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Glycosylation status of serum in inflammatory arthritis in response to anti-TNF treatment

Emily S. Collins¹, Marie C. Galligan²,³, Radka Saldeva³, Barbara Adamczyk³, Jodie L. Abrahams³, Matthew P. Campbell³, Chin-Teck Ng¹,⁴, Douglas J. Veale¹, Thomas B. Murphy², Pauline M. Rudd³ and Oliver FitzGerald¹

Abstract

Objective. Glycosylation is the most common post-translational modification and is altered in disease. The typical glycosylation change in patients with inflammatory arthritis (IA) is a decrease in galactosylation levels on IgG. The aim of this study is to evaluate the effect of anti-TNF therapy on whole serum glycosylation from IA patients and determine whether these alterations in the glycome change upon treatment of the disease.

Methods. Serum samples were collected from 54 IA patients before treatment and at 1 and 12 months after commencing anti-TNF therapy. N-linked glycans from whole serum samples were analysed using a high-throughput hydrophilic interaction liquid chromatography-based method.

Results. Glycosylation on the serum proteins of IA patients changed significantly with anti-TNF treatment. We observed an increase in galactosylated glycans from IgG, also an increase in core-fucosylated biantennary galactosylated glycans and a decrease in sialylated triantennary glycans with and without outer arm fucose. This increase in galactosylated IgG glycans suggests a reversing of the N-glycome towards normal healthy profiles. These changes are strongly correlated with decreasing CRP, suggesting a link between glycosylation changes and decreases in inflammatory processes.

Conclusion. Glycosylation changes in the serum of IA patients on anti-TNF therapy are strongly associated with a decrease in inflammatory processes and reflect the effect of anti-TNF on the immune system.

Key words: rheumatoid arthritis, psoriatic arthritis, inflammatory arthritis, N-glycosylation, galactosylation, sialylation, anti-TNF therapy, inflammation, CRP, IgG-GO.

Introduction

Protein glycosylation is the most abundant post-translational modification and it is estimated that >70% of human proteins are glycosylated [1]. Changes in serum glycosylation are early indicators of cellular changes in many diseases, including the inflammatory arthropathies (IA), and can provide useful diagnostic markers and insights into disease progression and pathogenesis [2]. Many glycans are involved in molecular recognition and specificity processes and have been shown to play important roles in the immune system including their involvement in leukocyte trafficking and T cell differentiation and receptor activation [3–7]. Modification of glycan structures can disrupt these sterically modulated interactions such as those between glycoproteins and lectins and cause disease-relevant changes, e.g. in signalling processes. Glycosylation changes have been reported for several proteins isolated from the sera of IA patients, including IgG, transferrin, haptoglobin, α1-acid glycoprotein and α2-macroglobulin, and have the potential to be important for the discovery of new biomarkers and/or new therapies [8]. Research into the changes in IgG...
glycosylation associated with rheumatic disease has extensively reported both a reduction in the subset of galactosylated IgG glycoforms (G1 and G2), often quantified as an increase in the percentage of agalactosylated (G0) IgG and also decreased galactosylation and sialylation in patients with RA compared with healthy individuals [8–10].

Population studies have shown that glycosylation on serum glycoproteins is age- and gender-specific, but is also affected by many environmental and biochemical factors such as diet, smoking, CRP or cholesterol levels [11]. IgG glycosylation can also vary with age (there is a significant decrease in galactosylation and increase in bi- and tri-glycosylation) [12–14], pregnancy (decrease of G0 glycoform) [15] and rheumatic disease, particularly with respect to the level of galactosylation in RA and PsA [16]. IgG-%G0 in RA correlates with disease activity and number of erosions, an indicator of disease progression, and it returns to the levels seen in healthy controls in remission [9, 17]. Decreased galactosylation of IgG in RA was correlated with markers of inflammation such as IL-6 and CRP [18]. However, to date, there have been limited efforts to screen this glycoform population directly from whole patient serum.

