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Chapter 1

Introduction

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New Thoughts on the Pathogenesis of Rheumatoid Arthritis.

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Rheumatoid Arthritis.
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A clinical perspective of rheumatoid arthritis.

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Patient-tailored therapy in rheumatoid arthritis: an editorial review.

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Adaptive immunity in rheumatic diseases: bystander or pathogenic player?
RHEUMATOID ARTHRITIS

Rheumatoid Arthritis (RA) is a chronic inflammatory disorder that typically affects cartilage and bone of small and middle-sized joints. Inflammatory cells invade the otherwise relatively acellular synovium, which leads to hyperplasia and formation of pannus-tissue. This infiltration causes destruction of cartilage, erosion of the adjacent bone and ultimately loss of function of the affected joint. Involvement of larger joints may also occur. Systemic inflammation, often going in parallel, can affect several organs (e.g. lungs, vessels and the hematopoietic system) and has long-term impact on organ function. Combined with inevitable side effects of yearlong anti-rheumatic medication (e.g. glucocorticoids) and the psychological burden of facing early invalidity and social instability, RA has, if insufficiently treated, important socio-economic impact and causes a reduction in life-expectancy of 7 years in average\(^1,2\).

For the clinician, there is considerable heterogeneity in both clinical picture and course of disease. Next to the characteristic signs and symptoms of RA, overlap with other rheumatic diseases can be observed (e.g. mixed connective tissue disease (MCTD)). In addition, other autoimmune diseases (e.g. Sjögren’s syndrome, autoimmune thyroiditis) may accompany RA. For reasons largely unknown, the course of disease is highly variable, ranging from mild cases with non-erosive, even sometimes spontaneously remitting disease, to severe, rapidly progressive and destructive arthritis\(^3\). Recent analysis of genetic risk factors and autoantibody responses together with data from clinical trials suggest, however, that the clinical entity RA might consist of pathogenetically distinct subgroups, which present with similar if not identical clinical phenotypes\(^4\). Different treatment strategies may need to be applied to patients within these groups.

For the immunologist, RA is considered an autoimmune disease by most, implying breakdown of immunological tolerance towards self at a given moment in a patient’s life. The trigger initiating this breakdown is so far unknown\(^5\). The presence of autoantibodies and slowly rising C-reactive protein-levels several years before onset of clinical symptoms indicate that the inflammatory process may be well underway long before patients first consult a physician\(^6\). Variations between ethnic groups in susceptibility to RA, heterogeneity of disease course and variations in clinical, radiological and laboratory findings within groups strongly suggest that multiple factors, both environmental and genetic, influence onset and progression of RA, presumably with different impact during different stages of disease development. Genetic variations, autoantibodies, cellular immune responses, hormones and gene-environment interactions are among the most studied factors contributing to RA development.
AUTOANTIBODIES AND THEIR ROLE IN THE DISEASE PROCESS IN RA

An array of antibodies targeting self-antigens (e.g. collagen type II, calreticulin, cathepsin, BiP, CH65, etc.) has been described in patients with RA\textsuperscript{7}. Demonstrating pathogenetic relevance for any of these reactivity’s, however, has proven difficult, last but not least because the clinical and radiological phenotype of RA can also develop in the absence of any of the autoantibodies known so far.

*Rheumatoid factor*

The initial notion that mechanisms of autoimmunity might underlie RA pathogenesis came from the discovery of autoantibodies targeting the Fc-part of human IgG (so called “rheumatoid factors” (RF)) in the blood of affected patients\textsuperscript{8,9}. RF, present mostly as IgM-RF, but detectable in subgroups of patients also as IgG- and IgA-RF, are thought to form immune complexes activating complement, which in turn leads to increased vascular permeability and the release of chemotactic factors recruiting immune-competent effector cells to the joint\textsuperscript{10}. The mere presence of RF, however, is insufficient to initiate arthritis development, as RF are also found in infectious diseases, autoimmune diseases other than RA and in up to 15\% of healthy, mostly elderly individuals. Thus, sensitivity and specificity of RF are, depending on the population studied, 60-70\% and 50-90\%, respectively. Despite this lack of specificity, RF are part of the former and new classification criteria for RA\textsuperscript{11,12}.

