoimmune inflammatory joint disease that affects women 2-3 times more often than men. In Sweden the prevalence is estimated to 0.6-0.8% and the annual incidence to 25 out of 100000 individuals. Age at onset varies but most of the patients debut in the age of 30-50 years. About two thirds of the RA patients present with a symmetric polyarthritis, involving several small joints of the hands and feet at diagnosis, in the remaining patients larger joints are predominantly involved. Typical initial findings include swollen and painful joints with restricted range of motion, especially in the morning, so-called morning-stiffness. With long standing inflammation the risk for joint destruction and immobility increases, which may require joint surgery. Immunological reactivity in RA seems not be restricted to joints as RA patients also may experience inflammatory symptoms from other tissues like skin, serous membranes, lung, blood vessels, eyes, exocrine glands and more. The immunopathogenesis of RA is complex and not fully understood, but both genetic and environmental factors are thought to contri-
bute to disease initiation and maintenance. Indeed, RA cases are accumulated in some families and smoking is regarded a risk factor. The major histocompatibility complex class II restricted genes of the human leukocyte antigen (HLA) family are overrepresented in RA, and especially HLA-DR1 and HLA-DR4 alleles predispose to an increased susceptibility and are associated with a more severe disease. Particular genes are the basis for the shared epitope hypothesis in RA, and the HLA-DRB1 allele is thought to be involved in the presentation of arthritogenic antigens.

A role of sex hormones in RA is also suggested, since women are overrepresented. The disease activity often decreases during pregnancy, and RA debut in women quite often coincides with menopause. In addition, men with RA often present skewed sex hormone levels and may experience an improvement when treated with testosterone (1).

Various cytokines are supposed to strongly contribute to the perpetuation of joint inflammation (2). With established disease the RA patients may experience long time increased mortality due to complications of osteoporosis, atherosclerosis, infections, and hematological malignancies involving B-cell populations (3).

Psoriatic arthritis (PsA) is a chronic inflammatory joint disease that occurs in approximately twenty-five percent of patients with psoriasis. Women and men are equally affected and the age at onset ranges from 30-50 years. Mostly the skin disease precedes the joint disorder with years. Joint symptoms are seen among all psoriasis subtypes (4). In Sweden the prevalence of PsA is estimated to 0.2-0.3% and the annual incidence 3-6 per 100000 individuals. PsA is a heterogeneous disease, and different subsets can be identified based on the affected joints; an asymmetrical mono-/oligoarthritic, a symmetrical (RA-like) polyarthritic, a distal interphalangeal arthropathy and an arthritis mutilans type may be distinguished from the psoriatic spondylarthropathy. A typical feature of PsA is the uniform swollen toe/finger which implies for concurrent inflammation of articular and periarticular components of the digit (dactylitis) (5). Enthesitis, which corresponds to inflammation at insertion sites of tendons and muscles, is commonly found but may cause a delay of the PsA diagnosis if other obvious signs of disease are absent. Arthritic symptoms in PsA are considered to be mild, but joint destructions occur especially in the polyarthritic and mutilans sub groups. Like in RA the PsA inflammation may involve other tissues than joints; ocular manifestations and low-grade inflammatory bowel disease are more common in the spondylitic subset. The immunological pathways leading to PsA are intensively studied but not fully understood. Approximately 40 percent of patients with psoriasis or PsA have a family history of these disorders in first degree relatives. Bacterial infections (e.g. streptococcus species) and trauma (Köbner phenomena) are proposed exogenous factors that may trigger relapses in both psoriasis and PsA. In contrast to RA,
PsA is associated with MHC class I restricted genes, and HLA-B13, HLA-B17, HLA-B57, and HLA-Cw*0602 expression are more frequently observed in PsA. Interestingly, PsA patients with axial disease not seldom present with HLA-B27 - the marker of ankylosing spondylitis (Mb Bechterew).

PsA patients have an increased risk to experience consequences of the metabolic syndrome, and like RA, cardiovascular disease is considered an important factor for the shorter life expectancy observed in PsA (6).

The polyarticular form of PsA resembles RA in many ways; women are probably affected more often than men in contrast to non-polyarticular PsA, and auto-antibodies are more frequently seen in this PsA subpopulation.

**Table 1.** Comparison of disease factors that highlights differences and similarities between RA and polyarticular PsA.

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>Poly-articular PsA</th>
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<tbody>
<tr>
<td>Rheum. factor</td>
<td>70-85%</td>
<td>5-10%</td>
</tr>
<tr>
<td>ICs in circulation</td>
<td>50-65%</td>
<td>~ 50%</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td>ACPA (50-60% in early RA), ANA (~ 40%)</td>
<td>ACPA (5-10%), ANA (~ 15%)</td>
</tr>
<tr>
<td>HLA</td>
<td>HLA-DRB1*0401 Shared epitope</td>
<td>HLA-DRB1<em>0402, HLA-Cw</em>0602 (psoriasis) HLAB27 (if spondartropathy)</td>
</tr>
<tr>
<td>Synovial histology</td>
<td>CD3+CD4+T-cells++, CD163+macrophages++, CD38+plasma cells++, CD83+dendritic cells++, neutrophils+, synovial vascularity +</td>
<td>CD3+CD8+(IL-17+)T-cells+, CD163+macrophages++, CD38+plasma cells+, CD83+dendritic cells+, neutrophils++, synovial vascularity +++</td>
</tr>
<tr>
<td>Good treatment effect with</td>
<td>NSAID, SASP, MTX, CyA, antimalarial, gold, leflunomide, steroids, anti-TNFα, anti-CD20, CTLA4, anti-IL6R, anti-IL1R</td>
<td>NSAID, SASP, MTX, CyA, antimalarial, gold, leflunomide, steroids, anti-TNFα, PUVA, retinoids, anti-IL12/23</td>
</tr>
<tr>
<td>Joint destruction</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gender-distribution</td>
<td>♀ &gt; ♂</td>
<td>♀ = ♂ (?)</td>
</tr>
</tbody>
</table>

The synovitis in RA and PsA

A joint comprises two compartments; the joint space between cartilage-covered articulating surfaces and the synovial tissue. Both compartments are affected in joint inflammation which the patient experiences as joint swelling. This swelling is caused by synovial hyperplasia and effusion of synovial fluid into the joint space. The inflamed joint fluid is characterized by low viscosity,
increased white blood cell count and increased protein content compared to a healthy joint fluid. Interestingly, patients with PsA have lower synovial fluid aggrecan concentrations but higher COMP concentrations compared to RA patients. This suggests different ratios of cartilage degrade and repair mechanisms in the diseases, favouring repair in PsA (7).

Indeed, in PsA radiological findings of osteophytes (i.e. bony hypertrophied structures that can be found close to joint spaces) indicate this. In inflamed synovial tissues, immunological active cells are accumulated and the same type of cells can be found in both diseases. By histopathologic grading patients with chronic RA and PsA do not differ significantly by the mean grades of synovitis scores (8).

However differences can be highlighted when the synovial tissues are examined in detail: the RA synovitis presents with increased numbers of T-cells, a trend towards more B-cells forming secondary germinal centers, a lower proportion of polymorphonuclear cells (PMNC) and less vascularisation compared to the PsA synovitis (9).

On the other hand, PsA and RA are comparable with regard to the number of fibroblast-like synoviocytes and macrophages (10). These observations implicate different local mechanisms that promote inflammation in RA and PsA. Cytokines are important factors that maintain and intensify the local and systemic inflammation in both RA and PsA (2, 11). The cytokine profile in PsA is characterized by the presence of Th1 cytokines and the monokines tumor necrosis factor alpha (TNF-α), and interleukin-1beta (IL-1β) - and very elevated levels of IL-10. Compared to RA these levels are increased. The rheumatoid synovitis is initiated and perpetuated by auto-reactive T-cells, which amplify the immune response by stimulating B-cells, other migrated cells of the innate immune system, locally situated fibroblasts, chondrocytes, and osteoclasts. In the inflamed RA-joint, various cells release cytokines, especially TNF-α, IL-1, and IL-6; these contribute to the maintenance of the synovial inflammation (12). In RA, an invading inflammatory tissue called the pannus allows an attack on cartilage and bone resulting in joint erosions. This pannus is considered a hallmark of RA.

Monocytes and macrophages

Monocytes and macrophages have important effector functions in acute inflammation, but they are also involved in promoting chronic inflammation in diseases such as RA and PsA. Monocytes are derived from bone marrow progenitors and are released into the circulation upon cytokine stimulation. From this monocyte blood pool tissue macrophages are recruited (13). The macrophages act as local producers of TNFα, antigen presenting cells (APC), and digest cell debris and immune-complexes (ICs). In the circulation, different monocyte subpopulations can be defined upon their expressions of CD14 (i.e.
Lipopolysaccharide (LPS) receptor) and CD16 (Fc gamma receptor III). When these markers are used simultaneously in flow cytometry three different monocyte subpopulations can be identified: CD14^-CD16^- (classical monocytes), CD14^-CD16^+ (intermediate monocytes), CD14^+CD16^- (non classical monocytes) (Figure 1).

Figure 1. Monocyte subset distribution by flow cytometry

The CD14^+CD16^- cells constitute approximately 90% of all monocytes. These monocytes have low antigen presenting capacity due to the low expression levels of MHCII, but seem to have significant ability to phagocytose and secrete immune regulatory IL-10. On the other hand, the CD14^+CD16^+ cells release mediators such as TNFα, IL-12 and IFNγ and therefore are considered pro-inflammatory (14). In between these two groups a CD14^+CD16^- cell population can be defined, which presents high expression levels of MHCII and receptors for TNFα. In inflammatory conditions such as RA the number of the latter monocyte subset is increased, and interestingly psoriatic patients with active generalized pustulosis also present elevated numbers of these monocytes (15-17). This intermediate monocyte subset is attributed an important role in joint destructive processes for an additional reason; osteoclast precursors (OCPs) are believed to be derived from these monocytes. The number of OCPs is increased in peripheral blood in patients with erosive PsA compared to PsA patients without erosions (18). The CD16 expression on these OCPs increases during osteoclastogenesis, therefore CD16 has been proposed a potential marker of OCPs in PsA (19). Monocytes and macrophages express various immunoglobulin (Ig) Fc receptors on their surfaces. In this...
manner, the humoral immune system encompassing antibodies affect the level of activation of monocytes and macrophages of the innate immune response.