Some of the most efficacious therapies for the inflammatory arthropathies RA and PsA include the biologic anti-TNF agents adalimumab (a fully humanized monoclonal antibody), infliximab (a chimeric human/murine monoclonal antibody) and etanercept, a fusion protein of the ligand-binding portion of the p75 TNF receptor (TNFRII) and the Fc fragment of human IgG1 [19]. Anti-TNF agents bind to and neutralize soluble TNF but exert different effects on transmembrane TNF-expressing cells [20]. Etanercept prevents the interaction of soluble and membrane-bound TNF with TNF cell surface receptors by forming a 1:1 complex with the TNF trimer, occupying two of three potential receptor binding sites [21]. Recent studies have examined the effects of infliximab on IgG glycosylation status in CIA and also in RA and SpA patients [22, 23]. The effect of MTX [24, 25], in combination with infliximab (a chimeric antibody anti-TNF) [24] and anti-TNF therapy (with etanercept, infliximab and adalimumab) [25], have also been studied in RA patients. These studies have found that the levels of IgG-G0 decrease with anti-TNF therapy [22–24]. As of yet, we have found no study that has examined the effects of etanercept or adalimumab on whole serum glycosylation status or the effects of anti-TNF therapies on PsA specifically (as opposed to generalized SpA).

We have previously developed a highly sensitive, high-throughput and quantitative hydrophilic interaction liquid chromatography (HILIC)-based analysis of the serum N-linked glycome [26]. This method allows investigation of the ratio G0/G1 from IgG glycans and the whole serum N-glycome to identify changes in glycosylation with anti-TNF treatment in RA and PsA patients. Royle et al. [26] first used it in 15 RA patients and 1 control and obtained good correlation ($R^2 = 0.83$) between %G0 from isolated IgG heavy chains and from the whole sera. An advantage of this over other methods is that it requires very small amounts (5 µl) of serum, does not require any pre-purification and allows a large number of samples to be measured in only a few days. Hence, it is eminently scalable for use in a clinical setting.

The objective of this study is to evaluate the effect of anti-TNF therapy on whole serum glycosylation in RA and PsA patients. These patients were commenced on anti-TNF therapies and were prospectively followed over 1 year. We also determine whether the alterations in the N-glycome of these patients reverse upon the treatment.

**Materials and methods**

**Patient samples**

Fasting blood samples were allowed to clot for up to 1 h and were then centrifuged at 2000 rpm for 10 min at room temperature. Samples were frozen at −80°C within 2 h of being taken. The patient cohort comprised 54 IA patients. Twenty-nine patients had RA and 25 PsA (supplementary Table S1, available at Rheumatology Online). All patients fulfilled either ACR (RA) or Classification Criteria for Psoriatic Arthritis (PsA) classification criteria. All patients were deemed to have active disease by their treating physician requiring the introduction of an anti-TNF therapy and all were previously naive to anti-TNF. Samples were collected with fully informed written consent according to the declaration of Helsinki following approval by the St Vincent’s Healthcare Group Ethics and Medical Research Committee. Patients were prescribed either of two anti-TNF therapies at the discretion of the treating physician, adalimumab (23 patients, 16 RA and 7 PsA) or etanercept (31 patients, 13 RA and 18 PsA). Two-thirds of patients were also taking concomitant DMARDs, with MTX use accounting for 90% of those. 72% of RA patients were RF positive (RF values were not available for three patients). Serum samples were collected at baseline, 1 month and 12 months after the commencement of therapy and the clinical progress of the patients measured every 3 months by collecting data including TJC, SJC, Global Health VAS, CRP, ESR, HAQ, stiffness (mins), pain scores and fatigue scores from which DAS28-CRP (hereafter referred to as DAS) was calculated. EULAR response criteria were applied to characterize patients into good responders at 1 year, as defined by a reduction in DAS of >1.2 and a final DAS of <3.2. All other patients were deemed inadequate responders. Disease characteristics across RA patients and PsA patients were generally very similar, including average disease duration, baseline DAS and 1 year DAS. There was a significant difference in the average age of patients.

