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

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CHAPTER 6

Interleukin-10 differentially modulates IgG Fc glycosylation in human B cells

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ABSTRACT

Immunoglobulin G (IgG) structure and function are greatly dependent on glycosylation of the Fc tail. However, the composition of these Fc glycans are altered in several physiological and pathological conditions, e.g. in rheumatoid arthritis. So far, it is incompletely understood how the glycosylation machinery of B cells is regulated, and whether and to what extent environmental factors relevant to the development/maturation of B and plasma cells can modulate the Fc-glycosylation of secreted IgG. As interleukin-10 (IL-10) is an important regulatory cytokine with immunosuppressive functions in autoimmunity and pregnancy, we here studied the effect of IL-10 on the Fc glycosylation of IgG1 spontaneously secreted by antibody secreting cells (ASC) and of IgG1 secreted by B cells (comprising the compartment of naïve and memory B cells depleted of ASC) upon stimulation. Single cell suspensions were prepared from human tonsils that were obtained from individuals undergoing tonsillectomy. Cells were isolated by fluorescence-activated cell sorting (FACS) based upon $CD3^{-}CD14^{-}CD19^{+}CD27^{-/+}CD38^{-/+}$ expression and incubated with recombinant IL-10. IgG concentrations were measured by ELISA and IgG Fc glycosylation patterns were analysed by mass spectrometry. Under the influence of IL-10, IgG1 derived from ASC showed reduced galactosylation and sialylation levels whereas bisecting GlcNAc levels were enhanced. In contrast, IgG1 derived from B cells depleted of ASC, i.e. from the compartment of naïve and memory B cells, displayed increased Fc-galactosylation and sialylation levels. These results indicate that IL-10 has different modulatory effects on IgG Fc-glycosylation depending on the B cell differentiation status and the presence of the stimulatory/local environment. As IgG Fc glycosylation has the ability to influence antibody effector functions, regulating IL-10 levels could be an interesting approach in the modulation of pathogenic immune responses.

INTRODUCTION

Antibody secreting cells (ASC), under which plasmablasts (PB) and plasma cells (PC), are differentiated B cells dedicated to secrete protective antibodies. Antibodies rely on glycans in the fragment crystallisable (Fc) tail for their stability and to exert Fc-mediated effector functions such as complement binding, antibody-dependent cellular cytotoxicity and Fc receptor binding [1, 2]. Immunoglobulin G (IgG) has two biantennary *N*-linked glycans at position Asn297 in the C_H2 region of the heavy chain [3]. These Fc glycans are commonly composed of N-acetylglucosamine (GlcNAc), core-fucose and mannose structures, and may vary in the amount of bisecting GlcNAc and galactose and sialic acid residues on the antennae [3]. As sialic acid can only be attached to galactose residues, the presence of galactose determines the possible sialic acid occupation. In general, the amount of galactose and sialic acid residues are considered indicative of an inflammatory "status" of IgG antibodies. Overall, the presence of agalactosylated antibodies is associated with increased inflammation, whereas galactosylated and sialylated IgG are thought to exert anti-inflammatory functions [4-6].

Changes in antibody Fc glycosylation are well known in both physiological and pathological conditions. During pregnancy, Fc galactosylation and sialylation levels increase, while after birth these levels decrease again [7, 8]. Also, vaccination-induced, antigen-specific IgG1 shows increased galactosylation and sialylation levels and decreased bisecting GlcNAc levels several days after vaccination, while total IgG Fc glycosylation patterns are not affected [9]. In rheumatoid arthritis (RA), a chronic autoimmune disease characterized by joint destruction and the presence of autoantibodies, anti-citrullinated protein antibodies (ACPA) were found to have altered Fc glycosylation profiles, in particular when isolated from the site of synovial inflammation [10]. Of note, such changes could first be noted in serum ACPA approximately three months before the onset of RA. At this time point, ACPA Fc galactosylation and sialylation levels declined while the degree of core-fucosylation increased [11]. These examples demonstrate that the Fc glycosylation of IgG antibodies can be altered under certain conditions, though it is incompletely understood how the glycosylation machinery is regulated and how antibody Fc-glycosylation can be fine-tuned such that particular biological effects can be obtained.

Based on the observations described, it is conceivable that micro-environmental factors including hormones or cytokines could play a role in the modulation of IgG Fc galactosylation. Indeed, dependent on the kind of stimuli received during the activation and differentiation of B cells, the IgG1 Fc glycosylation profile shifts towards a more pro- or anti-inflammatory profile [12-14]. Wang *et al.* showed by stimulating B cells in vitro

that CpG and IL-21 could enhance IgG1 galactosylation, whereas all-trans retinoic acid had a reducing effect on IgG1 galactosylation and sialylation levels [13]. However, these IgG1 Fc glycosylation profiles were derived from ASC that had been differentiated under the influence of several B cell stimulating cytokines including IL-2 and IL-10, which by themselves could already have affected glycosylation status. Hence, it is still not clear to what extent individual stimuli relevant to the development and maturation of B cells, or combinations thereof, can influence the Fc glycosylation of secreted IgG.