Igs and autoantibodies

Igs are “Y”-shaped globular glycoproteins that are produced by B-lymphocytes to bind and neutralize antigens. Four polypeptide chains form the basis for an Ig molecule; two identical heavy chains (either μ, δ, γ, ε or α chain) and two identical light chains (λ or κ chain). Each tip of the “Y”-shaped Ig is called the variable F(ab)-region - it consists of a binding site (paratope) that can recognize a unique antigen structure (epitope). The base of the “Y”, termed the constant Fc region, constitutes the binding sites for complement and the corresponding Fc receptor (FcR). The specific set of sugar moieties on the Fc part of the Ig will affect the Ig’s ability to promote pro- or anti-inflammatory actions; thus, the addition of terminal sialic acid to such a glycan reduces FcR binding and converts IgG antibodies to anti-inflammatory mediators through the acquisition of novel binding activities. Between the arms and the base of the “Y” the hinge region is situated. It allows certain flexibility of the F(ab)-arms to bind epitopes at different distances.

![Figure 2. The immunoglobulin structure](image)

Dependent on the type of the heavy chain, the Ig is termed IgM, IgD, IgG, IgE or IgA respectively. While IgM comprise a pentamer and IgA a dimer structure, IgG, IgD and IgE are present as monomers. The Igs exist in two physical forms; a soluble form that is released from the B-cell (i.e. antibody), or a membrane-bound form that is attached to the B-cell surface (i.e. B-cell receptor).
The Ig isotypes differ in their biological properties, functional locations and ability to interact with antigens and FcRs. The IgM isotype is found mainly in lymphatic and blood vessels and is involved in the first line defense in an immune response. It has great capacity to activate complement, and participates in agglutination and cytolitic reactions. IgD acts as an antigen receptor on naïve B cells, which have not yet been exposed to antigen. IgA is the most abundant Ig isotype and exists in two forms; a monomer and a dimer (also called secretory IgA). It maintains immunological protection in mucous membranes and in body fluids. IgE is involved in allergy and in the protection against parasites. IgG can be divided into 4 subclasses (IgG1, IgG2, IgG3 and IgG4) and is the main type of antibody found in blood and in tissues. The IgG subclasses are numbered according to their serum concentrations; IgG1 being the most abundant. The functional differences among the IgG subclasses arise from structural variations in the Fc region and in the hinge region. All four IgG subclasses act as opsonins - thus binding to and marking antigens. While only IgG1 and IgG3 are effective in activating complement, IgG2 does this only weakly and IgG4 not at all. Antibody responses to protein and viral antigens are mainly composed of IgG1 and IgG3, while antibody responses against bacterial polysaccharide antigens are mediated mainly via IgG2. In biopsies from RF⁺ RA synovial tissue, IgG secreting plasma cells can be found, (often together with IgA and IgM), and the predominant IgG subclasses are IgG1 and IgG3 (20). In inflamed PsA synovium, IgG and IgA secreting plasma cells are present in the same extent as in RA synovia, however the IgM-expression is lower (21).

Auto-antibodies (auto-ab) are Igs that are directed against self-antigens. Typical auto-ibs in RA are the rheumatoid factors (RF), which exist of all Ig isotypes. The RFs comprise antibodies against the Fc portion of IgG. Bound to IgG they form immune complexes (ICs), which are suggested to play important roles in RA inflammation. Raised amounts of RFs and ICs are detected in the synovial fluid and on cartilage in RA joints, and in the circulation (22-24). Also in PsA increased levels of circulating ICs, Igs and auto-antibodies (including low titers of RF) can be seen, especially in the polyarthritic subgroup (25-30). This highlights that the appearance of RFs is not specific for RA. In addition, RF may also be detected at low levels in infectious diseases (e.g. borreliosis and hepatitis C) and in other rheumatic conditions such as Sjögrens syndrome. However, in RA the RF titers are often higher, remain elevated, and correlate with joint disease progression and an impaired treatment efficacy (31).

Other autoantibodies frequently seen in RA are antibodies against cyclic citrullinated proteins (ACPAs). Together with RF, ACPA is included in the new 2010 RA classification criteria (32). The process of citrullination (or deamination) of a protein is enabled by specific enzymes, peptidylarginine deiminases (PADs). This can change the structure and the function of the protein.
Examples of citrullinated proteins that are found in RA synovial tissues are fibrin, fibrinogen, vimentin, alpha-enolase and collagen type II. PADs have been demonstrated in RA synovial membrane and joint fluid but also in other chronic inflammatory conditions such as multiple sclerosis, and in the respiratory tract of healthy smokers (33-36). Interestingly, citrullination may also be important for skin cornification processes, as several PADs are expressed in the skin of patients with psoriasis, especially in psoriatic uninvolved epidermis (37-39). Low serum levels of ACPA have been noted in psoriasis patients as well as in PsA (40, 41).

FcReceptors

FcRs are proteins that interact with the Fc part of corresponding Igs (42). They are involved in phagocytosis, internalization of immune complexes (ICs), cell activation, cytokine secretion and antibody-dependent cell-mediated cytotoxicity. The FcRs exist mainly in a membrane anchored form, but can also exist in a soluble form arising from active secretion or proteolytic cleavage of the membrane FcR. The FcRs are mainly expressed on hematopoietic immune competent cells, but have also been found on peripheral neural cells and endothelial cells. However, each cell type appears to have its own specific composition of FcRs (43, 44). In humans five main types of FcRs are defined: the FcRs for IgM (FcμR and Fcα/μR (CD351)), the FcR for IgD (FcδR (CD?)), the FcRs for IgG (FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16)), the FcR for IgE (FcεRI and FcεRII (CD23)), and the FcR for IgA (FcαR (CD89)). In addition, a neonatal FcR (FcRn) exists, which plays an important role in monitoring the IgG turnover. When membrane bound FcRs are cross-linked by ICs they will either promote activating (CD64, CD32a, CD16) or inhibiting (CD32b) immune responses, or both (CD89). This is mainly due to the different FcRs intracellular signaling subunits such as the FcR common γ chain (45, 46). Igs and ICs display different binding affinities to their corresponding FcR, and polymorphisms in Ig binding domains may be pivotal for this (47, 48). The FcγRs are low affinity receptors for IgG in ICs, except for CD64, which is a high affinity receptor that binds monomeric as well as polymeric IgG strongly; this implies that most of the CD64 receptors will be occupied by IgG in normal physiologic conditions. CD89 is a low affinity receptor of monomeric IgA, while IgA in ICs and polymeric IgA bind more strongly to this receptor (49).
<table>
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<tr>
<th>Isotype (heavy chain)</th>
<th>Subclasses (serum half-lifes, days)</th>
<th>Structure</th>
<th>Receptor interaction</th>
<th>FcR affinity</th>
<th>Complement activation</th>
<th>Intracellular signaling via</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM (μ)</td>
<td>none (10d)</td>
<td>monomer on B-cells, pentamer in circulation</td>
<td>FcμR, Fcα/μR</td>
<td>high: FcμR + Fcα/μR</td>
<td>classical pathway</td>
<td>association with CD79a+b ITAM ?</td>
</tr>
<tr>
<td>IgD (δ)</td>
<td>none (2d)</td>
<td>monomer</td>
<td>FcδR</td>
<td>high</td>
<td>none</td>
<td>association with CD79a+b ITAM</td>
</tr>
<tr>
<td>IgG (γ)</td>
<td>IgG1 (21d), IgG2 (21d), IgG3 (7d), IgG4 (21d)</td>
<td>monomer</td>
<td>FcγRI, FcγRIIa,b,c, FcγRIIa, FcRn</td>
<td>high: FcγRI + FcRn</td>
<td>medium: FcγRIIa</td>
<td>classical pathway</td>
</tr>
<tr>
<td>IgE (ε)</td>
<td>none (2d)</td>
<td>monomer</td>
<td>FcεRI, FcεRII</td>
<td>very high: FcεRI</td>
<td>low: FcεRII</td>
<td>none</td>
</tr>
<tr>
<td>IgA (α)</td>
<td>IgA1 (6d), IgA2 (6d)</td>
<td>monomer, dimer (also called secretory IgA; slgA)</td>
<td>FcαRI, FcαRII, Fcα/μR, plgR</td>
<td>low: FcαRI</td>
<td>medium: Fcα/μR</td>
<td>Mannose-binding lectin pathway ??</td>
</tr>
</tbody>
</table>
The expressions of leukocyte FcRs are influenced by various factors, such as pro- and anti-inflammatory cytokines, medication, hormones, and age (Table 3) (50-54). Interestingly, acute phase proteins like serum amyloid A (SAA) and C-reactive protein (CRP) also interact with FcγRs (CD64 and CD32a) as well as with CD89 (55-59). Both SAA and CRP enhance opsonin-mediated phagocytosis, and by masking autoantigens from the immune system or enhancing their clearance these acute phase proteins contribute to the prevention of autoimmunity. Released from hepatocytes upon IL6 stimulation acute phase reactants like CRP increases in serum in the early phases of inflammation. CRP is used in daily clinical practice for the evaluation of infection and inflammation in antibiotic therapies and in rheumatic diseases.

In RA, alterations of monocyte FcγR expressions have been reported. Most studies point to an up regulation of activating FcγRs. The reason for the diverging outcomes is likely due to multiple factors; patient cohorts with varying disease durations and/or disease activities, concomitant anti-rheumatic treatments (in- or excluding steroids), various auto-antibody statuses, and different age/sex-matched control groups. This underlines the importance of assessing homogenous patient populations that are naïve to immunosuppressive drugs in relation to age and sex-matched healthy controls.

**Table 3.** Examples in the literature of the in vitro effects of hormones, anti-rheumatics and inflammatory substrates on monocyte FcR expressions in humans.

<table>
<thead>
<tr>
<th></th>
<th><strong>Increase in FcR expression</strong></th>
<th><strong>Decrease in FcR expression</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>estrogen</td>
<td></td>
<td>CD16</td>
</tr>
<tr>
<td>steroids</td>
<td></td>
<td>CD32, CD64</td>
</tr>
<tr>
<td>methotrexate</td>
<td></td>
<td>CD32, CD64</td>
</tr>
<tr>
<td>vitamin D</td>
<td>CD89</td>
<td>CD64, CD32, CD16</td>
</tr>
<tr>
<td>TNFα</td>
<td>CD89</td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>CD89</td>
<td></td>
</tr>
<tr>
<td>IFNγ</td>
<td>CD64</td>
<td>CD89</td>
</tr>
<tr>
<td>TGFβ</td>
<td>CD64, CD16</td>
<td>CD89</td>
</tr>
<tr>
<td>IL-4</td>
<td>CD64</td>
<td>CD64, CD32, CD16</td>
</tr>
<tr>
<td>IL-6</td>
<td>CD64</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>CD64, CD32</td>
<td></td>
</tr>
<tr>
<td>metabolic syndrome</td>
<td>CD64, CD32</td>
<td></td>
</tr>
<tr>
<td>ageing</td>
<td>CD32a</td>
<td>CD32b (women)</td>
</tr>
</tbody>
</table>
Present investigation

Aim

The general aim of this work was to study the expression and function of monocyte FcRs in two different polyarthritides in relation to immunoglobulin levels and disease activity. Thus, patients with active PsA and early naïve RA were evaluated. Since FcR status could possibly be affected by other factors such as immune suppressive treatment, receptor turnover and age/gender - we were also interested in elucidating these parameters in relation to the FcR function. Hence, the early RA patients were evaluated before and after anti-rheumatic treatment, soluble forms of FcRs were analyzed, and the correlations of age and monocyte Fc receptor expressions in healthy women compared with healthy men were studied. Special emphasis was also put on differences in treatment outcome in the RA cohort which, in combination with data on the Ig background, could be used for the individual therapy decision. In addition, we were interested in evaluating the distribution of monocyte subsets in patients and healthy controls as different monocyte subpopulations can have opposing functions in inflammatory conditions regarding cytokine production, migratory ability and MHC expression (60). Finally we studied the effect and safety of long-term B-cell suppression in a cohort of autoantibody positive RA patients with advanced disease.

