# The Anti-Citrullinated Protein Antibody immune response and its effector functions in Rheumatoid Arthritis

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# CHAPTER 1
# Introduction

*Adapted from:*

**Variable domain glycosylation of ACPA-IgG: A missing link in the maturation of the ACPA response?**

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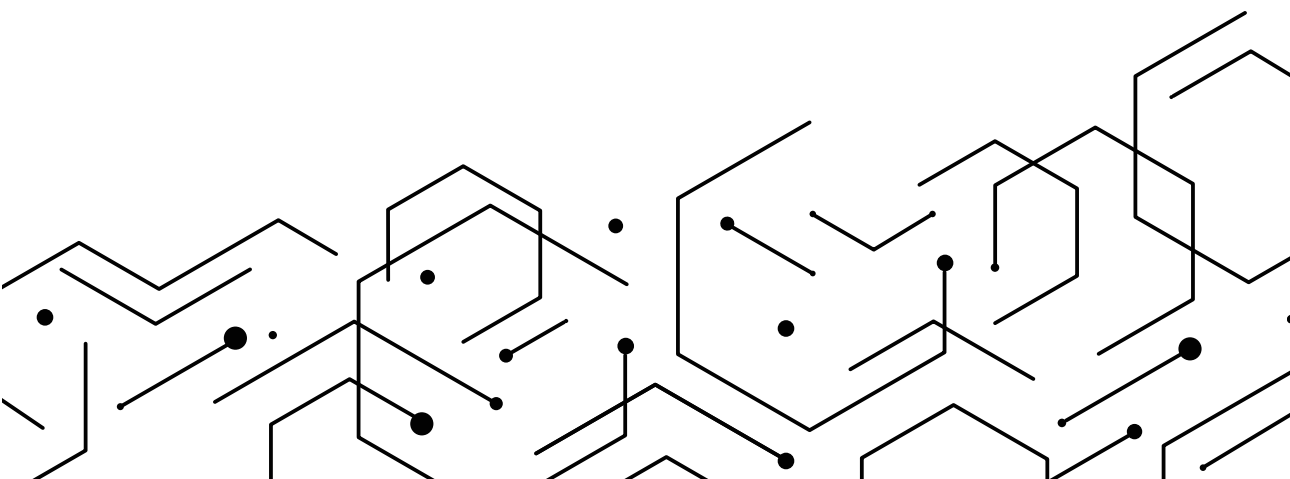

# RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by systemic and persistent inflammation of the synovium which can lead to joint damage, bone destruction and, ultimately, may cause disability and increased mortality [1]. Around 0.5-1% of the western population is affected by RA with a higher incidence among women and upon increased age [1, 2]. Although it is not yet understood what exactly drives RA pathogenesis, genetic and environmental risk factors are thought to contribute to the development of RA.

## Autoantibodies in RA

An important hallmark of RA is the presence of autoantibodies. Around 50-80% of patients with RA harbour either one or multiple types of autoantibodies [1]. Rheumatoid factors (RF) were the first autoantibodies discovered to correlate with RA and their presence has been included in the current RA classification criteria [3, 4]. RF are directed against the Fc tail of the immunoglobulin G (IgG) molecule and thought to exert pathogenic effector mechanisms via the formation of immune complexes, the activation of complement and the subsequent induction of inflammatory mediators [5]. However, RF are not highly specific for RA as they can also be found in other chronic or infectious diseases, and in healthy individuals [6]. In addition to RF, antibodies against posttranslational modifications of proteins can be found in the sera of patients with RA, such as Anti-Carbamylated Protein (anti-CarP) Antibodies, Anti-Acetylated Protein Antibodies (AAPA) and Anti-Citrullinated Protein Antibodies (ACPA). Collectively, these are termed Anti-Modified Protein Antibodies (AMPA). Anti-CarP antibodies recognize proteins which are carbamylated, a process leading to the chemical conversion of lysine residues to homocitrulline [7], while AAPA recognize the acetylation of lysine residues [8]. ACPA, instead, recognize proteins that are citrullinated, a process in which the positively charged amino acid arginine is converted to a neutral citrulline by peptidyl arginine deiminase (PAD) enzymes [9]. Importantly, ACPA are highly specific for RA and are strongly associated with the severity of arthritis, severe joint destruction during the course of disease and with low remission rates [10, 11]. Also histologically, ACPA-positive disease differs from ACPA-negative disease. Compared to the synovial tissue of ACPA-negative patients, the synovial tissue of ACPA-positive patients is reported to be characterized by a higher number of infiltrating lymphocytes, less extensive fibrosis and a thinner synovial layer [12]. Furthermore, ACPA can be present years before the first occurrence of clinical symptoms [13, 14]. In this phase, ACPA are considered risk factors for disease development, although not all ACPA-positive individuals will eventually develop arthritis. Still, based on the strong clinical associations between the presence