*Anti Citrullinated Protein Antibodies (ACPA)*

Citrullination is a process by which arginine residues in a given protein are post-translationally modified, in the presence of high calcium-concentrations, by an enzyme called PAD (peptidyl arginine deiminase). Under physiological conditions, it is believed that citrullination facilitates the degradation of intracellular proteins during apoptosis\textsuperscript{13,14}. In 1998, two antibodies present in serum of RA patients, anti perinuclear factor (discovered in 1964\textsuperscript{15}) and anti-keratin antibodies (first described in 1979\textsuperscript{16}) were found to recognize a common target: citrullinated filagrin\textsuperscript{17,18}. This observation, together with an unprecedented specificity of citrulline-specific antibodies for RA, has placed anti-citrullinated protein antibodies (ACPA) at the center of intense research efforts. Meanwhile, citrullin-specific reactivities against several proteins (e.g. fibrinogen, collagen, vimentin, enolase, and others) have been identified, and by the use of optimized assays ACPA are now detectable in 60-70\% of RA-patients, but hardly in other diseases or healthy subjects. Whether ACPA contribute to the disease process, and the possible pathogenetic mechanism of such a contribution, is matter of intense debate. A number of clinical associations and an increasing amount of experimental data, however, point in this direction.
Individuals with joint pain (arthralgia) that harbor ACPA in serum have an increased risk to develop arthritis. In patients with undifferentiated arthritis (UA), the presence of ACPA increases the risk for progression to RA, and lowers the chance for remission. ACPA positive RA patients suffer from more extensive joint destruction and more frequent extra-articular organ involvement than their ACPA negative counterparts. Also histologically, synovial tissue differs between ACPA positive and negative patients. Patients with UA benefit from treatment with methotrexate if ACPA positive, as they develop significantly less joint destruction in the first year than untreated controls, and progress to RA less frequently. For ACPA negative UA patients, methotrexate treatment was without effect on these parameters compared to placebo. A number of genetic factors, among which the so-called shared epitope (SE-) alleles, confer risk for RA development only to ACPA positive individuals, whereas they do not seem to influence the pathogenic process in ACPA negative disease. In fact, SE-alleles, a set of HLA-DRB1 molecules with a shared amino acid sequence formerly regarded as strong risk factors for RA, are risk factors only for the development of ACPA, without an independent risk effect on the development of RA itself.

Taken together, these observations support a model in which ACPA positive RA develops on a different pathogenetic background than ACPA negative RA. The ACPA immune response broadens shortly before onset of clinical disease, with an increasing number of citrullinated epitopes recognized and more ACPA isotypes generated. The question arises, however, as to the available scientific evidence that ACPA are the actual factors driving the disease process. This all the more, as patients can undergo complete, drug-free remission despite persistent, high ACPA serum titers.

ACPA pathogenicity

Citrullinated antigens are found in RA synovium, which is an important prerequisite for local ACPA pathogenicity. At the same time, ACPA levels are elevated in synovial fluid as compared to serum. In the mouse, antibodies to a citrullinated B-cell epitope of collagen type II (CII), which cross-react with citrullinated CII in human joints, were found to be arthritogenic. Monoclonal antibodies against citrullinated fibrinogen were found to enhance arthritis in a mouse model of pre-existing collagen-induced arthritis, and citrullinated human fibrinogen, but not unmodified fibrinogen, was able to induce arthritis in HLA-DRB1*0401 (DR4-IE) transgenic mice. In the latter experiment, no induction of arthritis was seen in wild-type mice lacking the transgene.

