Antibody Glycosylation in Rheumatoid Arthritis

How does autoantibody glycosylation affect Rheumatoid Arthritis?

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Summary

Rheumatoid Arthritis (RA) is an auto-inflammatory disease that affects approximately 1% of the population. Symptoms include tenderness, swelling and stiffness in joints. Though various treatment modalities are available for RA, a cure has not yet been found. This is due to the fact that, while many factors contributing to the disease progression are known, the initial cause is not. Recently, research in RA has focussed on anti-citrullinated protein antibodies (ACPAs). These are autoantibodies targeting citrullinated proteins, which are self-antigens. ACPAs are known to be present in 60-70% of RA patients and can be present years before the onset of the disease, without being pathologic. The hypothesis is that pathology is reached as a result of a process termed glycosylation. This is the addition of glycans, which are sugar groups. These glycans are believed to influence the structure of ACPAs in the constant, as well as in the variable regions, making them pro-inflammatory antibodies. The glycosylation of constant Fc antibody regions has been known for quite some time, but glycosylation of variable Fab regions is a relatively novel discovery. Antibody Fab glycans differ in composition from Fc glycans, comprising of a different set of sugar residues. Both types of antibody glycosylation are believed to originate during somatic hypermutation of B cells. This process is aided by T helper cells, which is also suggested to be a crucial factor. As of yet, it is however unknown how the initial break of tolerance to citrullinated proteins arises. A possible factor contributing to this process is the presence of foreign antigens, resembling citrullinated self-proteins, in conjunction with particular HLA haplotypes, which also points to a hereditary component of RA. Recent studies are rapidly providing new evidence supporting the hypothesis that ACPA glycosylation is an important determinant in RA. This process potentially contributes to disease pathogenesis though a lot is still to be learned before novel therapeutic targets can be considered.

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Introduction

Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease that affects approximately 1% of the population and generally develops between the ages of 40 and 70 years[1 2]. Patients with RA can experience tenderness, swelling and stiffness in joints, including knees, ankles, shoulders, wrists, fingers and toes. These symptoms are caused by inflammation of the synovial membrane, which is the lining of synovial joints[3]. Chronic inflammation can cause cartilage and bone destruction and may lead to joint deformities[4-7]. Various environmental factors have been shown to be involved in disease development, including inhaled pollutants, infections and nutritional choices[8-10] as well as genetic factors[8 11 12]. RA is categorized as an autoimmune disorder, since autoantibodies are present in RA patients that target post-translational modifications of proteins[13-16]. These autoantibodies possibly play a crucial role in RA development and progression.

Current treatment methods

Currently, RA is treatable rather than curable. Treatment methods are now mainly focussed on the reduction of the inflammatory reactions in joints[17]. Medications used for this are nonsteroidal anti-inflammatory drugs (NSAIDs) and steroid-based drugs in case of acute symptoms [7]. Another treatment option uses monoclonal antibodies (mAbs) against tumour necrosis factor (TNFs), such as infliximab[18]. Here, the cytokine TNF involved in inflammatory reactions is neutralized. Many patients seem to become resistant to this kind of therapy, however, or they don't seem to respond at all[17]. Other treatments are now specifically focussing on RA, rather than on the common chronic inflammatory symptoms. Drugs for this type of treatment are termed disease-modifying antirheumatic drugs (DMARDs)[19] and they are administered as early as possible after onset of the disease to prevent permanent tissue damage[20]. An example of a DMARD used in RA treatment is methotrexate[6 21].
As more becomes known about what causes RA, a new group of DMARDs arises. Since it is now well accepted that autoantibodies play an important part in the disease progression, new drugs inhibiting B cells and T cell co-stimulation have found their way into mainstream treatment options[17 19 22]. Examples of such treatments are monoclonal antibodies, such as Rituximab, that target CD20, a general membrane marker of mature B cells[17 22]. These drugs deplete B cells and inhibit antibody production[3]. Rituximab is used in the treatment of autoimmune diseases like RA, but it is also used for certain cancers.

Autoantibodies in RA

Proteins can be susceptible to posttranslational modifications, influenced by environmental factors mentioned previously[8 11]. Type II collagen and vimentin are proteins that are abundantly present in joint regions and these proteins are susceptible to citrullination[14 23]. In this process, an arginine amino acid in the protein's sequence is transformed to a citrulline. Upon recognition by the immune system, citrullinated proteins are regarded as foreign antigens. This triggers an immune response, including antibody production by B cells (Fig. 1). These antibodies are autoantibodies since they target self-proteins and they are termed anti-citrullinated protein antibodies (ACPAs)[13-16]. It has been shown that ACPAs are present in 60-70% of RA patients[4] and that they can be present in individuals years before the onset of RA[5 24 25].

However, the first autoantibodies found to be associated with the onset of RA were not ACPAs, but Rheumatoid factors (RFs)[24]. These antibodies target the Fc tails of IgG antibodies leading to the formation of immune complexes, inducing an inflammatory response. Rheumatoid factors are not exclusively present in RA, however, and they seem to be linked to other chronic inflammatory diseases as well. In contrast, ACPAs have been shown to be highly specific for RA[2 13].
ACPAs seem to play a crucial role in the onset and progression of RA. In vitro assays suggest that ACPAs can have direct effects on bone metabolism, are involved in the activation of immune effector cells and can trigger complement pathway activation[17].