of ACPA and disease, ACPA are now included in the 2010 EULAR/ACR classification criteria for RA [15]. All together, these observations have led to the general assumption that the immune response against citrullinated proteins may play a crucial role in RA pathophysiology. However, how the ACPA response originates and how ACPA exactly contributes to disease development is still an unknown aspect.

Similar to ACPA, also RF, anti-CarP antibodies and AAPA responses show clinical associations with RA with regard to joint damage and future RA development in arthralgia patients. However, compared to ACPA, less RA patients harbour these latter AMPA and little knowledge is currently available about these autoantibody systems in RA partly due to technical reasons (such as assay development and the availability of peptides). The clinical associations indicate that there is overlap between the different AMPA responses in RA patients, though it is yet unclear if and how these AMPA responses are exactly connected. In this thesis, studies are described addressing several aspects of the ACPA immune response as ACPA and/or ACPA-producing B cells show strong leads for involvement in RA disease development and can function as an autoantibody prototype to better understand the autoreactivity of B cells in RA.



## The ACPA B cell response

Antibodies are expressed and secreted by B cells as part of the humoral immune response. Mature naïve B cells leave the bone marrow and circulate through secondary lymphoid organs until they die or encounter their cognate antigen [16]. Activation of naïve B cells upon a first encounter with an antigen results in IgM antibody-secreting cells. Further proliferation and differentiation of these B cells is initiated via a second activation signal from T cells, which stimulates the processes of class switch recombination and affinity maturation. This can lead to the generation of plasmablasts and plasma cells that secrete high affinity class-switched antibodies (e.g. IgG, IgA, IgE). In addition, it can lead to the generation of memory B cells that recirculate through peripheral blood and secondary lymphoid organs. In RA, ACPA-secreting plasma cells can be detected in the inflamed synovium, in synovial fluid and in serum of ACPA-positive patients [17, 18]. ACPA are present in several isotypes including IgM, IgG and IgA [19]. Interestingly, a sustained and abundant presence of ACPA-IgM could be observed in the synovial fluid suggesting that there is a continuously stimulated, ongoing B cell immune response against citrullinated antigens [20]. In line with this hypothesis, circulating plasmablasts/cells in peripheral blood of ACPA-positive RA patients were found to spontaneously produce ACPA [21]. Further characterization of the ACPA immune response identified citrullinated-antigen specific B cells mostly as IgG- or IgA-expressing memory B cells and plasmablasts [22]. The B cell receptors of these ACPA-expressing B cells show a

high degree of somatic hypermutation [23, 24]. Thus, the immune response directed against citrullinated antigens in patients with RA generates a population of memory B and plasma cells with many features that are compatible with the concept of antigen-driven, T cell dependent maturation.

## MATURATION OF THE ACPA RESPONSE

### The two-hit model of ACPA-positive RA

The development of ACPA-positive RA is thought to be a multistep process which is described here by a two-hit model (figure 1) [25]. Environmental and stochastic events ("first hit") may drive the initial break of tolerance leading to ACPA production which can precede the onset of RA for years without signs of clinical symptoms [26]. Upon a certain second/additional trigger, citrullinated antigen-specific B cells may receive T cell help that induces the maturation and expansion of the initial response, thereby triggering the development of an "inflammatory" autoimmune response. The maturation of the ACPA response associates strongly with imminent onset of arthritis.