In the human, first evidence for ACPA-specific pro-inflammatory effects came from studies that observed stimulation of macrophages by ACPA-containing immune complexes. In addition, ACPA were found to activate the complement system. More recently, in vitro activation of basophils by ACPA of the IgE isotype was described. In addition, associations were noted between IgE and FceRI expression on synovial mast
cells, histamine levels in synovial fluid and ACPA positivity. As synovial mast cells are an important source of the proinflammatory cytokine IL-17, ACPA stimulated mast cell degranulation and cytokine production could contribute to local inflammation\textsuperscript{38}. More recent work has demonstrated \textit{in vitro} activation of human osteoclasts by antibodies to citrullinated vimentin, with increased bone loss in mice injected with these antibodies\textsuperscript{39}. This latter observation, which was not noted for non-specific IgG, so far most closely links ACPA to the pathological correlate of RA: bone erosions.

In summary, both clinical associations and an increasing number of experimental data support the hypothesis that ACPA contribute to synovial inflammation and joint destruction. The observation that RA can remit despite the presence of ACPA indicates, however, that the quality rather than the quantity of the ACPA immune response determines its pathogenicity. One important aspect of the immunogenicity of antibodies is determined by Fc-linked glycans.

**ANTIBODY GLYCOSYLATION AND ITS FUNCTIONAL CONSEQUENCES**

Human antibodies are glycoproteins with carbohydrate structures attached to the constant and, in some cases, to the variable region of the molecule. These glycans strongly influence the \textit{in vitro} and \textit{in vivo} biological characteristics of an antibody, such as serum half-life, binding to Fc-receptors, complement activation and interaction with lectins\textsuperscript{40,41}. Glycans attached to the Fc-tail of IgG-molecules have most extensively been studied.

**Fc-glycosylation of human IgG**

The Fc-tail of human IgG carries two complex-type N-glycans, each attached to one heavy chain at position 297 (asparagine) in the C\textsubscript{H}2 domain of the protein backbone (Figure 1A). The sugar chains are intercalated between the heavy chains, with which they non-covalently interact at several positions. This interaction maintains the three dimensional structure of the Fc-tail, which changes conformation and loses its function once the glycans are enzymatically removed\textsuperscript{42-44}. The glycan chains consist of a conserved biantennary, heptasaccharide core structure of N-acetylglucosamine (GlcNAc) and mannose residues (Figure 1B). Core fucose, additional (bisecting) GlcNAc, galactose, and a terminating sialic acid residue further modify this sequence. In total, these modifications yield more than 30 possible variants per glycan chain, of which glycoforms carrying either zero, one or two galactose residues (termed G0, G1, G2 glycoforms) are most abundantly found on human IgG (20–35\%, 35\%, and 16\% of all glycoforms, respectively). The degree to which Fc-linked glycans on IgG are sialylated, galactosylated, fucosylated or carry bisecting GlcNAc residues in a given individual depends on various
factors such as age, hormonal status, and the type of immune response during which the IgG molecule is produced\textsuperscript{45-47}.

Functionally, the Fc tail interacts with Fc receptors and binds the complement component C1q. In addition, it is the target of rheumatoid factors and as such involved in immune complex formation. Absence of galactose residues (G0) on the Fc-linked glycans is associated with concomitant absence of sialic acid residues and increases the affinity of the Fc tail for activating Fc gamma receptors (FcγR) expressed by immune cells\textsuperscript{49,50}. In addition, agalactosylated glycoforms on the Fc tail enhance the formation of IgG-containing immune complexes\textsuperscript{51}. Mainly for these reasons, a high abundance of G0-glycoforms on IgG is thought to correspond to pro-inflammatory properties of the molecule. In contrast, presence of galactose (G2) and sialic acid residues is believed to favor anti-inflammatory effector functions. Moreover, absence of core fucose residues leads to high avidity of the Fc-tail for binding to FcγRIIIa, which is based on a unique interaction of the afucosylated Fc-glycan with carbohydrates of the receptor\textsuperscript{52}. This interaction enhances antibody dependent cellular cytotoxicity (ADCC) by up to 100-fold, a finding exploited by glycoengineered therapeutic monoclonal antibodies\textsuperscript{52-54}.