**High-throughput N-glycan release and fluorescent labelling**

5 µl aliquots of each sample were reduced and alkylated before being set into SDS-PAGE gel blocks, washed and digested with PNGase F (Prozyme, San Leandro, CA, USA) for 16 h as previously described by Royle et al. [26]. The eluted glycans were labelled with 2-AB using...
Exoglycosidase digestion of 2-AB-labelled N-linked glycans

The 2-AB-labelled glycans were digested in 10 μl of 50 mM sodium acetate buffer, pH 5.5 for 18 h at 37°C, using enzymes of Arthrobacter ureafaciens sialidase (ABS, EC 3.2.1.18) in 1 U/ml and Streptococcus pneumoniae β-galactosidase (SPG, EC 3.2.1.23) in 0.1 U/ml (Prozyme, San Leandro, CA, USA). After incubation, enzymes were removed by filtration through a protein-binding Pall Nanosep 10 kDa filter (Pall Corporation, USA) [1] and the N-glycans were then analysed by HILIC.

Longitudinal data models

Linear mixed-effects models, as outlined by Pinheiro and Bates [28], were fitted to each of the transformed glycan peaks to evaluate its change over time. Multiple testing error was corrected for using the FDR approach [29]. In these models, we control for time, age, disease type, disease duration, gender, treatment type, log CRP, DAS (without CRP), responder and ESR. Interaction effects with time were also included (expanded details are available in supplementary data, longitudinal data models section, available at Rheumatology Online). The P-values provided are those from models re-fitted to include statistically significant terms only and were calculated using likelihood ratio tests.

Results

The N-glycans in serum samples from 29 RA and 25 PsA patients were analysed by HILIC and structural assignments were made using the software tool GlycoBase (glycobase.nibrt.ie) [30] and Royle et al. [26].

Undigested N-glycans were separated into 14 peaks (Fig. 1). The FG0/FG1 ratio (from the IgG N-glycome, contributing to the total serum N-glycome) of each sample was measured as the relative percentage areas of peak 1 (FG0) to peak 3 (FG1). These peaks contain the following IgG glycans: core-fucosylated, non-galactosylated glycans (Peak 1) and core-fucosylated, mono-galactosylated glycans (Peak 3), as shown in Fig. 1.

N-Glycans were then digested with ABS and SPG and separated into 14 peaks (Fig. 2). Total SLe6 levels were calculated based on peaks from this profile: SLe6 on tri- and tetraantennary glycans was measured as the relative percentage area of peaks 7 + 9 + 12 and total SLe6 on bi-, tri- and tetraantennary glycans (total) was measured as the relative percentage area of peaks 6 + 7 + 9 + 12. These features were measured and modelled separately, as peaks containing SLe6 on tri- and tetraantennary glycans contain only SLe6 glycans (A3F1G1, A4F1G1 and A4F2G2), whereas approximately half of the peak with biantennary glycans (A2F1G1) also contains high mannosylated glycan M6. All results were adjusted for age, gender and CRP where a significant relationship with the glycan peaks was observed.

Changes of glycosylation status with anti-TNF treatment

Glycosylation in all patients was measured at baseline and after both 1 month and 1 year of anti-TNF treatment. For some patients, serum samples were not available at all time points (n = 52 at baseline and at 1 month and n = 42 at 1 year). Logistic regression models were used to assess whether baseline glycosylation levels contain predictive information about whether patients have RA or PsA. Age and log CRP were controlled for in these models, as these covariates were found to be statistically significant. (The mean age of the RA patients was 57, while that of the PsA patients was 46.) The predictive ability of the undigested glycan profiles, the ABS digested profiles and the G0/G1 ratio was assessed and no significant effect was observed. Therefore, we conclude that there was no significant evidence from this study to indicate that glycosylation status could be useful for differentiation between RA and PsA.

Furthermore, from the linear mixed-effects models that were fitted to assess the changes in glycosylation over the course of treatment, an interaction effect between time and disease was included. This interaction was used to determine whether the change in glycosylation over the course of treatment differed between patients with RA and PsA. The interaction was not significant in any of the fitted models. Therefore, the observed data does not provide evidence that glycosylation levels changed.

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differently with anti-TNF treatment for patients with RA and PsA.