### The HLA association of ACPA-positive RA

The strongest genetic risk factor associated with RA is found within genes encoding certain HLA class II molecules, specifically HLA-DR. Within the third hypervariable region of the HLA-DRB1 chain there is a common amino acid sequence, known as the shared epitope, which has been identified as susceptibility factor for RA [27]. It is hypothesized that the positively charged amino acids of the shared epitope favour binding to negatively or neutrally charged amino acids, thereby preferring binding of citrullinated antigens over arginine residues [28]. Importantly, the shared epitope predisposition (HLA-SE) is only associated with ACPA-positive RA, not with ACPA-negative disease [29-31]. Moreover, HLA-SE alleles do not predispose to the initial break of tolerance towards citrullinated antigens, i.e. to the presence of ACPA, but only to ACPA-positive disease [31, 32]. As HLA are involved in the interaction between T- and B-cells, it is conceivable that T cells contribute to the maturation of the ACPA response, possibly via T cell help to citrullinated antigen-specific B cells. Supporting this hypothesis, unexhausted, activated T 'peripheral helper' cells were identified in the joints of RA patients which expressed factors enabling help for B cells, directed migration to the inflamed site and induced plasma cell differentiation [33].

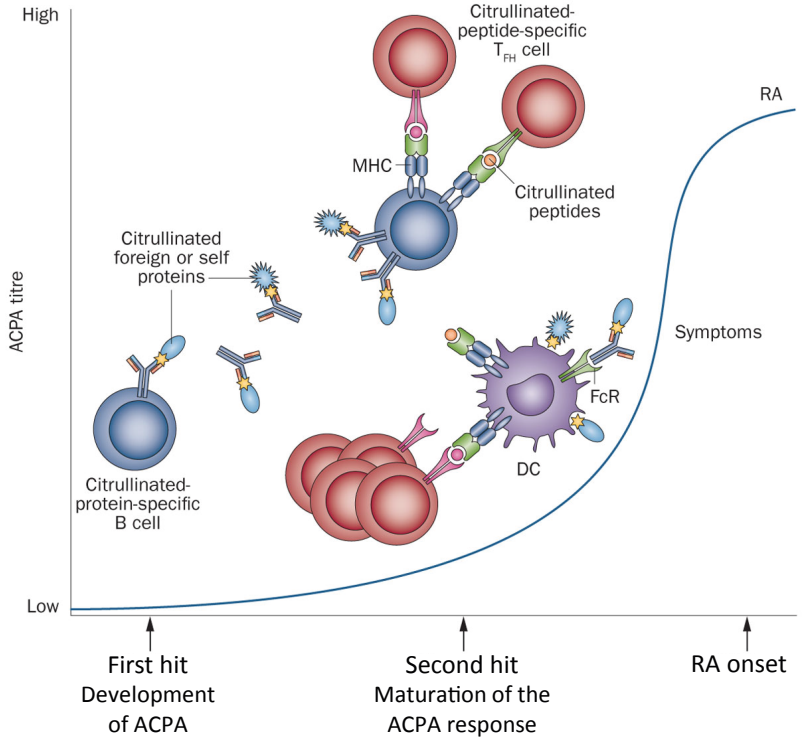


**Figure 1. Two-hit model of the ACPA response.** Initial break of tolerance caused by environmental and stochastic events may lead to the development of ACPA. Citrullinated proteins can be detected and processed by dendritic cells (DC), e.g. via Fc receptor (FcR) binding, and presented to T cells, which are then activated. It is only upon this trigger (second hit) that B cells producing ACPA receive T cell help and that the ACPA response matures, thereby initiating clinical onset of RA. Reprinted by permission from Springer Nature: Nature Publishing Group, Nature Reviews Rheumatology, *Coeliac disease and rheumatoid arthritis: similar mechanisms, different antigens*, Koning, F., R. Thomas, J. Rossjohn, et al., Copyright (2015) [25].