Interleukin 10 (IL-10) is an important regulatory cytokine with an immunosuppressive role in pregnancy and the ability to suppress inflammation [15-17]. For example, IL-10 was shown to suppress T cell proliferation and to prevent chronic arthritis development in an *in vivo* mouse model [18, 19]. Moreover, IL-10 is one of the cytokines closely involved in B cell survival, proliferation and differentiation into IgM- and IgG-secreting plasma blasts [20, 21]. As IL-10 has essential immunomodulatory functions, we hypothesized that it might influence the glycosylation status of antibodies secreted by B cells. To this end, we here investigated the *in vitro* effect of IL-10 on the Fc glycosylation of IgG1 spontaneously secreted by ASC and of IgG1 secreted by stimulated naïve/memory B cells that had been depleted of ASC.

MATERIALS AND METHODS

Tonsils

To obtain representative numbers of differentiated ASC, we isolated B cells from human tonsils as tonsils contain an increased amount of B cells compared to peripheral blood [22]. Tonsillar tissue was anonymously collected as rest material from individuals undergoing tonsillectomy at Leiden University Medical Center, The Netherlands. Tonsils were cut in small pieces and meshed over a 70 μm Falcon cell strainer to obtain a single-cell suspension. Cells were washed with serum-free X-VIVO 15 medium without gentamicin and phenol red (Lonza), as phenol red contains small traces of the hormone oestrogen, and put over a ficoll-paque gradient (LUMC pharmacy) to obtain the lymphocyte fraction. Erythrocytes in the lymphocyte fraction were lysed with lysis buffer (LUMC pharmacy) and tonsillar lymphocytes were washed and counted.

Flow cytometric cell sorting

Around $200x10^6$ tonsillar cells were stained for 30 minutes on ice with an antibody mix in PBS with 2% BSA (Sigma). The antibody mix included: CD3 pacific blue (clone UCHT1); CD14 pacific blue (clone M5E2); CD19 APC-Cy7 (clone SJ25C1); CD27 PE-Cy7 (clone M-T271); CD38 PerCP-Cy5.5 (clone HIT2); HLA-DR APC (clone L243), all BD bioscience.

$CD3^-CD14^-CD19^+CD27^{++}CD38^{++}$ antibody secreting cells (ASC) and $CD3^-CD14^-CD19^+$ cells (depleted of ASC) were isolated with a BD FACS Aria III cell sorter. ASC were further subdivided in PB and PC based on the expression of HLA-DR ($CD3^-CD14^-CD19^+$ $CD27^{++}CD38^{++}$ $HLA\text{-}DR^+$ PB and $CD3^-CD14^-CD19^+CD27^+CD38^+HLA\text{-}DR^-$ PC, respectively), and also isolated by cell sorting.

Cell culture

After isolation, ASC (PB and PC) were plated in 96-well flat bottom plates at a density of 40.000 (in case of total ASC) and 30.000 (in case of separated PB/PC) cells per well in 100 µl X-VIVO 15 medium containing 1% penicillin/streptomycin (100 U/ml; Lonza) and 2mM GlutaMAX (Gibco) in the absence of presence of IL-10 (25 ng/ml; Peprotech). Cells were incubated for 4 days at 37°C and 5% CO_2 after which supernatants were collected and stored at -20°C.

The $CD19^+$ population depleted of ASC was cultured at a density of 40.000 cells/well in a total of 200 µl X-VIVO 15 medium with 1% penicillin/streptomycin (100 U/ml) and 2mM GlutaMAX (Gibco). This volume contained 50 µl of $CD19^+$ B cells and 50 µl of 7500 rad irradiated human CD40 ligand (CD40L) expressing stromal cells (3000 cells/well). In addition, IL-10 (25 ng/ml; Peprotech) was added to the culture. After 7 days of incubation at 37°C and 5% CO_2, cell culture supernatants were collected and stored at -20°C.

Measurement of IgG

IgG concentrations in cell culture supernatants were measured using a Human IgG ELISA Quantitation Set (Bethyl laboratories) according to manufacturer's instructions. Supernatants were incubated at 1:10 and the HRP detection antibody was 1:20.000 diluted.