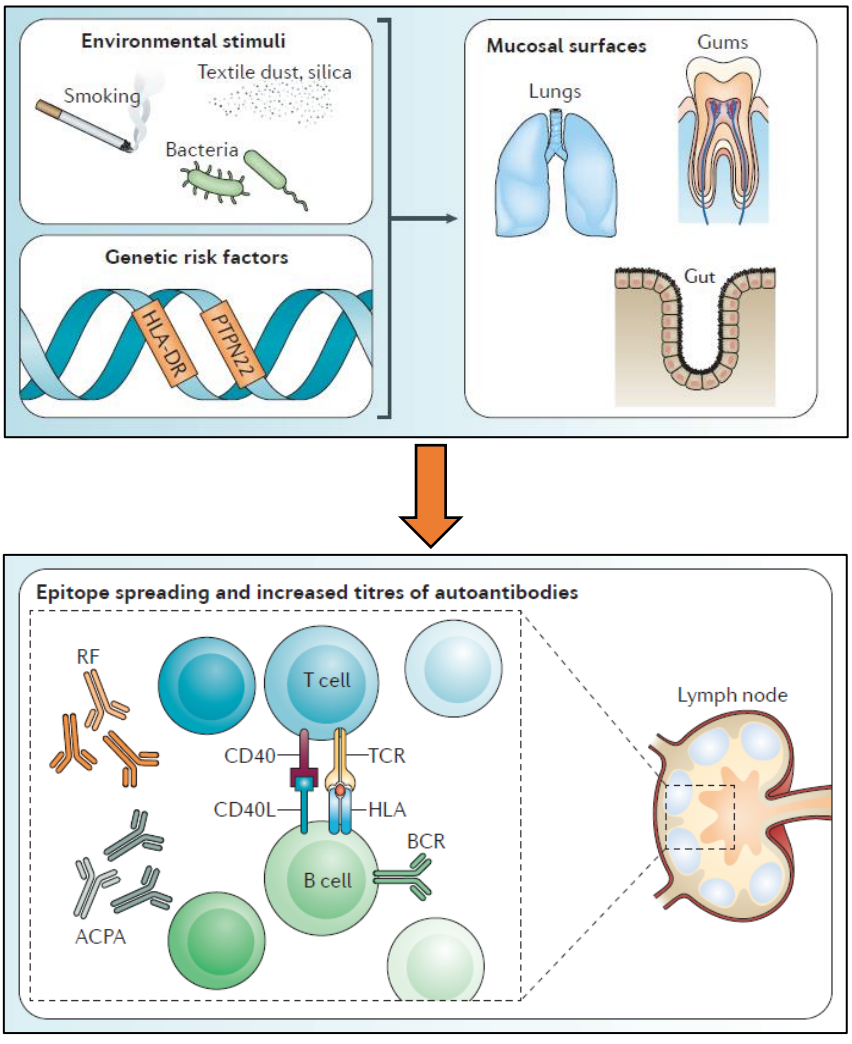

Figure 1. | ***ACPA production initiation in Rheumatoid Arthritis[15].*** *Environmental stimuli and specific HLA haplotypes contribute to the citrullination process and initiate. the production of ACPAs by B cells, a process that is supported by T helper cells.*

Antibody glycosylation

ACPAs are autoantibodies targeting citrullinated proteins and since IgG antibodies are the most abundant, studies have focussed mainly on this antibody isoform[26]. The effector function of antibodies is believed to be influenced by a process called glycosylation, which is the addition of carbohydrates to the antibody. Carbohydrates are sugar groups that can bind to antibodies by an N-glycosidic bond. Therefore, these groups are also referred to as N-glycans. N-glycans harbour a biantennary structure and their core structure consists of multiple N-acetylglucosamine and mannose residues[17] (Fig. 2). This is called the G0 glycoform. When glycans are attached to an antibody, they are bound to the nitrogen of the asparagine (ASN) domain. The attachment of these glycans can affect the antibody's affinity and avidity in regards to citrullinated proteins. Therefore, they are potential markers for the pathogenesis of RA[17 27].

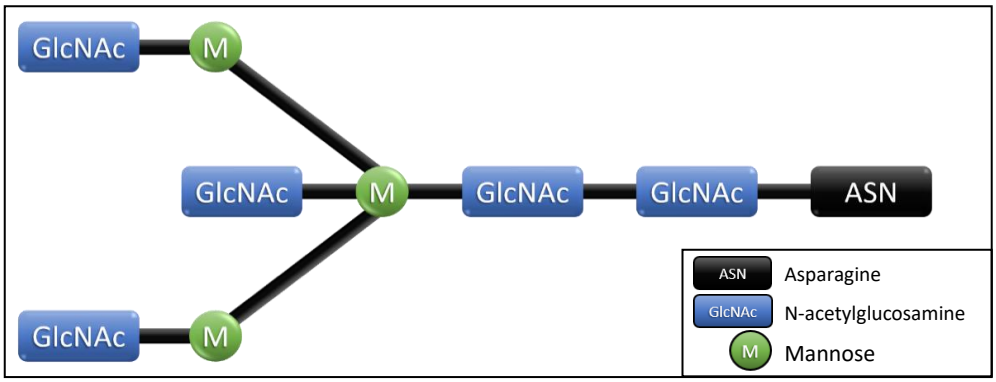

Figure 2 | ***Unglycosylated N-glycan structure.*** *The unglycosylated structure of glycans (G0) consists of N-acetylglucosamine and mannose. Glycans attach to the antibody's asparagine domain.*

Antibodies are made up of two identical heavy chains and two identical light chains, consisting of both constant domains (CH and CL) and variable domains (VH and VL)[3 4 28] (Fig. 3). In IgG antibodies, the region located below the antibody hinge region consists of four constant domains in total (two CH2 and two CH3 domains). Together they make up the fragment crystallizable (Fc) region that can bind to Fc-gamma receptors (FcγRs) on immune effector cells. Above the antibody hinge, two identical branches are located, both consisting of two constant domains (CH1 and CL) and two variable, antigen-binding domains (VH and VL). This region is called the fragment antigen-binding (Fab) region. Both the constant Fc and variable Fab regions of an antibody are susceptible to glycosylation and the effect on immune effector cells, and therefore (auto)immune responses, differs based on the glycan localisation[4 17 24 29-31].