Experimental methods

Subjects

Before taking part in the studies, written and signed informed consent (according to the Helsinki Declaration) was obtained from each participant. The local Ethics Committee in Uppsala approved the studies.

Thirty-three healthy controls (HC) (16 women/17 men, median age 53 years) were enrolled. All HC were blood donors at the Uppsala University Hospital.

Paper I: 23 PsA out-clinic patients visiting the Rheumatology Unit at Uppsala University Hospital (11 women/12 men, median age 55.5y) were studied. All
fulfilled the CASPAR criteria for PsA (5), and only patients with the polyarticular form of PsA were included. The reasons for this selection were the following: firstly, polyarticular PsA resembles RA phenotypically, however the two immunopathogeneses are different, and a comparison is therefore of great interest. Secondly, patients with polyarticular PsA more often suffer from joint destruction than other PsA subsets and require treatment with immunosuppressants similar to RA.

All studied PsA patients but one had a history of skin disease onset prior to arthritis. The average skin disease duration was estimated to 22.6 years, and the average arthritis duration was estimated to 8.3 years. Thirteen of the included PsA patients had had a history of rheumatic complaints more than 2 years. None of the patients fulfilled the definition of minimal disease activity (MDA) (61). At inclusion, 9 patients were treated with disease-modifying anti-rheumatic drugs (DMARDs; here methotrexate, sulfasalazine or leflunomide) and/or oral corticosteroids. Fourteen of the 23 patients were only taking analgesics and/or non-steroidal anti-inflammatory drugs (NSAIDs). None of the patients was treated with biologic drugs.

**Paper 2 and 3:** 20 out-clinic patients with newly diagnosed early RA visiting the Rheumatology Unit at Uppsala University Hospital (13 women/7 men, median age 53.5 years) were studied. All patients fulfilled the 1987 American college of rheumatology (ACR) criteria for RA (62), and their mean disease duration was 8.2 months. Nineteen patients were RF positive, while all patients were ACPA IgG positive. All patients were previously DMARD- and steroid-naïve. The patients were evaluated twice: at a first visit (FV) and at a follow up visit (FU) - after approximately 3-4 months of treatment. If not contraindicated, the patients were treated with methotrexate (MTX) (n=17) and prednisolone (PRED) (n=17) – according to national guidelines. The others received sulfasalazine (n=2) or chloroquine phosphate (n=1) in combination with PRED or intra-articular steroid injections.

**Paper 4:** 69 out-clinic patients with longstanding RA visiting the Rheumatology Unit at Uppsala University Hospital (56 women/12 men, median age 58.6 years) were studied. All patients had received at least one course of B-cell depleting therapy with rituximab (RTX). The patients had a mean joint disease duration of 17.4 years, and a high proportion had erosive disease (72%). All patients had fulfilled the 1987 ACR criteria for seropositive RA at time of diagnosis, and 66.7% were ACPA IgG positive at study entry. Sixty-two percent had tested at least one TNFα blocker prior to RTX. At time of assessment, 50 of the patients had concomitant DMARD treatment (i.e.: methotrexate, antimalarials, leflunomide or sulfasalazine). In addition, 40 patients were treated with low dose PRED or injectable steroids, while 9 patients were DMARD- and steroid-free. The evaluation of the patients was performed at the day of the following RTX-course.
Assessment of disease activity

The subjective assessments of disease activity comprised health assessment questionnaire (HAQ) forms and visual analogue scales (VAS) for morning stiffness, global health and pain (63). The stiffness values were calculated as the mean of the questions number 5 and 6 of the Bath ankylosing spondylitis disease activity index (BASDAI) (64). The HAQ, VAS scales and BASDAI are frequently used in daily clinical practice and are validated for the use in clinical trials.

The objective assessments of joint disease activity and treatment response included the evaluation of the current joint status by the responsible medical doctor (author). An online calculator was used for the determination of the disease activity score (DAS) (65). DAS is a common tool used to evaluate disease activity and treatment outcome in patients with PsA or RA. It takes into account the number of swollen and tender joints of 28 pre-selected joints ("DAS28"), the patient’s evaluation of global health, and a laboratory factor, either the erythrocyte sedimentation rate (ESR) or the C-reactive protein (CRP). A DAS value of 0-2.6 indicates remission, 2.6-3.2 a low disease activity, 3.2-5.1 a medium disease activity, and >5.1 corresponds to a high disease activity. For evaluating the individual treatment response, the European league against rheumatism (EULAR) response criteria for RA were used (66). These are based on the comparisons of pre- and post-treatment DAS28-ESR values:

Table 4. The EULAR response criteria for RA

<table>
<thead>
<tr>
<th>present DAS28 value</th>
<th>DAS28 improvement &gt; 1.2</th>
<th>DAS28 improvement &gt; 0.6 and ≤ 1.2</th>
<th>DAS28 improvement ≤ 0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 3.2</td>
<td>good response</td>
<td>moderate response</td>
<td>no response</td>
</tr>
<tr>
<td>&gt; 3.2 and ≤ 5.1</td>
<td>moderate response</td>
<td>moderate response</td>
<td>no response</td>
</tr>
<tr>
<td>&gt; 5.1</td>
<td>moderate response</td>
<td>no response</td>
<td>no response</td>
</tr>
</tbody>
</table>

The skin scoring of the PsA patients was performed by a trained senior rheumatologist using the psoriasis area and severity index (PASI) (67). The PASI is a composite index (range 0-72 points) that allows a scoring of the psoriatic skin erythema, induration and desquamation severity and also takes the proportion of the affected skin surface into consideration. A PASI score of 0 indicates a healthy skin, values < 10 correspond to a mild disease while a PASI of 72 is equivalent with maximal skin disease activity.
The laboratory assessment of disease activity comprised blood analyses of CRP and ESR, Ig isotypes and IgG subclasses, and autoantibody levels. Analyses of these parameters were performed with standardized methods at the Clinical Chemistry and Clinical Immunology Units at Uppsala University Hospital.

Isolation of peripheral blood mononuclear cells

Blood samples were drawn from patients and HC and peripheral blood mononuclear cells (PBMCs) were isolated from the EDTA-treated venous blood by Ficoll-Paque. The plasma was separated and frozen at -70°C for later analyses. All analyses of the FcR expression and function were performed on living cells, isolated on the same day or the day before, at the Department of Cell- and Molecular Biology at Uppsala University.

Analyses of FcR expressions

The PBMCs were washed in flow cytometry activating cell sorting (FACS)-buffer and stained with conventional fluorochrome or biotin-conjugated mouse anti-human FcR antibodies. The samples were assessed in a flow cytometer and analyzed by the FlowCellQuest software. The PBMCs were explored in a forward and side scatter diagram (Figure 3), where the monocytes were gated based on their greater size and granulation in comparison to lymphocytes. The monocytes were then further defined by their CD14 expression. Isotype control antibodies were used to define the negative population. Limits for the quadrant markers were set based on isotype controls and negative populations. The frequency of positive cells was analyzed in the PBMC gate, and each cellular monocyte Fc receptor expression was defined by it's mean fluorescence intensity (MFI).
Figure 3. Example of PBMC-gating (A) and double staining of gated PBMCs with mouse-anti human antibodies (B).

A: SSC = side scatter (corresponds to cell granularity), FSC = forward scatter (corresponds to cell size). B: The fluorescence intensity (MFI) is detected by 1) a fluorescein isothiocyanate (FITC)-conjugated mouse-anti human antibody (here CD16 FITC) in the FL1-H channel, and 2) a phycoerythrin (PE)-conjugated mouse-anti human antibody (here CD14PE) in the FL2-H-channel.

Isolation of monocytes using magnetic activating cell sorting

In order to achieve a pure monocyte population for the FcγR function experiments, the CD14 positive cells were extracted from the PBMCs using magnetic activating cell sorting (MACS) with anti-CD14 microbeads and LS columns. A standardized MACS protocol was used which was provided by the manufacturer (MiltenyiBiotec™) and had been evaluated in our group earlier (68).
Figure 4. The principle of MACS-sorting of CD14⁺ monocytes.

IC-binding by monocytes
To study the monocyte FcγR binding capacity of IC, MACS-purified monocytes from patients and controls were incubated with human IgG1 and IgG3 ICs in a rosetting assay. The ICs were made by incubating red blood cells (RBC) of an RhD⁺ blood donor with human IgG1 or IgG3 anti-RhD antibodies. Non opsonized RBCs were used as negative controls. After incubation at 37°C the obtained ICs were washed and diluted, put on ice and finally mixed with the MACS-sorted monocytes. After incubation the monocyte-IC pellet was re-suspended and stained to visualize the monocytes that had bound ICs (“rosettes”) in a light microscope (at 400x magnification). At least 100 monocytes per sample were evaluated, and all samples were blinded towards the examiner. A rosette was defined as a monocyte that had bound 3 or more erythrocytes. Only patient samples allowing duplicate evaluation were taken into consideration, and the mean of the duplicates was calculated. The IC-binding was defined by the percentage of rosettes (out of total cells) or by a rosetting index (i.e. % IgG1 rosetting divided by % RBC rosetting (negative control)). This index corrects for the background rosetting of RBC only.

TNFα production by IgG-stimulated monocytes
Monocytes can secrete cytokines upon activation (69). An important pro-inflammatory cytokine that is expressed in the psoriatic skin and in the synovial
tissues of PsA, as well in RA joints is TNFα. Anti-rheumatic treatments that address this cytokine have been effective in reducing signs and symptoms of psoriasis, PsA and RA (70, 71). To explore if FcγR-stimulated TNFα secretion was altered in PsA or RA, MACS-sorted CD14+ cells were stimulated with immobilized human IgG1 or IgG3 (resembling ICs). F(ab)²-fragments of IgG were used as negative controls, and lipopolysaccharide (LPS) or phorbol-12-myristate-acetate (PMA) as positive controls. [LPS and PMA activate intracellular signaling pathways via other mechanisms; LPS via Toll-like receptor 4, and PMA acts via protein kinase C-activation]. The monocytes were incubated with Igs or positive controls at 37°C over night. On the next day the cell culture supernatants were harvested (triplicate cultures pooled) and stored at -20°C for later analysis.