The \textit{in vivo} effects of different IgG Fc-linked glycoforms can be studied using recombinant monoclonal IgG molecules of defined specificity or by employing IgG molecules purified from pooled human plasma of healthy individuals. Such polyclonal IgG preparations (termed intravenous immunoglobulin, IVIG) are known for their important anti-inflammatory effects \textit{in vivo} and are used clinically to treat various autoimmune

\begin{figure}
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\caption{(A) Three dimensional structure of a human IgG\textsubscript{1} molecule with the two heavy chains depicted in dark and light grey. The glycan chains are intercalated in between the heavy chains (modified from\textsuperscript{48}). (B) Schematic depiction of a monosialylated glycan attached to one of the heavy chains at position 297. The dotted line shows the conserved core structure, which can be modified by fucose, galactose and sialic acid residues. Also disialylated glycoforms and glycans carrying an additional, bisecting N-Acetylgalactosamine (GlcNAc) residue can be found (not shown).}
\end{figure}
diseases. Using IVIG, a number of studies have proposed an important role of terminal sialic acid residues for IgG-mediated effector functions. In fact, removal of terminal sialic acid residues from IVIG-associated glycans has been reported to abrogate its anti-inflammatory activity in mouse models of autoimmunity. Conversely, enrichment of IVIG for sialylated molecules enhanced this property. In fact, it has been postulated that only a minor fraction of IgG molecules (~1-3%) within IVIG carries terminal sialic acid residues on their Fc-linked glycans and that this small fraction might account for the therapeutic effects observed. Accordingly, enrichment of the sialylated fraction by lectin affinity chromatography using Sambucus nigra agglutinin (SNA) or in vitro sialylation was found to result in a reduced dose requirement for the in vivo activity of IVIG. In line with this, a fully recombinant human IgG molecule with Fc-linked glycans terminating in sialic acid-galactose linkages recapitulated the in vivo anti-inflammatory activity of intact IVIG and enhanced its effect by 35-fold, compared with the activity of conventional IVIG.

It is important to note, however, that several aspects of the studies on IVIG described above are currently under debate. This is mainly based on the observation that lectin chromatography with SNA does not enrich for Fc-linked sialylated IgG, but for IgG molecules carrying sialic acid containing glycans in the Fab portion. In addition, the studies employed human IgG molecules to study effects in mice, indicating that it might not be possible to directly translate the findings to the human situation. Thus, although the relevance of the Fc-glycan for interaction of IgG molecules with FcγR is undisputed, the specific role of terminal sialic acid residues in this context requires further study.

In addition to FcγR mediated effects, Fc-glycans are also required for and modulate IgG-mediated complement activation. Although the ability of IgG to activate complement strongly depends on the IgG subclass, C1q binding to IgG and subsequent activation of the classical pathway was found to be most effective in the presence of G2-glycoforms. Mannose-binding lectin (MBL), on the other hand, binds to exposed, terminal mannose, fucose and GlcNAc residues on agalactosylated (i.e. G0 containing) IgG molecules in vitro, thereby initiating the lectin pathway of the complement cascade. Debate exists, however, as to the in vivo relevance of this finding with regard to IgG pathogenicity in autoimmune diseases, as IgG-G0 molecules in MBL-null mice (genetically deleted for MBL) did not lose their inflammatory potential in mouse models of immune-thrombocytopenia and arthritis, whereas their effects were abrogated in FcγR deficient mice.

IgG Fc-glycosylation in RA

In RA, early work has demonstrated aberrant glycosylation of the Fc-tail of serum IgG, which mainly lacks galactose and sialic acid residues as compared to IgG in healthy individuals. This hypogalactosylation (i.e. predominance of the G0 glycoform) and,
as a consequence, hyposialylation, of the Fc-tail associates with disease activity and can revert to normal levels during effective treatment, for example with tumor necrosis factor alpha inhibiting agents. A similar decrease in G0 content was observed in female RA-patients during pregnancy. The hypogalactosylation of IgG molecules in RA is likely to be regulated on the B cell level, rather than a result of enzymatic release of galactose residues post-secretion, as reduced expression of β-1,4-galactosyltransferase, the enzyme responsible for adding galactose residues to the Fc-linked glycan, was noted in B cells of patients with RA.