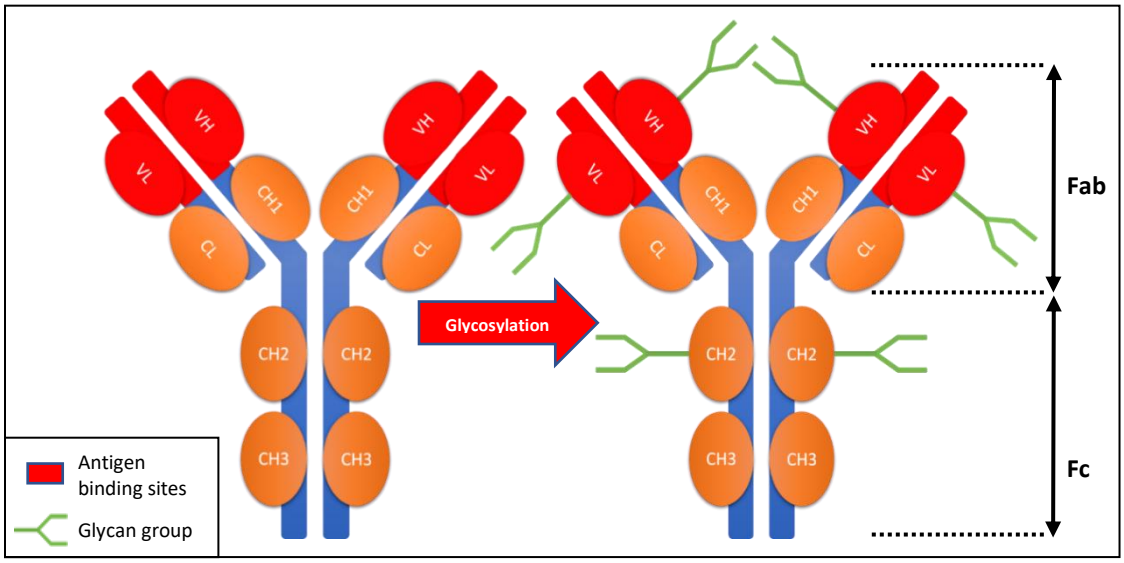

Figure 3 | **IgG *antibody domains and localization of N-glycans.*** *The IgG antibody consists of Fc and Fab domains, forming heavy and light chains. Glycosylation can take place on the CH2 domains of the Fc region and on the antigen-binding sites of the Fab region.*

In IgG antibodies, glycosylation of the Fc region has been shown to take place at the CH2 domain, located just below the antibody hinge region[17] (Fig. 3). Glycosylation of the Fc region is important for the effector functions of IgG via binding to FcγRs on immune cells and complement activation[3]. Accordingly, the removal of Fc glycans inhibits these functions.
Fab region glycosylation has been shown to take place on both the variable heavy chain domain (VH) and the variable light chain domain (VL). Fc glycosylation and Fab glycosylation can occur on an antibody at the same time, and certain combinations of Fab and Fc glycans can result in specific modulation of the antibody effector function. For instance, glycans can have an effect on antibody binding activity, antibody-mediated inhibition of protein-protein interactions, and antibody half-life (Fig. 4).

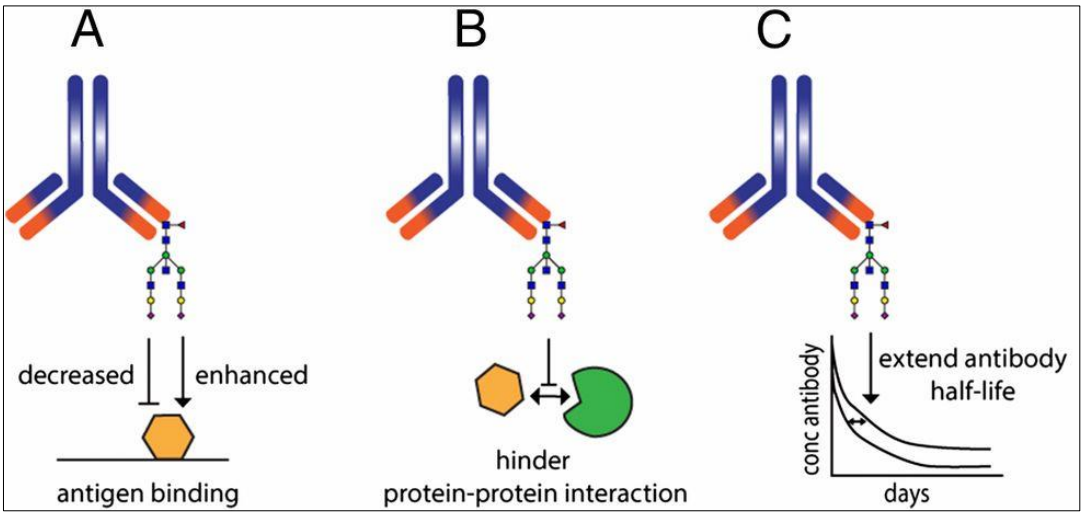

Figure 4 | ***Different effects of Fab glycosylation[29].*** *In the Fab region of antibodies, glycans can affect antigen-binding, protein-protein interactions and antibody half-life.*

Aim of this thesis

The aim of this thesis is to review our current understanding of the process of antibody glycosylation, its impact on antibody function and how antibody glycosylation contributes to the generation and function of anti-citrullinated protein antibodies (ACPAs) in RA. The mechanisms and factors involved in antibody glycosylation will be described including ACPA-production by B cells, the regulation of B cell activity and how this eventually might lead to the production of aberrantly glycosylated ACPAs associated with the development of RA.