Quantification of TNFα and soluble FcRs
To quantify the amount of TNFα in the supernatants, a TNFα-specific sandwich-ELISA was used. The supernatants or a recombinant human TNFα protein (standard) were incubated with an immobilized TNFα capture antibody in microtiter wells. After blocking and washing procedures, a biotinylated goat anti-human TNFα-antibody was added to the wells for the detection of bound TNFα. Following further washings, streptavidin-horseradish peroxidase or ExtrAvidin-peroxidase were added as detection enzymes. Finally, the chromogenic substrate tetramethylbenzidine (TMB) was added and the resulting enzymatic reaction stopped with 1M H2SO4. The colour reactions in the wells were analyzed at 450nm in an ELISA-reader and the results were compared to a standard curve with the recombinant TNFα. Data were analyzed with a SoftMaxPro4.8 software. A so-called stimulation index was calculated (e.g. IgG1-IC-stimulated TNFα-production divided with F(ab)²-stimulated TNFα-production). Such an index describes a factor of altered TNFα secretion in relation to background monocyte basal secretion of TNFα.

In addition, commercial sandwich ELISA kits were used for the quantification of soluble (s) human FcRs (sCD64, sCD16a, and sCD89) in the plasma of patients and controls.
The principle of a sandwich-ELISA

The principle of a sandwich-ELISA

1. Application of standard or supernatant to wells coated with capture antibody anti-X¹
2. Application of detection antibody anti-X²
3. Application of biotin
4. Application of streptavidin
5. Application of chromogenic substrate (TMB)
6. Application of sulfuric acid

A-E includes blockings, washes and incubation times.

**Figure 5.** The principle of a sandwich-ELISA. 1 application of standard or supernatant to wells coated with capture antibody anti-X¹ 2 application of detection antibody anti-X² 3 application of biotin 4 application of streptavidin 5 application of a chromogenic substrate (TMB) 6 application of sulfuric acid A-E includes blockings, washes and incubation times.

Statistical analyses

For all experiments a GraphPadPrism software was used for the statistical analyses and graph constructions. The MannWhitney U-test was performed for comparisons between groups, and the Wilcoxon signed-rank test was used for comparing RA matched samples. With the Spearman Rank correlation the statistical dependences between independent variables were assessed and further illustrated by regression analyses. These selections of statistical analyses were based on the recommendations of a university statistician. P-values < 0.05 were considered significant.
Results

Prior to the patient evaluations we characterized the HC group regarding FcR expressions and monocyte subset distribution. Healthy men and women displayed similar mean expressions (percentage and MFI) of monocyte FcαR and FcγRs. However, with age healthy women increased their expressions of CD64, CD32a and CD89 and showed a trend of increased expression of CD16. For healthy men no significant changes regarding these expressions could be seen except for CD89 which interestingly decreased with age. The monocyte expression level of the inhibiting CD32b was comparable between the genders and showed no age correlations (data not shown).
Figure 6. Age-correlated changes of monocyte FcR expressions in healthy women and men.
However, we have previously reported an age correlated decrease of CD32b expression on B-cells in healthy women that may be pivotal in the generation of autoreactive B-cell clones (72). Regarding the monocyte subsets we observed a trend of increased frequency of the CD14⁺CD16⁺⁺ non classical monocytes in healthy males compared to healthy females (Figure 7).

![Figure 7.](image)

Figure 7. Monocyte subset distribution in healthy women and men; CD14⁺CD16⁺⁺ (non classical monocytes), CD14⁺⁺CD16⁺ (intermediate monocytes), CD14⁺⁺CD16⁻ (classical monocytes).

Altogether these findings might shed some new light on why women in general outmatch men in autoimmune diseases and why men in general are more susceptible to infectious diseases (73).

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Paper 1. Up-regulation of CD64-expressing monocytes with impaired FcγR function reflects disease activity in polyarticular psoriatic arthritis.

In the first study we aimed to assess the monocyte FcR status and function in patients with active polyarticular PsA in relation to HC and to disease activity. Concerning the ongoing medical treatments, this cohort of 23 patients was heterogeneous, but all subjects had active joint inflammation as shown by their mean of DAS28 of 4.1 and DAS28CRP of 4.0. The mean PASI score was 4.1 - which corresponds to a mild skin disease.

We found that the PsA patients had elevated serum levels of IgG1, 2, and 3, and had increased numbers of CD64⁺ monocytes. The latter finding correlated positively with laboratory parameters of inflammation (CRP and ESR) and with DAS28. No group differences were observed comparing the expressions of the other FcγRs and the FcαR. Further, the PsA monocytes were highly loaded with IgG and this correlated with the serum level of total IgG. The PsA monocyte FcγR function was affected in terms of reduced IgG IC-
mediated TNFα release. Patients taking immunosuppressive drugs showed even less monocytic TNFα production. However, no obvious difference in the IgG1- and IgG3-IC binding capacity was noted comparing the PsA patients with HC. Interestingly, the monocyte FcγR function reflected the patients’ experience of disease activity as well as the doctor-observed signs of joint inflammation. PsA was also accompanied by an increase in the number of CD16 low expressing monocytes, which reflected the individual CRP and IgG2 levels. This implies that the CD16 low expressing monocyte subset is important in active PsA. Since immune responses to bacterial polysaccharide antigens (e.g. streptococcus species) mostly are of IgG2, and this pathogen is a known trigger of psoriasis flares, this result also suggests that IgG2-IC could play a role in PsA inflammation. The skin scores (PASI) in the patients were neither correlated with the FcR expressions or the FcγR function, the monocyte IgG load, nor the Ig isotype/IgG subclass levels. Therefore we concluded that our findings of increased CD64 expression and raised IgG levels in polyarthritic PsA are arthritis specific.

**Paper 2. Elevated Membrane and Soluble CD64: A Novel Marker Reflecting Altered FcγR Function and Disease in Early Rheumatoid Arthritis That Can Be Regulated by Anti-Rheumatic Treatment.**

Having observed alterations of Ig levels and FcγR function in a chronic seronegative joint-inflammatory disease like PsA - with even lower FcγR function in patients treated with anti-rheumatics - we were subsequently interested in evaluating untreated patients with a seropositive joint-inflammatory disease such as RA. We were particularly interested in exploring if a specific FcR could be linked to the RA pathogenesis. Consequently, 20 naïve and RF positive RA patients (with a mean DAS28 of 5.26) were studied before (at a first visit; FV) and after 3-4 months of anti-rheumatic treatment (at a follow up visit; FU) and compared with 33 age-matched HC. The individual treatment response was evaluated at FU – using the EULAR response criteria. Prior to treatment we found that the IgG1 and IgG3 serum levels were elevated in the RA patients, that these individuals presented with more CD64+ monocytes, and that the RA monocytes expressed more CD64 and cell surface-bound IgG than the HC monocytes. The FcγR function in the RA monocytes was more clearly impaired compared to the PsA monocytes shown in the previous study (Paper 1). Thus, the RA monocytes showed an overall reduced IgG1/IgG3 IC-binding, as well as a reduced IgG IC-stimulated TNFα secretion compared to HC monocytes. These alterations correlated significantly with different disease activity markers; patients with high number of swollen/tender joints/DAS28 or patients who reported poor global wellbeing presented mono-
cytes with the worst FcγR function. Interestingly, when grouping the patients’ results according to their clinical response to anti-rheumatic treatment it was found that the IgG1-IC-binding capacity in the monocytes from the non-responder patients at the FV was inferior to that of the good responders (Figure 8).

Figure 8: The IgG subclass specific immune complex binding capacity of monocytes reflects the later treatment response in early rheumatoid arthritis (g.r. = good responder, n.r. = non-responder).

This suggests that optimal IC handling is of great importance to prevent RA persistence.

Following anti-rheumatic treatment, a decrease of membrane CD64 was only observed in the good responders, who also preserved a high monocytic IgG load and up regulated the CD89 expression. Interestingly, the mean monocyte CD64 expression (MFI) before treatment in the good responders was higher than in the non-responders. However, the non-responders maintained their CD64 expression at FU. We therefore conclude that maintaining a high monocyte CD64 expression characterizes a state of persistent arthritis. At FU, the good responder monocytes were in an inactivated state, which could be demonstrated by the decrease of the ratio of activating and inhibiting FcγR expressions.
In addition, the analyses of the soluble FcRs sCD16a, sCD64 and sCD89 in the patient plasma revealed differences in comparison with HC. Upon anti-rheumatic treatment the levels of all 3 sFcRs decreased. What stood out was the elevated sCD64 in RA compared with HC and a matched reference group of 20 patients with active PsA. This indicated that sCD64 is specific to RA, and not to any type of polyarthritis. In contrast, sCD16a (which constitutes the soluble form of the monocyte-specific FcγRIIIa) was increased in both RA and PsA in comparison to the HC. This implies that sCD16a is an inflammatory marker, rather than a disease specific marker. In RA, it’s concentration reflected disease activity significantly as both subjective (i.e. patient reported pain and HAQ) and objective parameters thereof (i.e. SJC and DAS28) were correlated with sCD16a. We could not detect any difference in the sCD89 concentrations between patients (RA and PsA, respectively) and controls.

Moreover, we also observed a reduction in the serum CRP-levels in the good responders, who displayed higher pre-treatment CRP-levels than non-responders and effectively reduced their CRP concentration following treatment. The non-responders presented lower mean CRP levels initially, that did not change during treatment. This is interesting of two reasons and calls for further studies on the role of CRP in RA; firstly, the acute phase protein CRP is a ligand of FcγR and FcαR and could compete with IgG or IgA on available FcRs; secondly, CRP is released from the liver upon IL6 stimulation, and one could hypothesize that patients with low CRP could represent a RA subpopulation whose joint inflammation is driven by other cytokines than IL6, which may explain the poor treatment outcome after a combined methotrexate/steroid treatment.

Paper 3. Rheumatoid factor isotypes reflect the monocyte Fcγ receptor function and treatment outcome in early naïve rheumatoid arthritis.

The understanding of profound alterations in the FcγR expression and function in early RA pronounced further studies on Ig and auto-antibodies in the early RA cohort, especially in relation to treatment response, FcγR function and monocyte subsets.