From the undigested profiles, peaks 2 (M5, FA2B, A2G1), 5 (FA2G2, FA2BG2, FA2G1S1) and 11 (FA2G2S2, FA2BG2S2) significantly increased and peaks 12 (A3G3S2, A3BG3S2, A2F1G2S2), 13 (A3G3S3) and 14 (A3F1G3S3) significantly decreased (Table 1) over the course of treatment. These changes indicate an increase in core-fucosylated biantennary galactosylated glycans and a decrease in sialylated triantennary glycans with and without outer arm fucose.

The FG0/FG1 ratio showed a decreasing trend over the course of treatment with anti-TNF, as shown in Table 1. For the entire cohort of patients, regardless of their level of response to therapy, the FG0/FG1 ratio levels decreased significantly \( (P < 0.01) \) with median levels (0.93, 0.85, 0.73).

From the ABS + SPG digestions, peak 4 (M5, FA2B, FA3, A3B) significantly increased and peak 7 (A3F1G1) significantly decreased over the course of treatment, which is consistent with the changes observed in undisegested peaks 2 and 14 (Table 1). The finding of an increase in peak 4 is not inconsistent with a decrease in peak 7 and the undisegested peaks 12, 13 and 14. Peak 4 is a mixture of triantennary, biantennary and high mannosyl glycans while peaks 7, 12, 13 and 14 all contain tri-antennary glycans.

For total SLe\(^x\) (A2F1G1, A3F1G1, A4F1G1, A4F2G2) and on tri- and tetraantennary glycans (A3F1G1, A4F1G1, A4F2G2), relative amounts decreased significantly over the course of treatment (Table 1). Figure 3 shows boxplots of the percentage changes observed in these significantly altered glycan peaks after both 1 month and 1 year of treatment.

The glycosylation profiles were strongly dependent on CRP levels in general and thus CRP was controlled for in all models. Peaks 2 (M5, FA2B, A2G1), 5 (FA2G2, FA2BG2, FA2G1S1) and 11 (FA2G2S2, FA2BG2S2) significantly increased and peaks 12 (A3G3S2, A3BG3S2, A2F1G2S2), 13 (A3G3S3) and 14 (A3F1G3S3) significantly decreased (Table 1) over the course of treatment. These changes indicate an increase in core-fucosylated biantennary galactosylated glycans and a decrease in sialylated triantennary glycans with and without outer arm fucose.

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The glycosylation profiles were strongly dependent on CRP levels in general and thus CRP was controlled for in all models. Peaks 2 (M5, FA2B, A2G1) and 5 (FA2G2, FA2BG2, FA2G1S1) from the undisegested profiles and peak 4 (M5, FA2B, FA3, A3B) from the digested profiles decreased significantly with CRP \( (P < 0.01) \), whereas peak 14 (A3F1G3S3) from the undisegested profiles, the FG0/FG1 ratio, peak 7 from the digested profiles (A3F1G1) and SLex increased significantly with CRP levels (Table 1). In each of these cases, log CRP was highly significant, with \( P < 0.01 \). These results indicate a decrease in core-fucosylated biantennary galactosylated glycans and increases in agalactosylated glycans and glycans with outer arm fucose (SLe\(^x\)) with increasing CRP levels. As would be expected, levels of CRP decrease over the course of treatment and the changes in glycosylation appear to be concomitant with this. Interestingly, undisegested peaks 11 (FA2G2S2, FA2BG2S2), 12 (A3G3S2, A3BG3S2, A2F1G2S2) and 13 (A3G3S3) had no significant relationship with CRP, though peak 11 had a decreasing relationship and peaks 12 and 13 an increasing relationship with CRP (Table 1).

Patients were divided into responders or non-responders using the EULAR criteria: responders \((n = 39)\).
included all those with a good response; non-responders ($n = 15$) included all other patients with either moderate or poor responses. We did not observe a statistically significant difference in the change in glycosylation over treatment comparing responders vs non-responders. However, Fig. 4 shows that in general, the changes in glycosylation were less marked in non-responders. These differences may be driven by changes in CRP since many of the peaks were correlated with CRP levels. We found a significant difference in the CRP levels between responders and non-responders ($P < 0.01$). However, the change in CRP levels after 1 year of treatment was not significantly different for responders and non-responders ($P \geq 0.94$). This could be explained by the fact that only one patient showed an increase in CRP level after 1 year of treatment.