IgG purification and trypsin digestion

IgG from cell culture supernatants was purified with beads and digested with trypsin before IgG Fc glycosylation was analysed. CaptureSelect IgG-Fc (Hu) affinity matrix (Life Technologies) was added (10 µl/well) to 96-well Orochem filter plates (10-µm pore size; Orochem Technologies) and washed 3 times on a vacuum manifold with 200 µl PBS. Culture supernatant (95 µl) or control serum (2 µl) in 100 µl PBS was loaded on the beads and samples were incubated on a multiwell plate shaker for 1 hour on 1000 rpm. After washing 3 times with 200 µl PBS and 3 times with 200 µl milliQ, 100 µl of 100mM formic acid was added to the wells to detach the IgG from the beads. This was incubated for 5 minutes on a multiwell plate shaker at 1000 rpm and then eluted by centrifugation in a deepwell plate (Thermo Scientific). Samples were dried for 2.5 hours at 50°C by

vacuum centrifugation and reconstituted in 20 μl 50 mM ammonium bicarbonate in 15% ACN. Six hundred ng of tosyl phenylalanyl chloromethyl ketone (TPCK) treated trypsin, preconstituted in 20 mM acetic acid in a 10 ug/uL concentration and further diluted in MQ, was added to each sample, followed by a 5 minute incubation on a multiwell plate shaker. Wells were randomly checked for pH (>6) before overnight incubation at 37°C. The next day, samples were stored at -20°C until measurement by liquid chromatography mass spectrometry (LC-MS).

Fc glycosylation analysis

Fc glycosylation analysis was performed as described previously [23]. In short, the digested IgG samples were separated and analysed on an Ultimate 3000 UHPLC system (Dionex Corporation) coupled to a Maxis Impact HD quadrupole-time-of-flight mass spectrometer (MS) (Bruker Daltonics, Bremen, Germany) as described previously with minor modifications [24]. Extraction of tryptic glycopeptides by a C18 solid phase extraction trap column (Dionex Acclaim PepMap100) was performed after which separation was achieved on an Ascentis Express C18 nano-liquid chromatography (LC) column (Supelco, Bellefonte, PA) conditioned at 900 nl/min with 0.1% TFA (mobile phase A). The following gradient of mobile phase A and 95% acetonitrile (mobile phase B) was applied: 0 min 3% B, 2 min 6% B, 4.5 min 18% B, 5 min 30% B, 7 min 30% B, 8 min 1% B and 11min 1% B. The UPLC was interfaced to the MS with a CaptiveSpray ESI source and nanoBooster (Bruker Daltonics). Mass spectra were recorded from m/z 550 to 1800 at a frequency of 1 Hz. Quadrupole ion energy and collision energy of the MS were set at 2 and 5 eV, respectively. The total analysis time per sample was 13 min.

Statistical analysis

The quality of the mass spectra was evaluated as described previously [23] and is based on total signal intensities per glycopeptide cluster. Briefly, analyte curation was performed using the signal-to-noise ratio, isotopic pattern quality, and observed m/z deviation as obtained after data (pre-)processing with LacyTools [25]. The observed diantennary glycans were used to calculate the glycosylation traits by glycans using the following formulae:

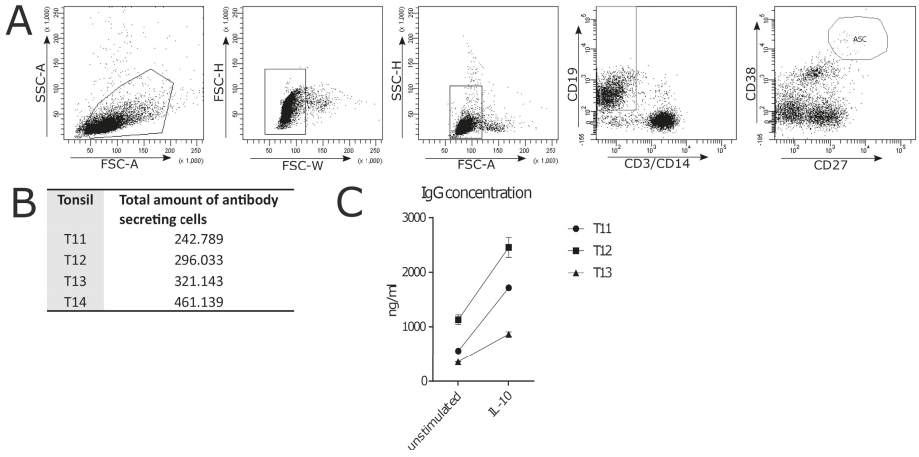
- Fucosylation: SUMIF(IgG1*F*)/SUMIF(IgG1*), where asterisks are wildcards, and F indicates fucosylation.
- Galactosylation: (0.5 × SUMIF(IgG1*H4*) + SUMIF(IgG1*H5*))/SUMIF(IgG1*), where asterisks are wildcards, H4 indicates the presence of one galactose (in total four hexoses in the glycan), and H5 indicates the presence of two galactoses.