## Expansion of the ACPA response

As described above, HLA shared epitope alleles are strongly associated with ACPA-positive RA but not with the presence of autoimmunity/ACPA in health [31, 32]. This suggests that HLA molecules contribute to the “second hit” rather than to the “first hit” of the two-hit model, thereby increasing the range and intensity of the ACPA immune response. A role for T cells as drivers of the activation of citrullinated antigen-specific B cells and of the maturation of the ACPA response is supported by an increase in ACPA titre, enhanced isotype usage and an expanded antigen recognition profile shortly before clinical onset of arthritis [14, 34-36]. However, ACPA are highly cross-reactive towards

different citrullinated proteins and, unlike antibodies against recall antigens, ACPA are of low affinity. This indicates that citrullinated antigen-specific B cells might not primarily be selected on affinity for their cognate antigens, but on different selection mechanisms in or outside of germinal centers [23, 37-39]. The nature of these alternative selection mechanisms is elusive. However, particular features of the ACPA-response, such as a remarkable degree of glycosylation of ACPA variable domains, might be involved in this process. This aspect will be discussed in detail below.

## BIOLOGICAL FUNCTIONS OF ACPA-IgG

In the past years, several *in vitro* studies have shown possible biological mechanisms of ACPA-IgG that could contribute to the inflammatory processes in RA. ACPA were found to induce effector functions such as the activation of classical and alternative pathways of the complement system [40], the induction of osteoclastogenesis and bone loss [41], stimulation of neutrophils, the formation of neutrophil extracellular traps (NETs) [42, 43], and the activation of various immune cells via Fc gamma receptors (FcγR).

### Fc gamma receptors

FcγR are common signalling receptors found in different combinations and levels on cells of the immune system. These immunoreceptors recognize the Fc tail of IgG antibodies and can in this way induce a wide range of cellular effector activities, as FcγR-expressing cells have different biological functions. FcγRI, FcγRIIA/C and FcγRIIIA contain immunoreceptor tyrosine-based activation motifs (ITAM) in their cytoplasmic tail and are therefore considered to be activating FcγR (figure 2). In contrast, FcγRIIb, an inhibitory receptor, contains an immunoreceptor tyrosine-based inhibition motif (ITIM) which can counteract signalling cascades of activating receptors. Of all FcγR, FcγRI is the only receptor able to bind monomeric IgG to binds its ligand with high-affinity [44]. High-affinity Fc receptors can bind the antibody in the absence of antigens, whereas low-affinity Fc receptors need multiple antibodies that have already bound antigen and formed immune complexes (IC) to trigger cellular activation. FcγRII and FcγRIII are low-affinity receptors that need the engagement of several receptors before an activating signal is triggered. In the case of RA and the ACPA response, an abundance of citrullinated proteins and ACPA-immunoglobulins have been identified in synovial fluid of RA patients. Therefore, it is likely that ACPA-IC can be formed locally in this compartment [20, 45, 46]. Moreover, the detection of circulating citrullinated fibrinogen-IgG IC has been reported in half of ACPA-positive RA patients [47]. Thus, it is likely that ACPA IC can trigger FcγR and induce FcγR-mediated effects in the in-vivo situation.

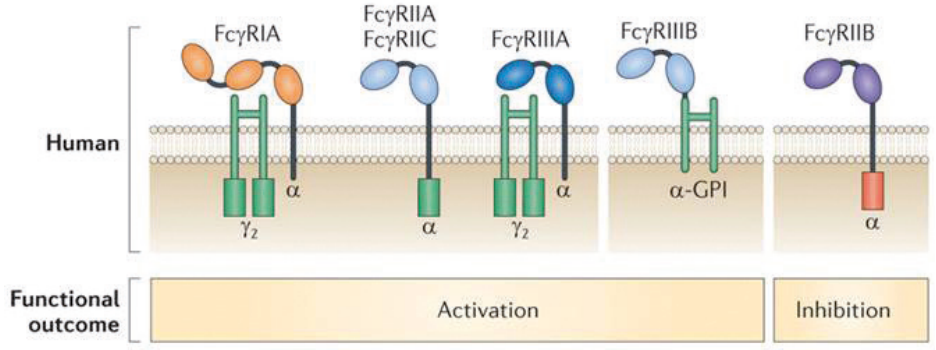


**Figure 2. Schematic overview of the different human FcγR.** FcγRI, IIA/C and FcγRIIIA/B belong to the activating FcγR, whereas FcγRIIB is the only inhibitory Fc gamma receptor. Reprinted by permission from Springer Nature: Nature Publishing Group, Nature Reviews Immunology, Intravenous immunoglobulin therapy: how does IgG modulate the immune system?, Schwab, I. and F. Nimmerjahn, Copyright (2013) [48].