Correlation of DAS score, CRP and glycosylation

CRP is a major component of the disease activity score (DAS). We evaluated whether glycosylation correlated with the DAS score to determine whether glycosylation could be used to monitor disease activity. Table 2 reports Pearson's correlation coefficients for glycosylation with log CRP and with DAS (without the CRP component) at baseline and after 1 year of treatment.

We found that that CRP was significantly correlated with DAS (without CRP) both at baseline ($r = 0.326$) and after 1 year of treatment ($r = 0.509$). Several glycan peaks were strongly correlated with log CRP at both time points. At baseline, no significant correlations between glycosylation and DAS (without CRP) were observed. However, after 1 year of treatment, a small number of glycan peaks had strong correlations with DAS (without CRP). Interestingly, this includes the FG0/FG1 ratio ($r = 0.466$). The DAS (without CRP) was more strongly correlated with CRP than with any of the glycan peaks. In general, the glycan peaks had a stronger correlation with CRP than with DAS (without CRP).

Discussion

It is clear from our results that anti-TNF treatment causes a decrease in FG0/FG1 ratio (indicating increased IgG galactosylation) in both RA and PsA patients. This increase in IgG galactosylation is in agreement with previous studies, where RA patients were treated with infliximab [22, 23], MTX or MTX in combination with remicade [24].

The exact mechanism of the fall in FG0/FG1 is unclear as it is not currently known precisely how TNF blockers affect IgG G0 concentration. Indeed, the exact mode of action of the various different anti-TNF therapies is not fully determined. IgG is secreted by B cells, which are also the site of the GTase activity responsible for IgG galactosylation [31]. The increased agalactosylation of IgG in IA is thought to be due to lowered galactosyltransferase...
<table>
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<tr>
<th>Peak ID</th>
<th>Predominant N-glycans</th>
<th>First quartile, median, third quartile, % glycans&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Effect of treatment on glycosylation (over time)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Effect of log CRP on glycosylation (model covariate)&lt;sup&gt;c&lt;/sup&gt;</th>
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<td></td>
<td></td>
<td>Baseline</td>
<td>After 1 month</td>
<td>After 12 months</td>
</tr>
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<td>Undigested profile</td>
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</tr>
<tr>
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<td>8.64, 9.59, 10.78</td>
<td>9.03, 10.12, 11.46</td>
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<tr>
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<td>P13</td>
<td>A3G3S3</td>
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<td>4.99, 5.57, 6.51</td>
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<tr>
<td>P14</td>
<td>A3F1G3S3</td>
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<td>6.46, 7.58, 9.63</td>
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<td>FG0/FG1 = log (P1/P3)</td>
<td>FA2/(FA2G1, FA2BG1)</td>
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<td>-38.82, -16.53, 3.14</td>
<td>-44.13, -31.63, -11.33</td>
</tr>
</tbody>
</table>

ABS+BTG digested profile | | | | | | | | |
| P4 | M5, FA2B, FA3, A3B | 8.34, 9.36, 10.32 | 8.80, 9.98, 11.24 | 9.58, 10.53, 11.54 | 0.0059 | <0.01 | -0.0387 | <0.01 |
| P7 | A3F1G1 | 4.50, 5.56, 7.05 | 3.76, 4.80, 6.32 | 3.55, 4.53, 5.50 | -0.0084 | 0.01 | 0.1075 | <0.01 |
| SLex on tri- and tetraantennary | A3F1G1, A4F1G1, A4F2G2 | 5.99, 6.88, 8.72 | 4.88, 6.20, 8.0 | 4.62, 5.81, 6.90 | -0.0051 | 0.01 | 0.0989 | <0.01 |
| SLex on bi-, tri- and tetraantennary (total) | A2F1G1, A3F1G1, A4F1G1, A4F2G2 | 8.24, 9.45, 11.56 | 7.37, 8.74, 11.1 | 7.20, 8.45, 9.65 | -0.0075 | 0.01 | 0.0659 | <0.01 |