Current knowledge on ACPA glycosylation in RA and contributing factors

Glycosylation of antibody Fc and Fab regions

The constant Fc region of an IgG antibody plays an important role in immune defence, mediating antibody-dependent cellular cytotoxicity, complement activation and phagocytosis[30]. Glycosylation is a crucial factor in the regulation of these functions, since this influences the conformation of the Fc tail of the antibody. As mentioned previously, Fc glycans are attached to the two CH2 domains in this region[3 31].
More recent studies have also noted the importance of glycosylation in the variable Fab regions of antibodies[4 17 24 29], where the antigen-binding sites are located. Recent studies have shown that Fab glycosylation occurs in approximately 90% of IgG ACPAs[13]. Whereas the level of glycosylation of the Fc regions of ACPAs are comparable with those on normal IgG antibodies, glycosylation of ACPA variable domains is much higher. Much less is known about variable domain glycosylation as opposed to constant domain glycosylation as its existence is a more recent discovery[24].
Antibody glycosylation can give way to both pro-inflammatory and anti-inflammatory activity, depending on the type and composition of the glycans[17]. Moreover, the combined glycosylation pattern of Fc and Fab regions determines antibody effector functions specific for that glycoform.

Types of antibody glycosylation

As stated earlier, antibodies can be glycosylated in different regions and glycosylation can attach on heavy chain as well as on light chain domains of the antibody by an N-glycosidic bond. There is, however, another variable that should be taken into account. It is important to note that besides the core G0 glycoform, N-glycans can contain different types of sugar residues, resulting in additional glycoforms. There are three main glycoforms, formed by the addition of either fucose, galactose, or sialic acid. These three types of residues can be present on glycans on the antibody's Fc regions as well as in Fab regions where they affect the antibody structure differently[17].

Fucosylation
Fucosylation is the addition of a fucose residue to an N-glycan and in RA, fucosylation has been shown to be increased[32]. The fucose residues are located at the base of the biantennary structure of the glycan[17] (Fig. 5). They are present on most IgG antibodies and studies in mice have shown that the presence of fucose residues influences the affinity for certain FcγRs and hence the antibody effector function[17 33]. In addition, studies with RA patients have shown that the total level of fucosylated IgG antibodies, lacking sialic acid and galactose, is increased during chronic inflammation[26 32]. This glycan conformation is believed to have a pro-inflammatory effect on antibodies and is termed the IgG G0F glycoform[26]. The presence of this glycoform also appears to be increased in diseases such as Crohn's disease, Systemic Lupus Erythematosus (SLE), and Sjögren syndrome but so far it remains unclear which extrinsic factors contribute to the production of the fucosylated IgG G0F glycoform of antibodies[17].

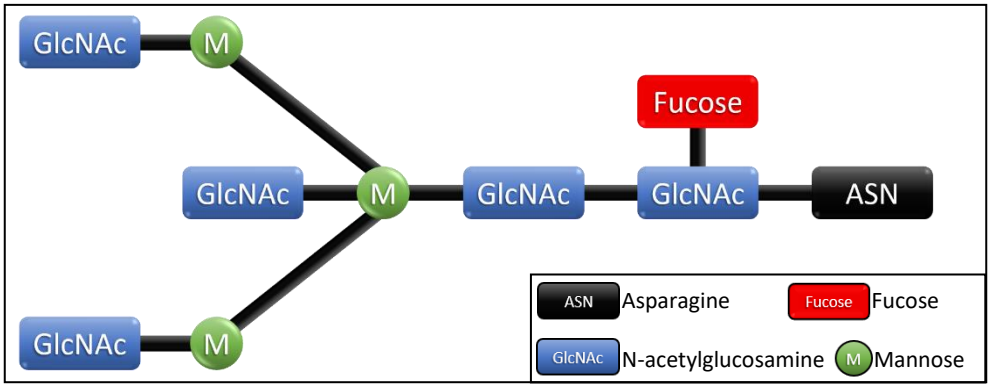

*Figure 5 | **Fucosylated N-glycan structure (G0F).** The fucosylated structure of glycans (G0F) consists of N-acetylglucosamine and mannose and harbours an additional fucose residue.*

Galactosylation

Galactosylation is the addition of a galactose residue to an N-glycan. A glycan can contain either one or two galactose residues, which are termed the IgG G1F or G2F glycoform respectively[26]. These residues are attached to the N-terminal forked ends of the structure (Fig. 6). On Fab domains, N-glycans appear to be highly galactosylated, as opposed to N-glycans on Fc domains. On Fc domains, the downregulation of galactosylated IgG glycoforms has been shown to be inversely correlated with upregulation of the G0F glycoform, which is possibly linked to the onset of RA[32]. On the other hand, the effect of extensive galactosylation of Fab domains has not yet been established. Galactosylation appears to be positively linked to disease alleviation, since treatment of RA with methotrexate shows increased levels of galactosylated antibodies[1]. This effect could however also be ascribed simply to the reduction of the G0F glycoform. It is not yet known what the precise function of this type of glycosylation is, though there is a general assumption that galactosylation might have an anti-inflammatory effect[26 34].

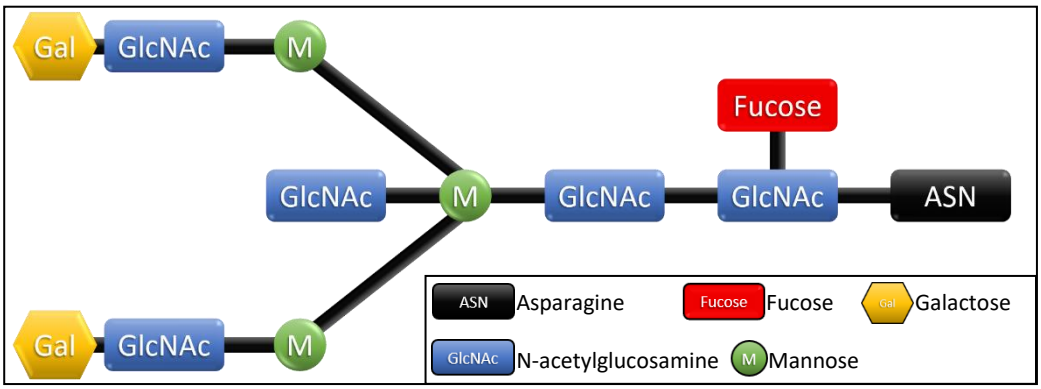

*Figure 6 | **Galactosylated N-glycan structure (G1F or G2F).** The galactosylated structure of glycans (G1F or G2F) consists of N-acetylglucosamine, mannose and fucose and harbours an additional galactose residue.*