In general, we saw higher mean pre-treatment auto-antibody levels (the IgM, IgG and IgA RF isotypes, and ACPA IgG) and Ig levels (total IgA, IgG, and IgG1,2,4) in the non-responders, who, however, maintained high levels of IgG3 and IgM RF at FU. Upon anti-rheumatic treatment all Ig isotypes, IgG subclasses and autoantibodies had decreased in the whole RA cohort. The elevated IgM RF, IgG RF and IgA RF levels in the early RA cohort were negatively correlated with the in vitro monocyte FcγR function: patients who pre-
sent high levels of these RF isotypes bound significantly less IgG1-IC. Interestingly, the same also applied to patients with high IgG3 serum levels. On the other hand, none of the RF isotypes reflected the IgG3-IC binding capacity. These data suggest that an impaired IgG1-IC handling is critical for continued arthritis and that high levels of RF isotypes contribute to this. Indeed, there was a negative correlation of high IgA RF with low monocytic TNFα release upon IgG1- and IgG3-IC stimulation.

Not only the RF isotype levels seemed to distinguish good responders and non-responders; a good treatment response was seen in patients who initially presented less simultaneously appearing RF isotypes. Single positive RF samples were only observed in good responders and comprised either the IgM or the IgA isotype, but not the IgG.

As monocyte subsets may have different roles in inflammation regarding their capacities to migrate, phagocytose, produce cytokines and present antigen we were also interested in evaluating these in relation to treatment response and RF isotypes. At FV, classical monocytes were elevated in early naïve RA compared to HC, especially in those individuals who later responded less to treatment. Non-classical monocytes were specifically increased in the good responders compared to the non-responders. High IgA RF levels correlated with low percentage of intermediate monocytes. There was also a trend for a correlation with more classical monocytes. We observed no association between IgM RF or IgG RF levels and the monocyte subsets. These data could imply that auto-antibodies may influence the monocyte subset distribution in early RA.

Paper 4. B-cell depletion in rheumatoid arthritis; conversion to RF negativity indicates a good treatment response but not an increased risk of infection.

In the fourth study we aimed to evaluate the effects of long-term systemic B-cell suppression in RA on disease activity, Ig serum levels and infection risk in everyday clinical practice. Thus, 69 RA patients who were treated with anti-rheumatics and/or steroids in combination with rituximab (RTX) were enrolled and their medical records were studied regarding current medications, contemporary diseases, number and types of infections and prescribed antibiotics, and episodes of leuco-/neutropenia and inpatient care. We found that RA patients who were IgM RF⁻ at evaluation had received more RTX, had lower disease activity and lower Ig levels than IgM RF⁺ patients. The total IgM levels reflected the SJC and the IgG3 serum levels.

No differences in the number of infections or prescribed antibiotics were observed when comparing RF⁻ with RF⁺ patients. On average, 1.2 systemic
antibiotics and 0.2 topical antibiotics had been prescribed per patient year during the RTX treatment. The most common infections were of the airways, urinary tract and skin. Individuals with contemporary diseases such as diabetes, asthma/COPD and secondary Sjögrens syndrome were more affected by infections.

One case of hepatitis B (HBV) reactivation was observed in a patient with known inactive HBV. This patient had successfully been treated with RTX during approximately 5 years but voluntarily paused the prophylactic antiviral medication for 3 months due to excellent general well-being. One death occurred due to tick borne virus encephalitis (TBE) in an otherwise healthy patient who had had great benefit of her RTX treatment for more than 5 years without complications or notable Ig isotype deficiencies. This patient had been vaccinated against TBE prior to RTX initiation; she got an insect bite approximately 2 weeks before the last RTX course was started, but this episode remained non-reported as no signs of infection developed. The TBE diagnosis could only be made at autopsy by the detection of viral RNA in brain tissue; titers of anti-TBE antibodies in serum and spinal fluid remained absent during hospitalization.

From this study we concluded that B cell depletion with RTX is effective in controlling inflammation in seropositive RA, and that IgM RF negativity could be a desirable treatment target. We also emphasized that primarily concomitant diseases and other medications rather than low Ig levels were decisive for the patients’ infection sensitivity.

![Figure 9. Calculated mean RABBIT risk scores in longstanding RA patients treated with rituximab. The patients are sub grouped according to their IgM RF status at evaluation.](image)

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The use of the RABBIT risk score - an easy-to-use tool - had limited value for identifying individuals with high infection risks: in some cases this risk score exaggerated and in other cases it underestimated the risk of infection. It should therefore not replace the physician’s careful risk assessment and judgement. To minimize infectious complications, patients with hepatitis B should be monitored closely during RTX therapy, and current insect bites - which could lead to TBE infection - should lead to a postponement of the RTX course. Furthermore, the recommendations of general and local vaccination programs should be followed prior to/during RTX therapy.
In this work I have explored the contribution of FcRs to joint inflammation in the most common chronic polyarthritides, RA and PsA, in relation to Igs, disease activity and treatment response. The patient populations and the matched HC were thoroughly characterized, and all participating patients suffered from active joint disease. The early RA study was unique, since the study design permitted comparisons of expression levels and functional data as well as before and after a period of anti-rheumatic treatment. Studying drug naive patients is of importance since several factors, beside the disease activity, can affect an FcR status.

Furthermore, the functional FcγR experiments in this work were performed on daily fresh MACS-purified monocytes, which rules out possible effects mediated by freezing/thawing or long time culturing. The monocytes are per se interesting to study since they provide the basis for tissue macrophages, dendritic cells and osteoclasts. Those cell types are accumulated in RA and PsA synovium and play important roles for the local cytokine production, antigen presentation and bone degradation that can be observed in inflamed joints. Indeed, an improved joint status both clinically and radiographically can be observed following adsorption aphaeresis of the granulocyte/monocyte subsets (74, 75), and in the K/BxN serum-induced arthritis model mice depleted of macrophages by clodronate liposome treatment are completely resistant to joint inflammation (76).

To define the different monocyte subsets we used the LPS receptor (CD14) and CD16. We observed that the CD14\textsuperscript{high} CD16\textsuperscript{low} expressing monocytes were increased in early RA and in PsA, and the CD14\textsuperscript{++}CD16\textsuperscript{{}} compartment was especially high in the non-responding RA individuals. Such classical monocytes express CD64, present high chemotactic and good phagocytic activity, have B-/T-cell suppressing properties and are effective cytokine producers (13). However, evaluations of monocyte subset distributions may be influenced by inflammation and treatment related factors. Thus, this could explain the somewhat different results we obtained in comparison with previous RA studies where intermediate monocytes were reported being increased. In those works the cohorts studied included immunosuppressed patients with longstanding disease and disease activity was not well-defined (77-79).

Previous published studies indicate that PsA would have autoimmune features evidenced by autoantibody production and IC formation (25, 27, 80-82). In the first study we were therefore interested in evaluating monocyte FcRs in
PsA in this context. Our findings of an increased polyclonal B-cell activity with raised IgG1, 2 and 3 subclass levels compared to HC, and the up regulation of the number of CD64⁺ monocytes with impaired FcγR-mediated TNF production support a role of IgG IC in PsA. In addition, the suspicion of polyarticular PsA resembling RA may be valid (I will come back to this observation later). However, in PsA the IC-binding capacity was preserved although the PsA monocytes were as heavily loaded with IgG as the RA monocytes. Though, there was a slight reduction in the TNFα production after IgG1 and IgG3 subclass stimulation of PsA monocytes. Since non FcγR-ligand triggered TNFα secretion was maintained in PsA we therefore suspect that the intracellular signaling pathways downstream of the FcγRs were affected. Interestingly TNFα may promote both pro- and anti-inflammatory reactions depending on which membrane-bound receptor it will interact with; soluble TNFα will act in a pro-inflammatory fashion via tumor necrosis factor receptor 1 (TNFR1)-binding/signaling while ligation of TNFα to TNFR2 will result in anti-inflammatory reactions. In this sense, monocyte suppression in terms of TNFα production may alter the balance of TNFR1 and 2 signaling thus counteracting inflammation (83). Interestingly, monocytes from the DMARD-treated PsA patients in paper 1 produced even less TNFα upon IgG subclass stimulation. This indicates that intracellular pathways beyond the FcγRs are not solely responsible for PsA inflammation. Since the PsA FcγR function only reflected subjective and objective signs of joint inflammation and did not correlate with the patients’ skin disease activity, I conclude that the findings of increased CD64 expression and raised IgG levels were specific for the arthritis component of the disease. In active polyarticular PsA, the increase in Ig production may be followed by monocytic IgG loading that could act as a tuning system for the inflammatory response but that also could lead to monocyte activation and differentiation via ICs binding to low affinity FcγRs. Interestingly and in contrast to the early RA patients, the PsA patients also presented elevated serum levels of IgG2, which may bind to specific FcRs that are not affected by IgG1 and IgG3 IC binding and were not tested in our study. IgG2 is the second most abundant IgG subclass in humans, and it is the predominant IgG subclass complexed in IgM-IC under physiological conditions. This predominance of IgG2 within IgM–IgG IC may be lost in autoimmune disease where IgG1 and IgG3 containing IgM–IgG IC dominate (84). IgG2, together with IgG4, is also considered a second line or late IgG subclass in the immune response. It has low complement binding properties compared to IgG1 and IgG3, and it is involved in immune responses to bacterial polysaccharide antigens such as streptococcal species. Interestingly, streptococci are known to initiate psoriasis flares (85). Therefore, recurrent bacterial infections or a defect in polysaccharide antigen elimination could be important for the PsA pathogenesis. Consequently, of future interest will be the evaluation of how IgG2-IC might affect the monocytic FcγR function in PsA.
In the early RA study the CD64 up-regulation came out much clearer than in the PsA study. Not only the number of CD64 expressing monocytes but also the CD64 expression per cell and the amount of released/shed sCD64 was increased in early RA compared to HC. Other membrane bound FcR expressions were not significantly altered. The importance of CD64 has been emphasized in animal models of arthritis; treatment with a CD64-directed immunotoxin inhibits arthritis progression and synovial signs of inflammation, and recombinant soluble human CD64 reduces signs of inflammation and decreases pro-inflammatory cytokine and autoantibody levels in the same animal models (86, 87). My results of the monocyte FcR expressions in RA differ somewhat from previous reports (Table 5):

Table 5. Reported monocyte FcR expressions and soluble FcRs in RA patients.

<table>
<thead>
<tr>
<th></th>
<th>CD64 (%)</th>
<th>soluble CD64</th>
<th>CD32 (%)</th>
<th>CD32b (%)</th>
<th>CD16 (%)</th>
<th>soluble CD16</th>
<th>CD89 (%)</th>
<th>soluble CD89</th>
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</thead>
<tbody>
<tr>
<td>Matt et al (PLOSone 2015)</td>
<td>↑ (↑)</td>
<td>↑</td>
<td>↔ (↔)</td>
<td>↔ (↔)</td>
<td>↔ (↔)</td>
<td>↑</td>
<td>↔ (↔)</td>
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Abbreviations. ↑ = elevated levels in RA compared with HC. ↓ = decreased levels in RA compared with HC. ↔ = comparable levels in RA and HC. n.r. = not reported. n.d. = not done.