<sup>a</sup>For detailed N-glycan composition of human serum, see Royle et al. 2008 [26]. |<sup>b</sup>The median (middle number) provides a measure of centrality, while the first and third quartiles provide measures of variability above and below the median, respectively. |<sup>c</sup>Structure abbreviations: all N-glycans have two core GlcNAcs; F at the start of the abbreviation indicates a core- fucose α1,6-linked to the inner GlcNAc; Mx: number (x) of mannose on core GlcNAcs; Ax: number of antenna (GlcNAc) on trimannosyl core; A2: biantennary with both GlcNAcs as β1,2-linked; A3: triantennary with a GlcNAc linked β1,2 to both mannoses and the third GlcNAc linked β1,4 to the α1,3 linked mannose; A4: GlcNAcs linked as A3 with additional GlcNAc β1,6 linked to α1,6 mannose; B: bisecting GlcNAc linked β1,4 to α1,3 linked mannose; Gx: number (x) of β1,4 linked galactose on antenna; F(x): number (x) of fucose linked α1,3 to antenna GlcNAc; Sx: number (x) of sialic acids linked to galactose. |<sup>d</sup>Positive coefficient for time indicates that the peak increases, whereas negative coefficient indicates that the peak decreases over time. |<sup>e</sup>All P-values were adjusted for age, gender and CRP where significant interaction occurred.
(GTase) activity and reduced B lymphocyte GTase activity has been demonstrated in RA patients [32–35]. Previously, sulphasalazine has been shown to affect the G0 concentration in RA patients by reversing this reduction in lymphocytic GTase activity [36]. TNF has been reported to have a regulatory role in B cell maturation and differentiation [37] and it has also been shown that TNF is able to affect GTase populations in bovine synoviocytes [38]. However, further study will be required to elucidate how TNF is able to affect GTase activity in B cells and exactly how this change leads to an altered G0 concentration.

We found no significant difference in glycosylation changes following treatment between responders and non-responders. However, changes in glycosylation were less marked in non-responders, which may be driven by changes in CRP since many of the peaks were correlated with CRP levels. This is in agreement with a previous study where N-glycan hypogalactosylation (G0/G1) did not distinguish RA patients as more likely to experience a favourable clinical response to MTX or anti-TNF therapy [25]. However, we found a correlation between DAS (without CRP) and FG0/FG1 at 1 year. This suggests that although FG0/FG1 does not seem to have any discriminative or predictive power at baseline, it could potentially be useful for distinguishing between active disease and remission. This is in agreement with Ercan et al. [25] who found significant correlation between the change in DAS and change in G0/G1 ratio over 3 months in two larger cohorts of solely RA patients. The failure to discriminate between responders and non-responders may relate to the relatively small numbers of patients studied, in particular the small number of non-responding patients. Alternatively, it may be that the altered glycosylation in serum is not a direct effect of TNF inhibition in patients experiencing clinical improvement as no differences between responders and non-responders were found earlier in larger cohorts of patients [25].
The HILIC methodology has the capability of relatively inexpensive high-throughput measurement of serum glycosylation, and while we were unable to predict response, the relationship between the various peaks and CRP suggests that this methodology could potentially become useful for a clinical setting, perhaps upon further resolution of the peaks to individual glycans or by including glycan peak values in a revised DAS score. Changes in the measured values of individual peaks may result from a change in the amount of glycosylation of individual proteins, a change in the relative abundance of those glycoproteins or availability of sugar nucleotide donors. All these causes of change are relevant to disease pathogenesis and none of these causes affect the suitability of glycan peak measurement for clinical use.

In contrast to other studies, we have examined glycosylation from total serum glycoproteins. Apart from the increased galactosylation on IgG, we have also observed an increase in core-fucosylated biantennary galactosylated glycans and a decrease in sialylated triantennary glycans with and without outer arm fucose on other serum glycoproteins in IA patients on anti-TNF treatment.

Some of these glycosylation features have been found to be altered in RA on acute phase proteins such as an increase in SLex and overall sialylation on AGP [39], an increase in highly branched glycans on transferrin [40] and an increase in fucosylated haptoglobin [41] compared with healthy controls. Fucosylated haptoglobin refers to outer arm fucosylation, as most of the fucose on haptoglobin is α1-3 linked, forming the SLex epitope [42].