Sialylation

Sialylation is the addition of a sialic acid residue to an N-glycan. These residues are added after galactosylation has taken place and they attach to the galactose residues at the N-terminus end of the structure (Fig. 7). Like galactosylation, sialylation is also inversely associated to the level of fucosylated glycans. In RA, reduced IgG sialylation has been demonstrated in mouse models as well as in patients[22]. Together with galactosylation, sialylation is an important factor in regulating the antibody effector function. IgG antibodies that lack sialic acids and simultaneously harbour lower levels of galactose residues on the CH2 domains seem to promote inflammation[26 34]. One explanation for this effect is that sialylation of the IgG antibody changes the conformation of its structure, resulting in a lower affinity for FcγRs, hence reducing inflammatory effector functions[28 35 36].

N-glycans on the Fab domains of ACPAs are not only highly galactosylated but also highly sialylated[31]. This is in contrast to glycans on the Fc domain, since in RA, Fc glycans are more prone to obtain the G0F glycoform. These observations support the idea that antibody glycosylation and changes in glycoforms are site-specific.

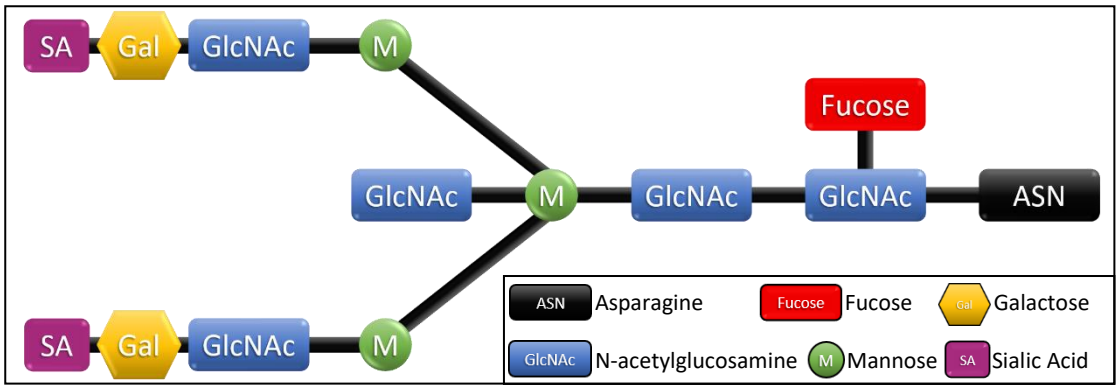

Figure 7 | ***Sialylated N-glycan structure.*** *The sialylated structure of glycans consists of N-acetylglucosamine, mannose, fucose and galactose and harbours additional sialic acid residues, attached to the galactose residues.*

ACPA development

It is known that ACPAs are present in a large proportion of RA patients (60-70%) and that they can remain inactive for years before the first symptoms of the disease occur[5 25]. It has also been shown that the glycosylation pattern of ACPAs is a driver for the progression of the disease[17 26 31 32 34-36]. The initial production of ACPAs seems to be the first step towards pathology, followed by a process in which several factors contribute to the development of symptoms. Currently, it is unknown what precedes this first step and leads to a break of tolerance to citrullinated proteins[13]. So, while the question remains as to why B cells start to produce these autoantibodies in the first place, recent studies have focussed on the steps following the initial break of tolerance as this may give clues to novel targets for treatment of RA.

HLA factor

As mentioned earlier, genetic factors contribute to RA[8 11 12]. This has been suspected for some time, since it was observed that first-degree relatives of patients had a higher risk of developing RA[37]. In RA, the main genetic component is the HLA gene region[38].
The most prominent alleles involved in RA are those coding for class II HLA molecules, specifically HLA-DR. Class II HLAs, coding for MHCII, are involved in antigen presentation and alterations in these alleles are known to be linked to a large range of autoimmune diseases, including RA[12 13 38 39]. An amino acid sequence, termed the HLA-shared epitope gene group (HLA-SE) is found in the HLA-DRB1 gene[24]. This sequence seems to be most commonly connected to the disease. The “shared epitope” refers to the shared amino acid sequence which is the same among all HLA-DRB1 regions. If someone carries the HLA-DRB1 gene, the risk of developing RA with ACPAs is increased[12]. The amino acids of the shared epitope are positively charged, which means they tend to bind to amino acids that are negatively charged. A hypothesis concerning citrullinated proteins is therefore that the shared epitope favours binding to citrulline residues, which are negatively charged as opposed to arginine[24 40].

B cell activity in ACPA production

The process of antibody production is initiated by antigen recognition[3]. Normally, these are foreign antigens that need to be removed and the correct immune response is initiated after B cells specific for the foreign antigen are activated. In the case of ACPA producing B cells, however, this antigen is a citrullinated protein. Citrullinated proteins are not uncommon in the human body and can therefore be regarded as self-antigens. When B cells recognize and bind to self-antigens, negative selection should take place during the maturation phase to prevent a break of tolerance. Negative selection promotes deactivation and eradication of these self-recognizing B cells to maintain tolerance. It appears, however, that the first step towards RA development is fuelled by self-reactive B cells specific for citrullinated proteins that escape negative selection, possibly because of the presence of Fab glycans and the subsequent masking of self-reactivity[13 55] (Fig. 8). As stated previously, the exact reasons for this breach of tolerance are not yet known, though recent studies suggest that the initial immune response is not directed against self-antigens directly, but against foreign proteins that exhibit similarities to citrullinated self-proteins[41-43].