These differences are most likely due to the combination of the following: inadequate age- and sex matching of control groups; simultaneous evaluation of early naïve RA individuals and RA patients with longstanding disease - involving seronegative patients and patients in remission; inclusion of RA patients with ongoing DMARD-, biologic- and steroid treatments; and substandard definitions of disease activity.
A good treatment response in our RA patients was reflected by a decrease in the surface expression of CD64 - and in general, lower sCD64 levels were observed at FU. These findings were specific for the early RA cohort, since HC didn’t present any signs of CD64 turnover and only few PsA patients had released sCD64 - to a very limited extent. What could be the role of the increase of membrane-bound CD64 and sCD64 in RA? I speculate in the following: during joint inflammatory conditions several pro-inflammatory cytokines could be responsible for the upregulation of membrane bound CD64 (see Table 3), however the increase in CD64 secretion in RA would be promoted by specific proteinases/shedases. The latter has been shown for CD16. The so-called ADAMTS-family (i.e. a disintegrin and metalloproteinase with thrombospondin motifs) of multidomain extracellular protease enzymes are potential candidates and are overexpressed in RA joints (88-91). This CD64 shedding could represent a control mechanism for handling the excessive IgG released during inflammation. It could also help increasing the size of circulating IC, and promote blocking of available Fc portions with sCD64 - thus protecting membrane bound FcγRs from being activated by IC. Large ICs are not as effective in promoting cytokine production as small ICs and bind easier to activating low affinity FcγRs, such as CD32 and CD16 (92, 93). Since only high-affinity CD64 is occupied with IgG in vivo, low-affinity FcγRs are readily available for ICs that are formed locally. The low-affinity FcγRs are supposed to be more suitable to enable antibodies to efficiently modulate adaptive immune responses than high-affinity FcγRs (47). One problem with surface CD64 upregulation and it’s IgG-loading could be important for the pathogenesis of arthritis or IC-mediated inflammation: IgG heavily loaded monocytes have been shown to increase their IC-binding capacity and pro-inflammatory property (94-96). This would contribute to ongoing inflammation and facilitates the repeal of peripheral autoimmunity.

In early RA we noticed that an unchanged CD64 expression, combined with a decrease in the monocytic IgG load, corresponded to a poor treatment outcome. Consequently, the CD64 “system” seemed to be overburdened in these non-responding individuals who also presented higher autoantibody levels. I speculate whether this finding reflects the spreading of the disease to other joints as one can assume that IC-handling was inferior in these patients. With this in mind it was interesting to proceed and evaluate the FcγR function in the early RA cohort. As suspected, both the capacity of IC binding and IgG subclass stimulated TNFα release was reduced compared to HC. Especially those RA patients who later would respond less to the anti-rheumatic treatment presented the lowest grade of FcγR function before the therapy was initiated. Since these individuals had comparable disease activity with the good responders, I hypothesize that not only the cytokine milieu, but also the impact of several FcγR ligands (Igs, auto-ab, ICs and acute phase reactants like CRP) could be responsible for this altered FcγR function. Consequently we analyzed
RF isotypes, ACPA and CRP, and we observed significant negative correlations of RFs with the FcγR function; especially a low IgG1-IC binding was related to high titers of 3 different RF isotypes, while monocyte TNFα production seemed to be dampened in high IgA RF individuals. Again the non-responders stood out, and they were overrepresented among the RA patients who had high autoantibody levels and had more concomitant RF isotypes. In RA there is a good correlation of the serum titers of RFs and ICs so we can assume that the non-responders had higher IC concentrations in the circulation than the good responders (97). A consequence of an impaired monocyte FcγR function in RA could be the dissemination of ICs from the blood to the joints. Probably the non-responders who presented higher mean levels of Igs and auto-abs were more affected by the impaired FcγR function. Indeed, the IC binding capacity of the non-responder monocytes at FV was inferior to that of the good responders (Figure 8); and an inferior IC handling was in general followed by more joint symptoms (98). In that sense, the migrating joint symptoms which many RA patients experience in the initial phases of the disease - or the temporary joint swellings which are reported by patients with palindromic RA - could be the result of such IC spreading events. In early disease the immune system would still be capable to cope with the ICs. Later however, “new” inflammatory sites provide the basis for additional antibody production and new IC formation and so the vicious circle is made permanent. In our study the low IgG1-IC binding capacity was related to high RF isotype levels, and IgG1 was elevated to a greater extent than the other IgG subclasses. I therefore conclude that an excess of IgG1 complexed with RF could play an important role for FcR mediated inflammation in RA.

The immunosuppressive state of the early RA monocytes that we noted could be the result of an increased IC handling via the inhibitory CD32b, resulting in ITIM-mediated down modulation of ITAM/common FcRα-chain-bearing FcRs and their intra-cellular signaling pathways. Indeed, only the good responders had up-regulated CD32b after the anti-rheumatic treatment. Consequently, their ratio of activating/inhibiting FcγRs was comparable to the HC at FU. Such a restoration of the CD32b expression is even more important for controlling the Ig production of B-cells. Although we did not per se analyze FcR-expressions on the patients’ B-cells, I assume that especially the good responders had managed to dampen their B-cell activation - as was proven by the significant decrease in their systemic Ig and autoantibody levels.

Interestingly, we noticed differences in the change of serum CRP between good and non-responders during the treatment period. Thus, only the good responders managed to reduce their CRP and that was from a high pretreatment level, suggesting an effect of the anti-rheumatic treatment on IL6 in these individuals (99-101). In the “DAS28-CRP” this acute phase reactant represents an objective laboratory marker of joint disease activity. The CRP analysis is commonly used for the evaluation of anti-inflammatory or antibiotic
therapies. However, when evaluating serum CRP one must remember that the result will only reflect a part of CRP’s total presence: CRP concentrations in tissues and on cell-surfaces (i.e. sites where CRP fulfills its tasks) can at best only be estimated (102). Interestingly CRP has been detected in the rheumatoid synovium, and there are indications that CRP contributes significantly to atherosclerosis by facilitating FcR interactions between cells (103, 104). Cardio-vascular disease (CVD) is the leading cause of death in RA and a high systemic inflammatory burden associated with RA appears to be a key driver for atherosclerosis. Consequently RA patients nowadays are screened for CVD worldwide (105). It is well known that CRP can compete with IgG and IgA on binding to available FcγRs and FcαRs. In this manner CRP can generate pro-inflammatory cytokines, thus enhancing an inflammatory response (55-59, 101). However, CRP may also exert a protective role against immune mediated inflammation; in animal models of autoimmune encephalitis or lupus nephritis administration of human CRP dampens the encephalitis or nephritis activity and severity in human CRP-transgenic mice (106, 107). With light of that, individuals with low CRP could therefore indirectly increase the availability of low affinity FcRs for pathological ICs.

Regarding the monocyte CD16 expression, we noticed trends of lower percentages of CD16⁺ cells and lower surface levels in PsA and in RA at FV compared to HC. This is in contrast to a previous study in which early RA patients (with lower mean levels of RF/ACPA and including autoantibody negative individuals) were reported having higher monocyte CD16 expressions than HC (108). The diverging results may be explained by differences in the study populations, in the autoantibody background pressure, in treatment strategies, in gating procedures, and in the use of different CD16 detecting antibodies. However, I note that that study reports on trends of poor treatment response in individuals who presented higher autoantibody levels. Our RA patients had an increased CD16 turnover evidenced by significantly elevated monocyte specific sCD16a compared to HC. At FU the number of CD16 expressing monocytes where marginally increased in the non-responders (from 42% to 54%) while the mean expression per cell had slightly decreased (118 MFI to 99 MFI). This was not observed in the good responders who rather presented stable CD16 expressions. Interestingly, the good responders presented signs of higher CD16 turnover (in terms of increased mean sCD16 levels) compared with the non responders both at FV (11861 vs. 7154 ng/ml) and at FU (6924 vs. 4689 ng/ml) (data not shown). This suggests that the potential of IC handling via this low affinity receptor was more optimal in the good responders during the observation period. Since the sCD16 levels were increased in both early RA and in the PsA cohort but also detectable in HC we concluded that this sCD16 elevation represents a diagnosis-unspecific inflammatory reaction involving IC in both diseases. In addition, we proposed
sCD16 analysis in RA for objectifying disease activity, as very distinct correlations with both objective (DAS28-CRP, ESR and the SJC) and subjective markers (patient reported pain and HAQ) of joint disease were obtained.

In RA, the occurrence of IgA RF is considered a negative prognostic factor, and in both psoriasis and PsA increased levels of circulating IgA-IC are observed (31, 109, 110). Consequently we were interested in studying monocyte FcαR expression in our patient cohorts. To our surprise we could not identify obvious differences between patients and controls, although trends of lower membrane CD89 levels and higher sCD89 levels were observed in PsA - as signs of a discrete increase in receptor turnover. The small number of patients observed and the heterogeneity of the patients’ ongoing treatments could have contributed to these non-significant differences. Interestingly, the monocyte CD89 expression correlated with the PsA patients’ reported morning stiffness. This could be interpreted as an indirect IL-6 effect since blood phagocyte CD89 expression has been shown to correlate with the serum levels of this cytokine, and IL-6 release undergoes a circadian rhythm - peaking at night (111, 112). In early RA, alterations in the CD89 expression were noticed during the treatment course, and symptom relief was accompanied by an increase in CD89MFI, especially in the good responders. Since monocyte CD89 expression correlates negatively with serum IgA levels in health and disease, we assume that with good response to treatment, a decrease of IgA-ICs binding to CD89 will discontinue receptor shedding into the circulation. Indeed, at FU the levels of sCD89 had decreased in the early RA cohort. I also suspect that the down regulation of surface CD64 in the good responders at FU could contribute to an increased availability of free FcRγ chains allowing more CD89 molecules to be expressed on the monocytes; surface expressions of CD64, CD16 and CD89 are dependent on the expression of the common FcRγ chain (113-115).

Regarding the immunosuppressed state of the RA monocytes in terms of reduced FcγR function, this could partly also have been managed by the FcαR. Thus this dual acting FcR promotes anti-inflammatory reactions when being triggered with monomeric IgA - resulting in inhibitory signaling through the FcαR1–FcRγ-chain (ITAMi). However, when cross linked by polymeric IgA/IgA-ICs CD89 will promote pro-inflammatory reactions. Such IgA aggregates induce the release of soluble CD89 (likely due to proteolytic cleavage), suggesting that the FcαRI has regulatory effector functions in IC mediated inflammation (45, 116-117).