These observations raise the question whether the disease activity dependent variation of IgG Fc-glycosylation in RA actively contributes to disease, or whether it merely reflects the inflammatory environment in which the IgG molecules are produced. Arguments for the latter concept are fuelled by the finding that hypogalactosylation of human IgG-Fc is not specific for RA, but also characterizes other autoimmune diseases and can even occur in the context of infectious diseases. The hypothesis of an active modulation of disease by G0-containing IgG, however, is supported by studies on the anti-inflammatory effects of sialic acid containing IVIG described above, and by animal studies. Specifically, in a murine passive transfer model of arthritis, agalactosylated IgG induced more severe arthritis than IgG without glycan modification, indicating that Fc-linked G0 glycoforms can indeed increase inflammation in this context. Moreover, deglycosylation of the Fc-tail abrogated arthritogenicity of monoclonal, collagen-specific murine IgG in a similar model. More recent data showed that the increase in IgG G0 glycoforms in RA could be detected several years before diagnosis, a finding that supports, but does not prove, the concept of a pro-inflammatory effect of G0 in human RA.

In summary, glycans on the Fc tail of human IgG have a strong influence on its biological function. Intriguing aberrations of Fc-linked glycans are noted in RA. Until now, it is uncertain whether these glycosylation changes are cause or consequence of inflammation.

THE ROLE OF REGULATORY T CELLS IN RA

Regulatory (Treg) T cells represent an important mechanism by which the immune system can control the development of autoreactivity. This is crucial, as autoreactive T- and B-lymphocytes can escape the classical checkpoint of central tolerance in bone marrow or thymus, which functions as the main barrier to eliminate autoreactive lymphocytes during their development. Accordingly, severe autoimmunity including arthritis develops in the absence of regulatory T cells, both in mice and humans.

The population of CD4 expressing human Treg cells is heterogeneous; it comprises a subset with imprinted regulatory functions (“naturally occurring” Treg cells) derived
from the thymus, and a set of peripheral T cells that can acquire them (“adaptive” or “inducible” Treg cells)\textsuperscript{74}. Both types are considered to be “regulatory” based on the capacity to effectively inhibit proliferation and cytokine secretion of effector T cells in culture. CD4\textsuperscript{+} Treg cells are classically identified based on the expression of high levels of CD25 and the transcription factor FoxP3, and of low levels of the \(\alpha\)-chain of the IL-7 receptor (CD127). The expression of FoxP3 is stable in natural Treg cells due to epigenetic imprinting, while inducible Treg cells express FoxP3 transiently\textsuperscript{75,76}. More recently, the transcription factor and regulator of FoxP3 expression Helios was identified as phenotypic and functional marker of natural, but not adaptive, Treg cells\textsuperscript{77}. In vitro, Treg cells are characterized by low proliferation rates, low production of IL-2 and by secretion of TGF-\(\beta\), IL-10, IL-35, perforin and granzymes\textsuperscript{78,79}.

**Regulatory T cells in RA**

The role of Treg in RA pathogenesis is unclear. Unlike in the mouse, human natural and adaptive Treg are difficult to differentiate from activated T cells without regulatory functions, as markers such as CD25 and FoxP3 are inducible upon stimulation. Because of this, the number and functional integrity of regulatory T cells in RA are subject to debate\textsuperscript{80}. In the mouse, depletion of CD25\textsuperscript{+} FoxP3\textsuperscript{+} Treg can enhance arthritis, while adoptive transfer of Treg can ameliorate disease\textsuperscript{81,82}. As these experiments, together with arthritis development in Treg deficient mice and humans shows the general capacity of Treg to modulate arthritis, both functional Treg deficiency or resistance of effector cells to Treg-mediated suppression could operate in RA.