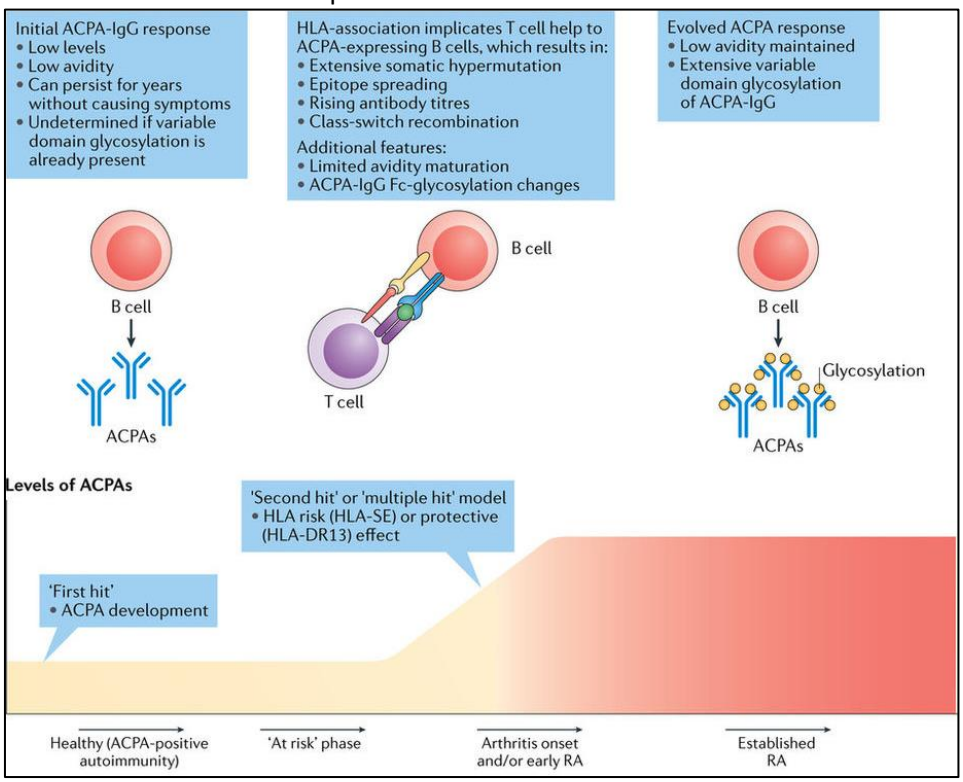

*Figure 8 | **Hypothesized progression of RA[13].** After initial production of ACPAs by B cells, ACPAs can be present years before occurrence of a "second hit". Here, the HLA phenotype mediates the shift of ACPA-positive autoimmunity to ACPA-positive RA. B cells produce pathological ACPAs containing glycosylation on their Fc domains as a result of somatic hypermutation in germinal centres. In the evolved ACPA response during established RA, Fab domains are also glycosylated, though it is unknown when this process occurs.*

Some studies have put forward the hypothesis of the active involvement of T helper and/or autoreactive T cells, complementing B cells, to play a role in the generation of ACPAs[44-46]. The fact that HLA is a strong genetic risk factor for RA development supports this assumption, allowing B cells to present specific peptides to autoreactive T cells[39].

CD4+ T helper cells are involved in the maturation process of B cells, which takes place in the germinal centres of lymph nodes. Here, somatic hypermutation takes place, where B cells are modified by affinity maturation and isotype switching, making an immune response more antigen-specific (Fig. 8). In the case of RA, this is also where Fc glycosylation changes of ACPAs are believed to occur. Here, N-glycosidic sites are formed on the ACPA Fc and Fab domains, making them susceptible for N-glycan attachment[47 48]. This is the stage where the risk of developing RA transforms

to the actual onset of the disease which includes the addition of different glycosylation sites on the IgGs variable domains[13]. Another study claims that ACPA variable domain glycosylation does not randomly occur during somatic hypermutation, but that the process is more regulated[48]. The study suggests that specific compositions of the glycosylation sites confer a selective advantage to ACPA+ B cells, allowing these B cells to evade negative selection during the maturation phase. Though the underlying mechanisms are as of yet unknown, a hypothesis is that variable domain glycosylation masks self-reactivity by displaying a low affinity for self-antigens in germinal centres[13 48 55].

T cells and cytokines

T cell help, in part via secretion of specific cytokines, is important in the activation and differentiation of B cells. In addition, T cell derived cytokines likely play a role in modulating the antibody glycosylation process as well. The major drivers of the inflammatory immune response against extracellular bacteria and fungi are the Th17 cells, a subset of CD4+ T cells[3 13 49]. These cells also play a role in autoimmune inflammatory diseases. In RA, they are believed to be associated with both the disease onset and progression[50].

The main cytokines that are produced by Th17 cells are IL-17, IL-21, and IL-22[3 49] (Fig. 9). A study claims that in autoimmune diseases, including RA, IL-17 promotes survival of naïve and memory B cells, preventing them from going into apoptosis[51]. This suggests that Th17 cells could promote the presence of B cells producing ACPAs. In addition, IL-21 acts on B cells by promoting their differentiation into antibody-secreting plasma cells. This process is aided by CD40 signalling and toll-like receptor (TLR) activation. There are however cytokines from the tumour necrosis factor family (TNF) that can bypass the requirement for CD40 and TLR signalling. An example of such a cytokine is B cell activating factor (BAFF). Especially BAFF and IL-17 appear to be involved in promoting proliferation, differentiation and survival of B cells by taking over the functions of these signalling factors[49 51]. Moreover, it has been shown that inhibiting IL-17 with monoclonal antibodies (anti-IL-17-mAb) can prevent the progression of RA in mice[41].