Having noticed that a disturbed balance between FcγR status and function, Ig levels, and surface IgG load was important for the treatment outcome in early RA, I was then interested in evaluating the effects of long-term B-cell suppression on Ig levels, disease control, and disease activity. Various conventional RA treatments are known to reduce serum Igs to various degrees; I chose to study patients on treatment with a specific anti-B-cell therapy, RTX. I also intended to identify possible biomarkers of RA disease control. Besides
CRP and ESR no specific biomarkers are in use in clinical practice, however previous work report on RF being probably better than ACPA for evaluating joint disease activity (118-122).

In the 4th study I enrolled initially RF positive RA patients with longstanding disease who had on average tested more than one other biologic drug (most commonly a TNFα blocker) before RTX was started. Since B-cells and B-cell products are involved in the defence against external pathogens and an impaired B-cell function is reported in conjunction with RTX treatment, I also intended to study these patients’ accumulated risk for infectious diseases (123, 124). All 69 patients had received at least one course of RTX. At assessment, 57% of the patients proved IgM RF negative. In retrospective, these seronegative patients had received more RTX, had lower current disease activity, and presented with lower Ig isotype levels than the seropositive patients. I therefore concur with a previous report that proposed IgM RF as a good marker of disease activity (122), but I also want to stress that IgM RF negativity could be a desirable laboratory treatment target for inflammation control in RF⁺ RA. This is supported by the fact that RTX seems to affect autoantibody producing B-cells more than physiological protective antibody secreting B-cells (125), and therefore RTX not necessarily must be associated with an increased infection risk.

The RA patients’ total IgM levels reflected their SJC and IgG3 levels, and a trend of negative correlation of the patient reported wellbeing (PG) with IgG3 was observed indicating a possible protective role of IgG3 in RA inflammation. Interestingly short-lived antibodies such as IgM (which represents the first line humoral immune response), and IgG3 (which is the first appearing IgG subclass in a humoral immune response) have the highest affinity for C1q (126, 127). A rise in both these Igs corresponded to B-cell recovery after the last RTX course. This could be considered a readiness to deal with the increased amounts of complement containing ICs which is observed in RA - and which reflects disease activity (128).

The long-term B-cell suppressive effect of the anti-rheumatic treatment resulted in lowering IgG1 below normal levels in some of the RA patients. When studying these low IgG1-individuals (defined as having an IgG1-level less than 5g/L) I found that they had significantly higher disease activity (p = 0.043, data not shown) than the high IgG1 individuals (defined as having an IgG1-level of more than 5g/L). In that sense preserving a normal IgG1-level could be considered to have a protective role in RTX-treated RA, while too low IgG1 levels (not managing to block enough activating CD64 receptors) would predispose to an increase in available binding sites for pathological IgG containing ICs: IgGRF, whose reactivity is particularly directed to the IgG1 and IgG3 subclasses, could then exert prolonged IC mediated inflammation via CD64 (129-131).

In the medical records airway infections were most commonly noted, followed by infections of the urinary tract, skin, gut and gynecological sphere.
No differences in the number of infections or prescribed antibiotics were observed comparing RF⁻ with RF⁺ patients. This suggests that RTX is a safe drug, even after long-term regular treatment, and my data are consistent with the work of others (132-134).

However, the seronegative patients were overrepresented among those who presented with low Ig levels and low white blood cell counts. The patients - who in retrospective - had had any kind of Ig deficiency were also more often admitted to hospital (p<0.05, students T-test), had more urinary tract infections, and had had more antibiotic treatments compared to patients who never showed signs of any Ig deficiency (135). Especially, lowered levels of IgG1 (this Ig is mainly involved in the response to protein antigens), IgG2 (this Ig is produced in response to polysaccharide antigens) and IgA (this Ig protects the mucosal surfaces) reflected previous high consumption of antibiotics (136). One can therefore assume an association of increased infection risk with a prolonged B-cell suppression.

Could this be related to the RTX therapy solely? The immunosuppressive effects of RTX include delayed-onset leucopenia, hypogammaglobulinemia, and probably also decreased T-cell immunity. Ig deficiencies in general occur more often in autoimmune diseases, and hypogammaglobulinemia may also be caused by other medical conditions and several anti-rheumatics (137, 138). In my RTX-study I observed that concomitant immunosuppressive treatments and contemporary diseases (such as diabetes, chronic airway diseases and secondary Sjögrens syndrome) were more important for the increased risk of infections observed in this RA cohort. These observations are in line with previous reports (139-141), and I conclude that RTX treatment is safe as long as concomitant diseases and infections are identified and treated properly. The RABBIT risk score did not thoroughly identify the high consumers of antibiotics, and it’s use in clinical practice is therefore of limited value.

To summarize, interactions of FcR and Ig/IC are important for inflammatory events in RA and perhaps also in the polyarticular form of PsA. These interactions lead to an altered FcγR function which is more pronounced in RA than in PsA. In both diseases an upregulation of CD64 is correlated with disease activity and in RA a significant increase in CD64 turnover speaks for a specific role for this receptor in RA inflammation. Future treatment options in RA might include supporting/normalizing the FcγR function in general or CD64 in specific. Today suppressing B-cell activity is an effective and relatively safe way to tackle the problem from the opposite side.
Future perspectives

I am eagerly awaiting further studies on the role of CD64 in the other subtypes of PsA and in early naïve seronegative RA. An interesting way forward would be to mimic the CD64 receptor turnover in the good responders: perhaps soluble CD64 could be a future treatment option in seropositive RA. In the meantime I believe controlling auto-ab production to minimize the effect of pathogenic ICs is of great importance and I feel reassured that long-term B-cell depletion still is an effective and a safe principle for achieving this.

It would also be of great interest to study CRPs contribution to the RA inflammation, particularly its interactions with the FcRs and their function. In vitro/healthy conditions CRP binding to IgG occupied FcRs is reduced, while IgG binding to the same receptors is not affected by prebound CRP. Consequently, it takes an increase in serum CRP to fulfil its immune-modulative effects via FcRs. Animal studies provide evidence of a protective role of CRP in autoimmune disease by enhanced IC clearing. Thus CRP-bound IC have low pro-inflammatory potential in vitro, compared with IgG-ICs. (142-145). The possible role of IgG2-IC in PsA inflammation is another interesting topic to go on studying since IgG2-IC previously were identified as ligands of the high-affinity FcγR CD64 if present in the form of a small or large IC (146).
Sammanfattning på svenska

Ledinflammationer (artriter) förekommer i alla åldrar och utgör medparten av patientunderlaget på reumatologiska kliniker runt om i landet. Dessa sjukdomstillstånd kan till sin natur vara akuta/övergående såsom vid reaktiv artrit, eller kroniska. De två vanligaste kroniska artriterna är reumatoid artrit (RA) och psoriasisartrit (PsA). Medan RA är en sjukdom som främst drabbar kvinnor och som oftast kännetecknas av autoantikroppssproduktion (får de s.k. reumatoida faktorerna samt antikroppar mot citrullinerade proteiner) hos majoriteten av patienterna, är PsA en mer heterogen sjukdom som kan uppträda hos patienter med hudsjukdomen psoriasis. Flertalet undergrupper av PsA har identifierats, dessa skiljer sig från varandra genom olika ledengagement, könsfördelning, genetisk predisposition och ärflichetsaspekter.

Den polyartikulära formen av PsA liknar i viss mån RA i ett immunologiskt perspektiv, men även utifrån ledengagementet: framförallt småleder i händer och foter drabbas, ofta symmetriskt. Trots att PsA per definition beskrivs som en seronegativ artritsjukdom (avseende RF förekomst) enligt de nya internationellt gällande klassifikationskriterierna (CASPAR), ses som vid RA en ökad förekomst av immunkomplex (IC) i blodcirkulationen hos psoriatiker. Eftersom immunglobuliner (Ig) i IC via interaktion med specifika receptoror (s.k. Fc receptoror, FcR) på immunkompetenta cellers ytor kan aktivera inflammatoriska processer i vävnader ställde vi oss primärt frågan om FcR kunde vara involverade i PsA patogenesen. Det är sedan tidigare känt att det för varje Ig isotyp (IgM, IgD, IgG, IgE och IgA) finns specifika FcR. FcR för IgA benämns FcαR (CD89), och för IgG finns tre olika typer av FcγR (FcγRI (CD64), FcγRII (CD32) och FcγRIII (CD16)) som interagerar olika starkt med IgG subklasserna (IgG1, IgG2, IgG3, IgG4). Utifrån celltyp och distribution av de olika FcR avgörs en cells förmåga att reagera på Ig/IC utifrån dess FcR uttryck.

I det första arbetet studerades 23 patienter med aktiv polyartikulär PsA avseende FcR uttryck och funktion i förhållande till sjukdomsaktivitet och i jämforelse med en kontrollgrupp bestående av 33 friska ålders- och könsmatchade blodgivare (= HC). Analyserna utfördes på dagsfärsk blodprover, och resultat av ovan nämnda FcαR och FcγR uttryck samt FcγR-funktion på monocyter ligger till grund för de slutsatser vi dragit. Monocyter studerades primärt pga. följande orsaker: för det första utgör dessa celler ursprunget för vävnadsmakrofager som ansvarar för en stor del av den lokala cytokin-produktionen, antigenpresentationen och vävnads-remoduleringen i inflammaderad vävnad; för det
andra är dessa celler väl tillgängliga genom blodprovstagnings och kan lätt identifieras utifrån membranspecifika ytstrukturer såsom CD14 och FcR. Monocyternas FcR uttryck analyserades med flödescytometri efter infärgning med FcR specifika antikroppar. FcγR funktionen utvärderades med 2 metoder: kapaciteten att binda IC (med hjälp av rosetting-teknik) samt TNFα-produktion efter stimulering med immobiliserat IgG1 eller IgG3 (med hjälp av en TNFα specifik sandwich-ELISA).

I det första arbetet noterade vi att 1) PsA patienter uppfattar ett högre antal monocyter som uttrycker CD64 men att 2) CD64-uttrycket per cell var jämföbar med de friska kontrollerna. Vi såg inga statistiska skillnader avseende uttrycken för de övriga FcR när vi jämförde PsA med HC. Vi kunde dock se att 3) den ökade mängden cellmembran-bundet IgG vid PsA kunde härledas till CD64⁺ monocyter, och att 4) IgG subklasserna 1, 2 och 3 var förhöjda vid PsA. Dock var FcγR funktionen vid PsA jämföbar med friska kontrollerna avseende IC-inbindning men försämrad när det gällde IC-medierad TNFα-produktion, den senare skillnaden sågs tydligare hos de patienter som hade en pågående antireumatisk terapi. Dessa resultat korrelerade med objektiva och subjektiva markörer för ledsjukdomsaktivitet men inte med patienternas psoriasis-aktivitet (som definierades med hjälp av s.k. PASI-score). Vi drar därför slutsatsen att det förändrade FcγR status vi noterar hos PsA patienterna är artrit-relaterat men att detta i sig inte har en övertygande hämmande effekt på FcγR funktionen.