As CD25 and FoxP3 expression in human T cells cannot be equalized with suppressive function, reports on Treg in RA range from decreased numbers to increased frequencies, and from impaired to enhanced suppressive functions\textsuperscript{83-86}. In addition, differences were reported between Treg cells in peripheral blood and synovial fluid\textsuperscript{85}. Elegant flow cytometric studies combined with functional and epigenetic data have shown that human Treg cells can be subdivided in resting naïve and activated effector Treg cells based on the expression of CD25 and CD45RA, and in a population of FoxP3 expressing, CD25\textsuperscript{+} but CD45RA\textsuperscript{−} non-Treg cells\textsuperscript{87}. This more subtle delineation of Treg cell populations, however, has not been used in most studies.

Of interest, impaired Treg cell function has been reported under the influence of TNF-\(\alpha\), which was reversible by anti-TNF treatment\textsuperscript{84,88}. This observation is plausible, as Treg cells express TNFR-II, which makes them susceptible to the deleterious effects of TNF-\(\alpha\). In fact, treatment with TNF-antagonists gave rise to a newly generated, functionally distinct Treg-cell population that secretes TGF-\(\beta\) and IL-10. However, more recent data suggest that also this notion might be debatable, as TNF was also found to promote Treg cell function\textsuperscript{80}. 
Taken together, it is currently unclear to what extent Treg cells are defective in RA, or why functional Treg are insufficient to control the disease.

**OUTLINE OF THIS THESIS**

Based on the observations and considerations described above, this thesis investigates several aspects of immunological disease mechanisms that are of relevance to the inflammatory immune response in rheumatoid arthritis. Specifically, three main research questions triggered the experiments presented and form the outline of this thesis:

1. Do regulatory T cells feature anti-inflammatory properties besides the inhibition of effector T cells, which could help explain their therapeutic effectiveness in a murine model of established arthritis?

2. Are there specific features of the ACPA immune response that could contribute to inflammation in RA, and can analysis of these features help in understanding the characteristics of ACPA producing B cells and their development?

3. Do certain genetic variants that associate with RA susceptibility contribute also to disease progression, as evidenced by the rate of joint destruction in RA?

**Part I** was triggered by the observation that adoptive transfer of regulatory T cells during the effector phase of murine collagen-induced arthritis significantly decreased inflammatory disease activity, without affecting the levels of circulating antibodies (chapter 2). This was unexpected, as effector T cells, the primary target of Treg cells, were thought to be involved primarily in the initiation phase of this otherwise antibody-driven disease model. At the same time, this finding suggested that Treg cells possess means to dampen inflammation beyond the inhibition of effector T cell function. In the light of the role of TNF-α in RA, our studies revealed that Treg cells can express and shed a soluble receptor for TNF-α, TNFRII. This TNFR-shedding was capable of inhibiting an early, TNF-mediated inflammatory response in the mouse, which demonstrated the in vivo relevance of this functional aspect of Treg. Importantly, the feature of TNFR-shedding could be shown for murine as well as human Treg cells. In conclusion, the first part of the thesis demonstrates the identification of a mechanism underlying anti-inflammatory properties of regulatory T cells.

**Part II** is dedicated to the quality of the ACPA immune response and its potential contribution to inflammation in RA (chapters 3 – 6). As described above, ACPA are detectable
in similar levels in patients with active and inactive disease\textsuperscript{91,92}, indicating that the quality rather than the quantity of the ACPA immune response determines its pathogenicity.

We studied both features of the ACPA Fc tail as well as characteristics related to antigen binding via the variable region.