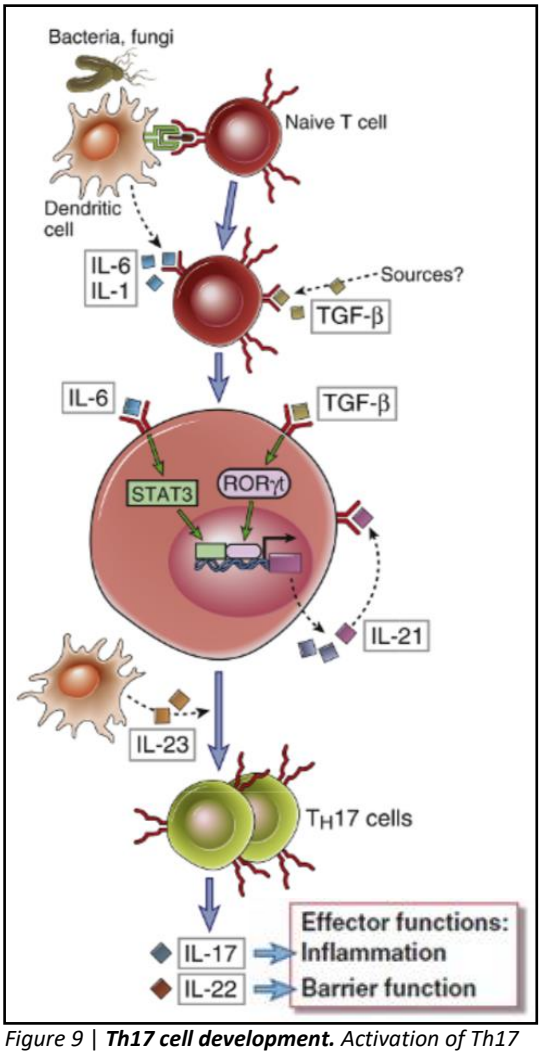

*Figure 9 | **Th17 cell development.** Activation of Th17 cell in response to antigens, its subsequent proliferation and IL-production[3].*

As mentioned, cytokines likely contribute to the process of antibody glycosylation. An example of this is IL-23, which is produced by dendritic cells and aides the proliferation of Th17 cells[52] (Fig. 9). It has been shown that IL-23-activated Th17 cells can accumulate in germinal centres of lymphoid organs, where they secrete IL-21 and IL-22. This can cause downregulation of sialyltransferase expression, diminishing the presence of sialic acid on IgG antibody Fc regions[13 52]. As described previously, the lack of sialylation in the Fc region is considered a marker for a pro-inflammatory immune response. In RA, the level of IL-23 is increased and it has been shown that Il-23 deficient mice display a decreased activity of IL-17 and decreased levels of IL-17 and IL-22 in joint regions[53]. As of now, there are several hypotheses that attempt to explain how ACPA production is initiated, while others focus more on the steps that follow after this, which in itself might lead to treatment options.

Discussion/Conclusion

Findings and implications

In this thesis I discussed our current knowledge on the mechanisms and factors involved in antibody glycosylation, how glycosylation impacts antibody function, and how changes in antibody glycosylation might contribute to the pathogenic potential of ACPAs as prominent autoantibodies in RA.
In RA, the presence of anti-citrullinated protein antibodies, termed ACPAs, have been shown in 60-70% of patients[4 13]. Citrullination can occur under the influence of environmental factors[8 11 12] and such post translational modifications may trigger a break of tolerance to self-antigens and lead to autoimmune disease such as RA. This does not mean, however, that the mere presence of ACPAs is sufficient to develop RA, since it has been shown that ACPAs can be present for a long time before the first symptoms occur[5 15 24 25]. Different effects of ACPAs in joint regions have been shown[17], but what triggers ACPA production in the first place is largely unknown[13].

Much attention has been given to the potential role of glycosylation of IgG ACPAs in RA, which is the addition of N-glycans. The hypothesis is that glycosylation plays a role in antibody affinity and avidity for Fc receptors and self-antigens[16 17 27], depending on the glycan composition present on antibody Fc regions and Fab regions respectively[4 17 24 29]. Multiple variants of antibody glycosylation have been described and the glycosylation patterns differ between the different antibody domains. Three types of residues are described that appear to have the greatest effect on antibody function[17]. These residues are fucose, galactose and sialic acid.

In the Fc region of antibodies, it has been shown that N-glycans can reside at the constant CH2 domains, attached to the nitrogen of asparagine (ASN)[4 17 24 28 30 31]. Here, it has been demonstrated that the addition of a galactose and sialic acid residue at these sites reduces antibody effector functions and has an anti-inflammatory effect[1 13 36]. One hypothesis regarding this effect is that sialylated antibodies display a lower affinity for FcγRs of inflammatory effector cells. Galactosylation appears to be a necessary process preceding sialylation, since sialic acids bind to the galactose residues at the N-terminus of the glycan[13]. However, little is still known about the effects of galactosylation on its own and it would be of interest to study this further. These studies should also take into account the difference between the addition of either one or two galactose residues, termed the G1F or G2F glycoform respectively, since these might affect the antibody's antigen-binding site differently.