An increase in sialylation, branching and SLex epitopes is connected to chronic inflammation, which accompanies RA [43]. Our results in both RA and PsA patients show a decrease in these glycan features implying a decrease in the inflammatory processes. Indeed, pro-inflammatory cytokine TNF signals activation of the NFκB pathway which is involved in autoimmune inflammatory response [44]. With treatment using anti-TNF antibodies, this pathway is inhibited, the chronic inflammation is...
suppressed and therefore inflammatory processes are decreased.

### Rheumatology key messages

- Glycosylation in the serum of IA patients on anti-TNF therapy is strongly associated with inflammation.
- Changes in patient serum N-glycome reflect the effect of anti-TNF on the immune system.

### Acknowledgements

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### TABLE 2 Correlation of DAS with CRP and glycosylation

<table>
<thead>
<tr>
<th>peak ID/correlation with</th>
<th>DAS (without CRP)</th>
<th>log(CRP)</th>
<th>DAS (without CRP)</th>
<th>log(CRP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS (without CRP)</td>
<td>—</td>
<td>0.326*</td>
<td>—</td>
<td>0.509**</td>
</tr>
<tr>
<td>Log(CRP)</td>
<td>0.326*</td>
<td>—</td>
<td>0.509**</td>
<td>—</td>
</tr>
<tr>
<td>Undigested peaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Logit peak 1</td>
<td>0.076</td>
<td>0.373**</td>
<td>0.258</td>
<td>0.492**</td>
</tr>
<tr>
<td>Logit peak 2</td>
<td>0.08</td>
<td>0.017</td>
<td>0.079</td>
<td>0.131</td>
</tr>
<tr>
<td>Logit peak 3</td>
<td>-0.148</td>
<td>-0.362**</td>
<td>-0.321*</td>
<td>-0.173</td>
</tr>
<tr>
<td>Logit peak 4</td>
<td>0.004</td>
<td>-0.39**</td>
<td>-0.297</td>
<td>-0.323*</td>
</tr>
<tr>
<td>Logit peak 5</td>
<td>-0.092</td>
<td>-0.489**</td>
<td>-0.493**</td>
<td>-0.364*</td>
</tr>
<tr>
<td>Logit peak 6</td>
<td>-0.009</td>
<td>-0.228</td>
<td>0.189</td>
<td>0.153</td>
</tr>
<tr>
<td>Logit peak 7</td>
<td>0.139</td>
<td>-0.093</td>
<td>-0.126</td>
<td>-0.081</td>
</tr>
<tr>
<td>Logit peak 8</td>
<td>-0.212</td>
<td>-0.637**</td>
<td>-0.311</td>
<td>-0.642**</td>
</tr>
<tr>
<td>Logit peak 9</td>
<td>0.059</td>
<td>-0.068</td>
<td>-0.112</td>
<td>0.016</td>
</tr>
<tr>
<td>Logit peak 10</td>
<td>-0.073</td>
<td>0.109</td>
<td>0.019</td>
<td>-0.169</td>
</tr>
<tr>
<td>Logit peak 11</td>
<td>0.092</td>
<td>-0.154</td>
<td>-0.022</td>
<td>0.184</td>
</tr>
<tr>
<td>Logit peak 12</td>
<td>-0.001</td>
<td>-0.113</td>
<td>0.334*</td>
<td>0.086</td>
</tr>
<tr>
<td>Logit peak 13</td>
<td>-0.065</td>
<td>-0.195</td>
<td>0.167</td>
<td>-0.211</td>
</tr>
<tr>
<td>Logit peak 14</td>
<td>0.193</td>
<td>0.562**</td>
<td>0.046</td>
<td>0.387*</td>
</tr>
<tr>
<td>log(FG0/FG1) = log(P1/P3)</td>
<td>0.177</td>
<td>0.628**</td>
<td>0.466**</td>
<td>0.611**</td>
</tr>
<tr>
<td>ABS + BTG digested peaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Logit ABS 1</td>