## ACPA induced Fc gamma receptor-mediated effector functions

In line with the considerations above, most effector functions described for ACPA so far are related to FcγRIIA-mediated cellular activation. FcγRIIA is the most widely expressed FcγR and can be found on neutrophils, eosinophils, platelets, mast cells, monocytes, macrophages and dendritic cells [49]. Mast cells, for example, were shown to be activated by plate-bound ACPA, thereby stimulating IL-8 secretion which could be inhibited by blocking FcγRIIA [50]. In addition, ACPA-containing IC were found to induce TNFα production by activating macrophages and monocytes of healthy individuals and RA patients [42, 51-54]. Most of these studies highlighted the role of FcγRIIA in this context, as blocking this receptor resulted in a reduction of TNFα secretion, whereas blocking FcγRI or FcγRIII had no effect. This ACPA-induced TNFα production could be enhanced even further in the presence of IgM-RF, presumably through the (additional) formation of larger immune complexes [55, 56]. Together, these *in vitro* studies clearly show that ACPA can stimulate cytokine-dependent inflammation, which may also occur in the synovial fluid of RA patients and thereby contribute to disease pathogenesis. Although ACPA, and likely also citrullinated antigens, can be present in high concentrations, it does not always induce active inflammation as evidenced by the presence of ACPA many years pre-disease without any clinical indications and the high ACPA serum titres in patients that underwent complete, drug free remission [13, 57, 58]. These observations suggest that the quality rather than quantity of the ACPA is an important contributor for the induction

of inflammatory disease, raising the question whether there are molecular features of ACPA that can determine and contribute to inflammatory processes in RA.

## GLYCOSYLATION OF ACPA-IgG

### IgG Fc glycosylation

Stability and biological activity of antibodies are influenced by *N*-glycosylation of the antibody Fc region [59, 60]. This feature has most extensively been studied for human IgG molecules. Removal of these carbohydrate structures from the antibody can result in a loss of conformation and a loss in function, indicating that these structures have important immunomodulatory functions [60]. *N*-glycans can be incorporated within proteins when the *N*-glycosylation consensus sequence Asn-X-Ser/Thr (N-X-S/T, where X≠P) is present. IgG molecules carry such sequences in the Fc region and have two biantennary *N*-linked Fc glycans attached to conserved asparagine residues at position 297 in the $C_H2$ region of the heavy chain [61]. These IgG Fc glycans consist of a *N*-glucosamine structured core with mannose residues. Next to that, the glycan structure can be further decorated with a core-fucose residue, bisecting GlcNAc and different amounts of galactose and sialic acid residues (figure 3). Around 35% of the IgG Fc glycans in healthy subjects contain, on average, no galactose residues (G0), 35% have one galactosylated arm (G1) and 16% of the IgG Fc glycans terminate in a galactose residue (G2) on both arms [61]. The other 14% of the IgG-Fc tails contain sialylated G1- and G2-type glycans. These sialic acid residues can only be incorporated in the presence of a galactose residue. The degree of galactosylation and sialylation of human IgG Fc-glycans is strongly associated with biological effector functions that are generally considered “pro-inflammatory” and “anti-inflammatory”, although some caution needs to be taken when using this classification.

Glycans are highly dynamic and may display a modified oligosaccharide composition dependent on physiological and pathological conditions. During physiological conditions such as with increasing age, pregnancy and vaccination the degree of N-glucosamine, galactosylation and sialylation may vary [62-65]. In pathological conditions, antibody Fc glycosylation levels may express a more “pro-inflammatory” phenotype with lower galactosylation and sialylation levels, and increased core-fucosylation; this phenotype associates with increased inflammation, a hallmark of cancer, viral infections and autoimmune diseases [66]. Previously, we and others have demonstrated that ACPA-IgG in RA have a “pro-inflammatory” Fc glycosylation pattern with reduced galactosylation and sialylation levels, if compared to non-ACPA IgG [67, 68].