Fc regions of ACPAs harbour the G0F glycoform, which comprises fucosylation in combination with the lack of both galactose and sialic acid. ACPA Fc glycosylation is believed to be associated with pro-inflammatory effector functions[26]. This contrasts with the anti-inflammatory effect of the addition of galactosylation and sialylation. It appears therefore that the antibody's affinity for certain Fc receptors of inflammatory effector cells might increase due to conversion to the G0F glycoform. Since glycosylation of the Fc region is a common occurrence in antibodies, this is not a distinctive feature of ACPAs in RA. The main difference with other antibodies is that ACPAs have extensive variable Fab domains glycosylation.
In the Fab region, glycosylation can occur at the VH and VL domains of the Fab heavy and light chains respectively[4 17 24 29]. In recent years, more and more importance has been ascribed to the glycosylation of variable domains. It is important to examine how the structure of this region can be influenced, since this might affect the affinity and avidity of the antigen-binding sites. On Fab glycans in ACPAs, it has been shown that a high number of galactose residues and sialic acid is present. This

contrasts the glycosylation profile of Fc domains, where these types of glycans are lacking, possibly promoting inflammation[1 13 26 31 36]. Research should elaborate on how these different glycan compositions in Fab and Fc regions affect antigen binding and immune effector function to citrullinated proteins respectively.

It has not yet been established whether there is a difference between heavy and light chain glycosylation of the variable antibody region in ACPAs. As mentioned previously, there is evidence of a distinction between the extent of glycosylation in constant and variable regions[4 16 17 24 27 29]. However, this does not take into account possible differences between heavy and light chain glycosylation within variable domains. Future studies might address this and investigate whether differences in glycosylation patterns exist between the heavy and light chains of the variable regions of ACPAs and whether this impacts their function

It has been shown that specific HLA class II haplotypes constitute a risk factor for developing RA[12 13 38]. These alleles code for the MHCII receptors that present antigenic peptides. This might be an indication of an alteration in antigen recognition, resulting in self-antigen targeting. It appears that the first step towards eventual RA is fuelled by the appearance of self-reactive B cells specific for citrullinated proteins that can escape negative selection. A question to be answered is therefore why B cells producing ACPAs can do this. A possibility is that they are not eradicated because of the presence of Fab glycans, masking their self-reactivity[13 55].
Several studies offer another hypothesis that tries to explain how certain B cells suddenly come to recognize self- antigens[41-43]. The idea is that foreign antigens exist that are similar to citrullinated self-proteins. Upon encountering these antigens, the B cell would then be activated and produce antibodies. After eradication of the foreign antigens, the B cell might not be able to make a distinction between the original foreign antigen to which it reacted and citrullinated self-proteins, leading to more ACPA production and autoimmunity. This hypothesis does not take into account, however, why ACPAs present before the onset of disease do not attack citrullinated proteins and why they can be silent for a long time before glycosylation drives initiation of RA. A suggestion is that ACPAs might not be pathogenic in their initial form, but rather become pathogenic with glycosylation.

Other thoughts on the initial production of ACPAs are that T cells play a role in B cell stimulation at the point of mutual antigen recognition. Here, the HLA haplotype might play a role, as this process is driven by MHCII recognition and presentation. Also, T cells that are autoreactive for citrullinated self-proteins themselves might contribute to the inflammatory process in RA.
Besides the role of T cells, there also seems to be an increase of IL-23 levels, produced by dendritic cells, which leads to the proliferation of Th17 cells. These Th17 cells then initiate the inflammatory response in RA[53]. IL-23-activated Th17 cells have been shown to accumulate in germinal centres of lymphoid organs. There, they secrete IL-21 and IL-22, which is believed to cause downregulation of sialyltransferase expression, diminishing the presence of sialic acid on IgG antibody Fc regions[13 52]. What lies at the root of the IL-23 increase and whether it also affects IgG antibody Fab glycosylation is not yet known.

Possible therapeutic options

In recent years, our knowledge on antibody glycosylation in general and ACPAs in particular has increased considerably, potentially offering new targets for treatment.
One option is to target the source of the autoantibodies, that is the (ACPA producing) B cell. It has been discovered that BAFF and IL-17 are involved in promoting proliferation, differentiation and survival of B cells that produce ACPAs. Normally, ACPA producing B cells should not be present, but if they are, this implicates a break of tolerance. For this reason, BAFF inhibitors and inflammatory cytokine inhibitors are tested for clinically[49 51]. These treatments do not always seem to work, however, and resistance to therapy is a risk factor here, as is the case for the TNF inhibitor infliximab for instance[17 18]. Another idea is to focus on the process of glycosylation of ACPAs. Because glycosylation patterns can be so diverse and can differ in glycoform as well as in site localization, it is certainly no easy task to think of treatment options regarding this[27]. There are, however, some suggestions put forward in studies that can give some direction to this search. In ACPA Fc regions, the IgG G0F glycoform could be targeted and maybe the processes of galactosylation and sialylation can be triggered, reducing the inflammatory response. In the ACPA Fab regions extensive sialylation can be detected which appears to be an ACPA-exclusive trait. When targeted, the glycans might be removed using enzymes that promote deglycosylation[17]. In any case, they might be used as a marker for detecting ACPAs, so the antibodies could be completely immobilized, and deactivated, or removed all together. Also, citrullinated self-proteins could be targeted by antibodies that might hinder the attachment of ACPAs, so these proteins will not be affected and tissue damage might be averted.

Another option regarding antibody glycosylation profiles is that glycan groups might be extracted or engineered, so they can be used to remodify ACPAs[54]. Since there has been a suggestion for N-glycosidic sites emerging in ACPAs during somatic hypermutation of B cells, this might be a process where different glycan groups with a higher affinity for these sites can be added which do not promote inflammatory responses. This would, however, require a lot more knowledge on the glycosylation process. In this respect, it should be determined precisely when glycosylation occurs, making a distinction between Fc and Fab regions, and when these glycosylation patterns might be subject to change.

The glycosylation of ACPAs in RA is studied extensively and the current believe is that this is fundamentally involved in disease progression. Recent studies provide new evidence on the subject and our knowledge on the processes and factors involved is expanding fast. There is, however, a lot still to be learned before the process of antibody glycosylation can be considered a target for (therapeutic) modulation. Future studies can widen our understanding of the precise mechanisms underlying ACPA glycosylation in RA and bring us one step closer to new therapeutic approaches.

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