<td>0.082</td>
<td>0.056</td>
<td>-0.413**</td>
<td>-0.23</td>
</tr>
<tr>
<td>Logit ABS 2</td>
<td>-0.207</td>
<td>-0.085</td>
<td>0.066</td>
<td>-0.345*</td>
</tr>
<tr>
<td>Logit ABS 3</td>
<td>0.009</td>
<td>-0.347*</td>
<td>-0.13</td>
<td>-0.057</td>
</tr>
<tr>
<td>Logit ABS 4</td>
<td>0.164</td>
<td>0.064</td>
<td>-0.029</td>
<td>0.326*</td>
</tr>
<tr>
<td>Logit ABS 5</td>
<td>0.068</td>
<td>-0.031</td>
<td>0.498**</td>
<td>0.228</td>
</tr>
<tr>
<td>Logit ABS 6</td>
<td>0.054</td>
<td>0.008</td>
<td>-0.246</td>
<td>-0.077</td>
</tr>
<tr>
<td>Logit ABS 7</td>
<td>0.13</td>
<td>0.425**</td>
<td>0.084</td>
<td>0.308</td>
</tr>
<tr>
<td>Logit ABS 8</td>
<td>0.155</td>
<td>0.401**</td>
<td>0.22</td>
<td>0.46**</td>
</tr>
<tr>
<td>Logit ABS 9</td>
<td>0.035</td>
<td>0.24</td>
<td>0.315</td>
<td>0.085</td>
</tr>
<tr>
<td>Logit ABS 10</td>
<td>-0.115</td>
<td>0.132</td>
<td>0.138</td>
<td>0.04</td>
</tr>
<tr>
<td>Logit ABS 11</td>
<td>0.161</td>
<td>-0.146</td>
<td>-0.345*</td>
<td>-0.234</td>
</tr>
<tr>
<td>Logit ABS 12</td>
<td>0.098</td>
<td>0.359**</td>
<td>0.067</td>
<td>0.238</td>
</tr>
<tr>
<td>Logit ABS 13</td>
<td>0.157</td>
<td>0.135</td>
<td>-0.126</td>
<td>-0.24</td>
</tr>
<tr>
<td>Logit ABS 14</td>
<td>-0.111</td>
<td>0.055</td>
<td>0.058</td>
<td>0.137</td>
</tr>
<tr>
<td>Logit ABS 15</td>
<td>0.011</td>
<td>0.248</td>
<td>0.202</td>
<td>0.301</td>
</tr>
<tr>
<td>Logit SLex on bi- , tri- and tetraantennary (total)</td>
<td>0.120</td>
<td>0.369**</td>
<td>0.043</td>
<td>0.237</td>
</tr>
<tr>
<td>Logit SLex on tri- and tetraantennary</td>
<td>0.124</td>
<td>0.418**</td>
<td>0.123</td>
<td>0.300</td>
</tr>
</tbody>
</table>

Correlation coefficients vary from -1 to 1. Values close to 1 imply a strong increasing linear relationship; values close to -1 imply a strong decreasing linear relationship. Values close to 0 imply no correlation. Table shows Pearson correlation coefficients for glycosylation with log CRP and DAS (without CRP), both at baseline and 1 year after anti-TNF treatment.

*Significant P-value < 0.05; **highly significant P-value < 0.01.
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Supplementary data

Supplementary data are available at Rheumatology Online.

References

17. Parekh RB, Roitt IM, Isenberg DA et al. Galactosylation of IgG associated oligosaccharides: reduction in patients with adult and juvenile onset rheumatoid arthritis and rela-
18. Troelsen LN, Jacobsen S, Abrahams JL et al. IgG glyco-
19. Moss ML, Sklar-Tavron L, Nudelman R. Drug insight: tumor necrosis factor-converting enzyme as a pharma-
25. Ercan A, Cui J, Hazen MM et al. Association of IgG glyco-
27. R-Development-Core-Team. R: A language and environ-
28. Pinheiro J, Bates D, DebRoy S et al. The R Core team. R: A language and environ-

www.rheumatology.oxfordjournals.org