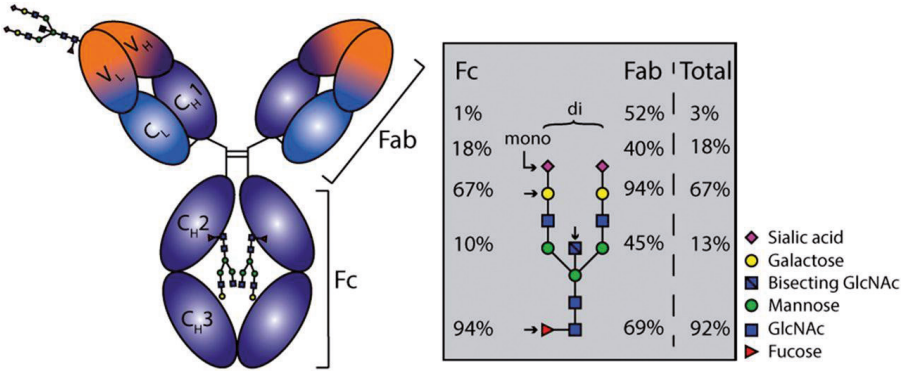


**Figure 3. Depicted is an IgG molecule (left), which consists of a Fab (variable part) and Fc region (constant part of the antibody).** IgG molecules have two glycans in their Fc region and may additionally have glycans in the Fab domain. On the right a fully decorated N-glycan is depicted consisting of different glycan residues. The amount of glycan residues may differ dependent on their region of incorporation in the IgG molecule, as indicated with the percentages. Reprinted by permission from: The Emerging Importance of IgG Fab Glycosylation in Immunity, van de Bovenkamp F.S., Hafkenscheid L., Rispens T., Rombouts Y. The Journal of Immunology, vol. 196, February 15, 2016, pp. 1435-1441. Copyright 2016. The American Association of Immunologists, Inc. [69].

The first observations that the glycosylation of IgG in RA patients is different from serum IgG from healthy individuals came from Parekh et al in the 80s [70]. Later, it was found that specifically ACPA-IgG have an altered Fc-glycosylation pattern characterized by low galactosylation and sialylation levels compared to serum IgG [67]. This effect is more pronounced at the site of inflammation, for which synovial fluid can be seen as a proxy. As ACPA can be present long before disease manifestation, it was also the question when upon disease development this change in ACPA Fc-glycosylation pattern occur. Interestingly, galactosylation levels of ACPA-IgG decreased around 3-4 months before RA onset, while the degree of fucosylation increased [71]. This effect was paralleled by an increase in systemic inflammation. While it needs to be determined whether and how this altered Fc-glycosylation profile impacts the biological effector functions of human ACPA-IgG, recent evidence from murine studies suggests that such Fc-glycosylation changes can indeed induce the onset of arthritis. Diminished sialylation in the IgG Fc region of mice, induced by IL-23 dependent mechanisms, enhanced the inflammatory and arthritogenic activity of these antibodies, inducing the onset of arthritis in mice [72]. Together, these data may suggest a pro-inflammatory role of ACPA Fc-glycosylation in the onset of RA. In the human situation, however, a clear causal relationship still has to be determined.

## Functional consequences of IgG glycosylation

As briefly described above, IgG glycosylation is closely involved in several antibody-mediated effector functions such as binding to FcγR. In addition, antibody half-life in serum, activation of complement and the interaction with lectins can be affected and modulated by glycans [73]. Changes in IgG glycosylation may therefore have important functional consequences in the biological characteristics of an antibody. A relevant example hereof is that antibodies lacking core-fucosylation in the IgG-Fc have an increased affinity for the glycosylated FcγRIIIA, which results in an enhancement of antibody-dependent cellular cytotoxicity (ADCC) [74-76]. Moreover, galactose and sialic acid residues have been reported to induce an anti-inflammatory response of IgG in mice by modulating the interaction with the humanized lectin DC-SIGN and the lectin Dectin-1, receptors binding to glycan residues [77, 78]. As ACPA-IgG has an altered glycosylation profile compared to that of non-ACPA IgG, it might well be that its glycosylation could induce antibody effector functions that contribute to the pathogenic behaviour of ACPA-IgG.