Based on the observation that the Fc tail of IgG molecules in RA lacks galactose and, consequently, sialic acid residues, and that Fc-linked glycans can modulate immune responses, we hypothesized that ACPA might differ from non-specific IgG molecules in their Fc glycosylation profile and thereby have the potential to enhance inflammation. To study Fc glycosylation antigen-specifically, we first developed a method for isolating ACPA from small quantities of human serum and combined it with a high throughput analysis of Fc glycopeptides by mass spectrometry (Chapter 3). This methodology allowed us, for the first time, to study glycan residues linked to the ACPA Fc tail, and to compare them to those found on the Fc tail of non-specific total IgG of the same patient. When applied to serum and synovial fluid samples of ACPA positive RA patients, the analysis revealed that ACPA indeed exhibit a specific, pro-inflammatory glycan profile in that they significantly lack sialic acid and galactose residues (Chapter 4). Importantly, we found differences in the Fc glycosylation profile of ACPA in serum and synovial fluid within the same patient, which was not the case for non-specific IgG. In line with the initial hypothesis, ACPA in synovial fluid were highly agalactosylated. As such, this finding represents evidence for qualitative differences of ACPA in different compartments, and indicates that ACPA producing B cells might possess specific functional characteristics, which are distinct from “conventional” B cells.

In this context, little is known on the origin and development of ACPA-specific B cells. Most B cells mature in germinal centers, where they receive help from follicular helper T cells to undergo class switch recombination and affinity maturation\textsuperscript{93}. ACPA of all Ig isotypes have been detected in patient sera, supporting the notion that ACPA producing B cells originate from germinal center reactions\textsuperscript{28,37}. Importantly, during conventional immune responses, only B cells with B cell receptors of high affinity for the antigen receive appropriate survival signals required to differentiate into memory B or plasma cells. To gain further insight into specific features of ACPA that might relate to aberrant B cell development, we studied the avidity of ACPA in comparison to the avidity of antibodies against recall antigens such as tetanus (Chapter 5). Surprisingly, ACPA were found to be mainly of low avidity, irrespective of the degree of class switch recombination that the ACPA specific B cells had undergone. Also during the course of 5 years, we did not detect affinity maturation within individual patients. This observation supports the notion of a developmental difference between “conventional” and ACPA producing B cells, but the underlying mechanism remains unknown.

Finally, another aspect of ACPA pathology relates to the antigens recognized. The ACPA response is polyclonal and generates multiple specificities that recognize various
citrullinated proteins\textsuperscript{94}. This has fuelled the hypothesis that certain reactivity’s might be more specific for, or more relevant to the disease process than others. As most currently used detection assays use citrullinated antigens designed to detect as many ACPA positive individuals as possible, yielding high sensitivity of the assay, these do not take into account potential subgroups of patients in which the ACPA recognition profile might associate with clinical features of the disease. As destruction of the affected joint is the prominent feature of RA, we addressed this issue by analyzing whether certain fine specificities exist within the repertoire of citrullinated antigens that are specifically pathogenic by promoting enhanced joint destruction over time (Chapter 6). Of interest, no fine-specificity associated with the rate of joint destruction within the ACPA positive subgroup, indicating that recognition of citrullinated antigens in itself, but not the recognition of specific citrullinated proteins, is of primary relevance to RA disease pathology. Moreover, it suggests that analysis of the ACPA recognition profile within ACPA positive individuals does not identify patients specifically at risk for progressive disease.

Part III, in keeping with risk factors for joint destruction, analyzes the contribution of genetic variants located in the 6q23 region to the rate of joint destruction in RA (Chapter 7). This region had previously shown association with RA susceptibility in several studies, but the underlying mechanism for this effect, as for many genetic risk factors, remained unknown\textsuperscript{95-97}. Of interest, the association was only found in the ACPA positive subgroup, in line with observations on other RA-associated risk factors including the shared epitope alleles. The variants are located close to the gene encoding TNFAIP3, a negative regulator of NFκB involved in TNF-receptor mediated signaling. In our study, we observed that carriers of two single nucleotide polymorphisms displayed increased joint destruction over time. This observation refines the understanding of potential effects mediated by this genetic locus and represents the first description of a risk factor outside the HLA-region that could be linked to disease outcome.

Chapter 8 provides a summary of the work presented and a discussion of the results in the context of current literature.
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