Sedan gick vi vidare och studerade en grupp patienter med nydiagnostiserad autoantikroppspositiv tidig RA. Dessa individer hade aldrig haft behandling med specifika antireumatiska läkemedel. I denna studie ville vi kartlägga tidig RA patienters FcR uttryck och funktion före och efter standardbehandling med methotrexate och prednisolon, två läkemedel som ofta används i det initiala skedet vid RA. För att närmare utröna vilka förändringar av FcR status under behandlingens gång som skulle kunna tolkas som gynnsamma eller ogynnsamma utvärderades även patienternas behandlingssvar vid återbesöket med hjälp av EULARs respons-kriterier för RA. Sålunda kunde vi dela in RA kohorten i good, moderate respektive non-responders vid återbesöket efter 3 månaders behandling. Retrospektivt kunde sedan jämförelser mellan dessa responsgrupperns utgångsläge avseende FcR status och funktion göras. Vi fann att tidig RA utmärkte sig av 1) en ökning av antalet CD64⁺ monocyter, vilka även hade 2) en ökad täthet av CD64 samt 3) mer IgG på sina ytor jämfört med monocyter från friska kontroller. Vidare noterade vi att 4) FcγR funktionen var nedsatt både avseende IC inbindning och IC stimulerad TNFα-produktion, detta var alltså ett tydligare resultat än i studien med PsA patienterna. Ffa patienter som svarat dåligt på den givna behandlingen hade vid studiestart sammant FcγR funktion. Även vid tidig RA avspeglade förändringar av FcγR status och funktion patienternas sjukdomsaktivitet, men några statistiskt säkerställda skillnader i uttrycken för de övriga FcR detekterades inte vid jämförelse med HC. Eftersom CD64 vid tidig RA var mer påtagligt påverkat än
vid PsA beslöt vi oss även för att analysera lösliga FcR (sCD64, sCD16a och sCD89) i plasma hos båda patientgrupperna och HC. Vi kunde då tydligt se att sCD64 var kraftigt förhöjt vid tidig RA men inte vid PsA och HC. Detta visar på en ökad CD64-turnover vid RA, och vår slutsats är att förhöjda nivåer av membranbundet och lösligt CD64 talar för aktiv RA.

Vidare sågs en förhöjd turnover av den monocyte-spezifika lågaffinitets-receptorn CD16a i form av en ökning av sCD16a hos PsA och RA patienter jämfört med HC. Detta tolkar vi som uttryck för en inflammations-spezifisk mekanism som säkerligen avspeglar ett ökat omhändertagande av IC vid dessa båda sjukdomstillstånd (och som förstärks av observationer av icke-signifikant låga CD16 nivåer på PsA- och RA-monocyter jmf med HC). Eftersom sCD16a samtidigt på ett mycket påtagligt sätt korrelerade avkallade med både objektiva, subjektiva som med serologiska parametrar för sjukdomsaktivitet i tidig autoantikroppspositiv RA så föreslår vi att analys av densamma bör ingå i utvärderingen av initial antireumatisk terapi. För sCD89 sågs inga skillnader mellan patientgrupperna eller jämfört med HC. Däremot hade RA patienterna effektivt minskat mängden av samtliga lösliga FcR vid återbesöket vilket avspeglar en nedsatt FcR turnover som resultat av behandlingen. När vi sedermera jämförde RA patienter som svarat bra rättigt på behandlingen så förstärktes CD64s roll vid tidig RA: de patienter som vid andra besöket i princip var symptomfria hade tydligt reducerat CD64-uttrycket på sina monocyter - och detta från en hög initial nivå. Dessa patienter hade även bibehållit mängden ytbundet IgG vid återbesöket. Patienter som inte hade någon nytta av behandlingen ökade istället antalet CD64⁺ celler och uppreglade CD64 tätheten per cell - från en initialt lägre nivå - under behandlingens gång. Dessa non-responders minskade även mängden ytbundet IgG. Tyvärr närmare skillnader avseende men avseende CD64 uttrycket kunde ses vid jämförelse av respons-grupperna: 1) endast good responders uppreglade CD89 efter behandlingen - detta tolkar vi som en effekt av minskad belastning av IgA mot denna receptor samt en ökad tillgänglighet av FcγR1Ib (CD32b) var högre hos good responders än hos non-responders efter behandlingen - detta tolkar vi som en effekt av minskat IC omhändertagande via denna receptor pga. ett bra behandlingssvar, och detta karakteriserar även ett tillstånd av monocyte-inaktivering (vilket vi kunde åskådliggöra med ett s.k. ratio av aktiverande FcR/inhiberande FcR).

Eftersom akutfasproteiner såsom CRP också binder till FcR (både till FcγR och FcαR) och CRP därigenom teoretiskt skulle kunna utgöra en konkurrent till Ig och IC om fria FcR, var vi även intresserade av att jämföra respons-grupperna avseende CRP nivåer. Vi noterade då att good responders hade högre CRP vid behandlingsstart och att dessa individer lyckades minska dessa signifikant medan non-responders bibehöll en låg CRP-nivå genom hela observationsperioden. Non-responders verkade sålunda varken ha haft någon hjälp av CRP eller av sina FcR för att hantera IC eller Ig under studiens gång.
Följaktligen så beslöt vi oss i det tredje arbetet för att vidare kartlägga tidig RA patienterna avseende Ig isotyper, IgG subklasser, RF isotyper samt ACPA IgG före och efter behandling och ställt i relation till deras individuella FcγR funktion som vi tidigare hade analyserat i papper två. Målet var att upptäcka skillnader mellan responsgrupperna som skulle kunna förklara de olika behandlingsutfallen.

Vi kunde notera att tidig RA kännetecknades av förhöjda IgG1 och IgG3 nivåer, att non-responders överlag har högre medelhöjder av Ig isotyper, IgG subklasser, RF isotyper och ACPA jämfört med good responders. Non-responders uppvisade oftare flera samtida RF isotyper och lyckades inte reducera IgM RF eller IgG3 nivåerna efter behandlingen. Det senare resultatet är i sig intressant då IgM och IgG3 är de Ig som besitter störst förmåga till komplement-aktivering. Utifrån våra resultat skulle man kunna dra slutsatsen att IgM RF är ett objektivt mått på ihållande (kronisk) artrit. Vidare noterade vi att IgM RF, IgG RF och IgA RF korrelerade negativt med RA monocyternas förmåga att binda IgG1-IC men inte IgG3-IC, och att höga IgA RF-nivåer motsvarade monocyters försämrade förmåga in vitro att producera TNFα efter IC-stimulering. Slutsatsen av denna studie blev att nivåer och sammansättning av RF är avgörande för behandlingsresultatet om tidig RA patienter behandlas med methotrexate och prednisolon och detta avspeglar sig i patienternas förändrade monocyt FcR uttryck och FcγR funktion.

Utifrån resultaten från 2a och 3e studien är det uppenbart att en dämpad B-cells funktion i RA patienter kan minska Ig produktionen och följaktligen den FcR medierade inflammationen. Vid behandling av RA med olika antireumatiska läkemedel ses en minskning av Ig nivåer och därav följer troligtvis även en minskning av cirkulerande IC. Ett biologiskt läkemedel som specifikt kan påverka B-cells homeostasen är rituximab (RTX). Detta läkemedel ges som infusion med viss regelbundenhet inom reumatologisk öppenvårdsverksamhet. RTX minskar mängden CD20⁺ B-celler genom lys eller apoptos och med detta medföljer en minskning av B-cells produkter och B-cellsfunktion. Inför arbete 4 ställde jag frågan om långvarig anti-B-cellsterapi kunde påverka RA patienters Ig nivåer och om dessa Ig förändringar var jämförbara med de vi hade sett i den tredje studien. Jag var även intresserad av långtidseffekten av RTX på patienternas immunförsvar mot infektioner. Sålunda studerades 69 patienter med ursprungligen RF⁺ RA som hade en pågående behandling med RTX (i kombination med eller utan DMARD/kortikosteroider). Jag fann att patienter som var IgM RF⁻ vid bedömning hade erhållit mer RTX och hade lägre sjukdomsaktivitet än IgM RF⁺ patienter. Patienternas totala IgM nivåer korrelerade med sjukdomsaktiviteten och med IgG3, detta är jämförbara resultat med den 3e studien. Utifrån journalstudier fick jag fram att i snitt 1.4 antibiotikabehandlingar per RTX-år hade förskrivits till dessa patienter och att infektioner i luftvägar följt av urinvägar samt hud var de vanligast förekommande. Patienternas infektionsbenägenhet verkade snarast hänga ihop med
andra samtida sjukdomstillstånd såsom kronisk obstruktiv lungsjukdom, diabetes och sekundärt Sjögrens syndrom, då sådana patienter var överrepresenterade bland antibiotikaförbrukare och inneliggande sjukhusvård pga. infektionstillstånd. Användningen av en matematisk riskanalys för infektioner (s.k. RABBIT risk score) kunde inte på ett övertygande sätt identifiera de patienter som retrospektivt hade drabbats mest av infektioner. I denna RTX-studie uppdagades ett fall av hepatitis B (HBV) reaktivering hos en patient som självvalt gjort uppehåll med sin antivirala medicinering och ett dödsfall pga. TBE-infection; båda individerna hade tidigare haft en mycket god effekt/inga biverkningar av sina fleråriga RTX-behandlingar. Patienten med HBV normaliserade sin leverfunktion efter återinsättning av antiviralt läkemedel. Vid tidpunkten för dödsfallet hade patienten med TBE inga detekterbara antikroppar i blod eller ryggmärgsvätska trots att hon tidigare hade vaccinerat sig mot TBE. Vi rekommenderar därför noggrant anamnestagande avseende aktuella insektsbett, att vedertagna vaccinationsrekommendationer följs och att patienter med kroniska infektionssjukdomar följs rigoröst inför/under behandling med RTX.

Sammanfattningsvis har denna avhandling visat att FcR har en viktig roll vid kroniskt inflammatoriska ledsjukdomar. Hos reumatiker syns CD64 ha en mer framträdande roll än som tidigare antagits, men om dess roll hos RF negativa RA patienter också är viktig för sjukdomen återstår att studera. Ifrån djurmodeller av RA känner vi till att terapier med CD64 toxin eller lösligt rekombinant hCD64 på ett övertygande sätt har kunnat reducera försöksdjurens ledinflammationsaktivitet. För framtiden kan man därför hoppas att våra resultat kan bidra till att utveckla läkemedel som direkt eller indirekt kan påverka artrit-patienters FcγR funktion i allmänhet eller CD64:s funktion i synnerhet. En effektiv kontroll av patienters systemiska B-cellsaktivitet angeriproblemet från andra sidan (genom minskning av receptor-ligander), vilket i dagsläget kan uppnås relativt säkert genom återkommande B-cells depletioner.
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