## ACPA Fab glycosylation

In contrast to the constant amino acid sequence of the IgG Fc tail, the sequence of antibody variable domains, which form part of the antigen-binding fragment (Fab)-domain, are generated during recombination of V-, D- and J-gene segments during B cell development. At later stages, these recombined sequences are further modified by the process of somatic hypermutation. Hence, there is an almost innumerable variability of amino acid sequences in this region of the antibody molecule. Notably, only few human genes encoding V-segments of the antigen-binding fragment also harbour *N*-glycosylation consensus sequences [69]. These allow for the presence of *N*-glycans in the Fab domain. Moreover, Fab glycosylation can be found in the antibody variable domain as a consequence of somatic hypermutation which, during antibody maturation, can generate *de novo N*-glycosylation sites [79]. Still, glycosylation of the Fab-region of IgG is generally low in the healthy situation, with 15-25% of serum IgG molecules carrying *N*-glycans in this region (figure 3). In certain disease situations, however, *N*-glycosylation motifs are more commonly observed in immunoglobulin variable regions, in particular in patients with follicular lymphoma, diffuse large B-cell lymphoma and Burkitt's lymphoma [80, 81]. Interestingly, also IgG of RA patients have elevated levels of Fab glycans compared to IgG from healthy donors [82].

In this context, it is interesting that an unusually high degree of Fab-glycosylation has recently been found on ACPA of the IgG isotype. These Fab glycans were found on both heavy and light chain variable domains [83]. In fact, it was estimated that more than 90%

of ACPA-IgG molecules carry Fab glycans [84]. ACPA-IgG isolated from synovial fluid, the site of inflammation, could even exceed 100% Fab glycosylation implying that multiple glycans can be attached to the variable domain [84]. By sequencing the B cell receptor of ACPA-expressing B cells, it was shown that introduction of *N*-glycosylation sites in the ACPA-IgG variable domain occurs in a selective process during somatic hypermutation and is not a random accumulation of mutations [24]. Detailed analysis of the glycosylation profile further revealed that these ACPA-IgG harbour *N*-linked glycans that are highly sialylated. This glycosylation profile of ACPA-IgG, which comprises the full range of bi-antennary glycan residues, could indicate that ACPA Fab glycans are relatively easily accessible to glycosyltransferases. Although a comparable degree of Fab glycosylation could not be identified for several other autoantibodies, it is yet unknown whether this abundant presence of Fab glycans is a molecular feature specific for ACPA or that it extends to other autoimmune responses as well [83]. Potentially, Fab glycans have an essential function in ACPA-positive RA, given their abundant presence on ACPA-IgG. Therefore, dedicated research is needed to determine the specificity of Fab glycans for ACPA and to determine the function of ACPA Fab-glycosylation in the context of RA pathogenesis.



## THESIS OUTLINE

Despite considerable advances in our understanding of relevant disease processes operational in RA, the involvement of autoantibodies and autoantibody-expressing B cells in this disease is still incompletely understood. Based on the findings and considerations described above, the studies described in this thesis were initiated to explore the ACPA immune response and its biological effector functions to eventually provide a better understanding of the origin and function of ACPA, ACPA glycosylation and B cells in RA pathogenesis.

**Chapter 2** describes studies of the ACPA B cell response in synovial fluid. In a previous study by our department, circulating ACPA-producing B cells had been identified in the peripheral blood of patients with ACPA-positive RA [21]. However, as the synovial fluid is the primary site of inflammation in RA, we set out to phenotypically and functionally characterise ACPA-expressing B cells in the synovial compartment. We observed a high frequency of ACPA-IgG secreting plasmablasts/ plasma cells in the synovial fluid, largely exceeding frequencies of those from peripheral blood. Interestingly, we observed that synovial fluid mononuclear cells could provide a microenvironment in which ACPA-secreting plasmablasts/ plasma cells could continuously produce ACPA over an extended period of time. Thus, work presented

in chapter 2 provides evidence for the differentiation and persistence of the ACPA response at the site of inflammation.

Next, we focus on the glycosylation of the ACPA variable domain. As discussed, almost 90% of the ACPA-IgG molecules have Fab glycans which are highly sialylated [84]. However, it was unknown if all ACPA isotypes and IgG subclasses carry these highly sialylated Fab glycans. This information was deemed important to understand the development of Fab glycosylated ACPA and their potential role in RA pathogenesis. In the studies described in **chapter 3,** we therefore explored the presence of glycans in the variable region of ACPA-IgM and various IgG subclasses. We hypothesized that ACPA-IgM would lack additional Fab glycans, as glycans in the variable domain are predominantly introduced upon somatic hypermutation. Indeed, we did not observe additional Fab glycans on ACPA-IgM molecules, in contrast to ACPA-IgG. These results indicate that extensive Fab glycosylation is a specific feature of ACPA-IgG in RA and provide an argument for the involvement of T-cell help in the generation of Fab-glycosylated ACPA-IgG.

We further studied Fc-mediated biological effector functions of ACPA in relation to their Fc glycan composition, and investigated whether the inflammatory environment influences Fc glycosylation patterns of antibodies. As described, ACPA are abundantly secreted as IgG molecules and thought to form immune complexes which can interact with immune cells. As such, FcγR-mediated effector mechanisms are presumably most relevant in the context of synovial inflammation. To identify potential effector mechanisms of ACPA-IgG that could contribute to RA pathogenesis, we performed studies analysing the binding of ACPA-IgG to individual FcγR, which are described in **chapter 4**. We generated ACPA immune complexes and used a standardised *in vitro* system of single FcγR-transfected CHO cell lines. We observed that most ACPA-IgG immune complexes bind to FcγRI and extended our research further to IFN-γ stimulated neutrophils, as activated neutrophils express this receptor and are highly abundant in RA. Blocking studies revealed that almost 30% of the binding of ACPA-IgG immune complexes to activated neutrophils was mediated by FcγRI. These observation suggest a role for FcγRI expressed by activated neutrophils as relevant receptor for ACPA-IC in the context of synovial inflammation. The studies described in **chapter 5**, focussed on the interaction of ACPA-IgG and IgM rheumatoid factors. IgM-RF are thought to prefer binding to IgG molecules with low Fc galactosylation levels [85, 86]. Since ACPA-IgG display low levels of Fc galactosylation [67], we investigated whether RF preferentially bind ACPA-IgG over non-ACPA IgG. In this study, we show that IgM-RF does not prefer binding to ACPA-IgG or IgG with low galactose content over galactosylated IgG. This study indicates that potential conformational changes induced by the presence of Fc-galactosylation does not influence RF binding and suggests that ACPA-IgG are not necessarily better targets for RF

than non-ACPA IgG. Finally, in **chapter 6** we describe investigations into the influence of the inflammatory environment of B cells on the Fc glycosylation patterns of secreted antibodies. Changes in antibody Fc glycosylation profiles can have direct consequences on antibody effector functions leading to enhanced inflammation and disease aggravation. Therefore it is important to know whether and to what extend Fc glycosylation profiles can be regulated. As terminally differentiated cells and main producers of antibodies, it would especially be interesting to observe if IgG secreted by plasma cells are still capable of changing its antibody characteristics. The answers to these questions are pivotal to our understanding if and how inflammation during the course of a disease could be modulated. We examined the effect of IL-10, relevant for development/maturation of B cells, on the Fc glycosylation of antibodies produced by antibody secreting cells (ASC) and stimulated B cells depleted of ASC. We observed that in the presence of IL-10, ASC can still modulate their oligosaccharide content. Surprisingly, this change in glycan composition was opposite to the antibody Fc glycosylation modification observed with IL-10 stimulation of naive and memory B cells, indicating that the change in Fc glycosylation is not only dependent on the environmental stimuli but also on the differentiation status of the B cell.

**Chapter 7** provides a summary of the results and discusses the relevance of ACPA and ACPA glycosylation in RA pathogenesis.

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