- Bisection: SUMIF(IgG1*N5*)/SUMIF(IgG1*), where asterisks are wildcards, and N5 indicates the presence of a bisecting GlcNAc.
- Sialylation: (0.5 × SUMIF(IgG1*S1) + SUMIF(IgG1*S2))/SUMIF(IgG1*), where asterisks are wildcards, S1 indicates the presence of one sialic acid, and H5 indicates the presence of two sialic acids.

Differences in IgG concentrations and glycosylation levels between unstimulated and IL-10 stimulated B cells were calculated for significance using the Wilcoxon matched pairs signed rank test.

RESULTS

Tissue-derived environmental factors such as cytokines can influence Fc glycosylation profiles of IgG produced by *in vitro* stimulated B cells [13]. As the IgG molecules analysed in the published study originated from *in vitro* generated ASCs, and as stimulatory cytokines were used to differentiate the cells, it remained unclear whether the regulatory cytokine IL-10 would have direct effects on the Fc glycosylation profile, and whether IL-10 could modulate the Fc glycosylation pattern in *in vivo* differentiated ASCs. Thus, we here examined if the IgG1 Fc glycosylation profile of *in vivo* differentiated ASC can be modified under the influence of IL-10. To obtain sufficient amounts of ASC, we isolated ASC from human tonsils by FACS based on CD19, CD27 and CD38 expression (figure 1A, B), and cultured the cells for 4 days in the presence of IL-10. Hereafter, secreted IgG (figure 1C) was captured and analysed for its Fc glycosylation profile by LC-MS. As there are four subclasses of IgG that each differ in their glycosylation patterns, we chose to focus on IgG1 which is the most abundant form of IgG. Under the influence of IL-10, IgG1 Fc-galactosylation and sialylation levels were roughly 20% lower than those found on IgG1 spontaneously secreted by unstimulated ASC, whereas the abundance of bisecting GlcNAc increased by 30% (figure 2A, B). As ASC include both plasmablasts and plasma cells, we also specifically differentiated between plasmablasts and plasma cells based on HLA-DR expression to investigate whether this effect was observed for both cell subpopulations (supplementary figure 1A, B) [26]. IgG1 from plasmablasts and plasma cells showed a similar trend in modulation of Fc galactosylation with IL-10 stimulation (supplementary figure 1C, D). These results indicate that the Fc glycosylation profile of IgG1 antibodies secreted by ASC, including both plasmablasts and plasma cells, can indeed be modified in the presence of IL-10.



Tonsil	Total amount of antibody secreting cells
T11	242.789
T12	296.033
T13	321.143
T14	461.139

Figure 1. Isolation and analysis of IgG1 Fc glycosylation patterns on IgG1 produced by antibody secreting cells. Antibody secreting cells, as indicated in red, were sorted by FACS based on the expression of CD19+CD3-CD14-CD27++CD38++. Here, a representative FACS plot of the cellular suspension of one tonsil is shown (A). The total amount of ASC obtained after sorting is indicated in (B) n=4. After culturing the ASC for 4 days in the presence of absence of IL-10, total IgG content was measured (C) n=3, P=0.25. IgG concentration measurement was not determined for T14.

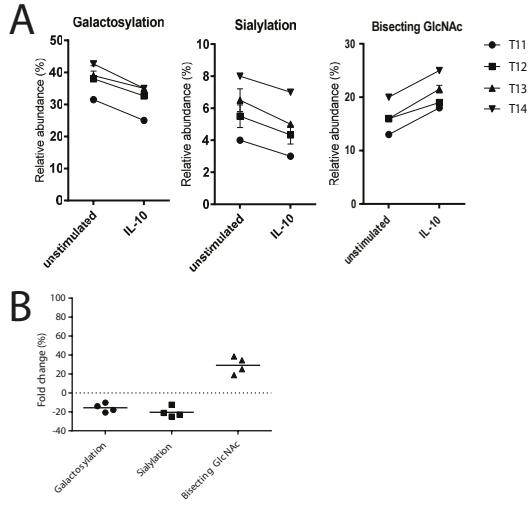


Figure 2. Fc glycosylation patterns of IgG1 produced by antibody secreting cells can be modified under the influence of IL-10. (A) The relative abundance of IgG1 Fc galactosylation, sialylation and bisecting GlcNAc is shown for ASC cultured with or without IL-10 (n=4). No statistical differences between unstimulated and IL-10 stimulated conditions were measured using the Wilcoxon matched pairs signed rank test. (B) The IgG1 Fc galactosylation, sialylation and bisecting GlcNAc expression profiles of IgG1 from ASC cells cultured in the absence and presence of IL-10 were compared and calculated in a fold change (%) (n=4). Black line indicates the mean percentage of each Fc profile and the dotted line indicates where the fold change is zero.

To investigate whether the effects of IL-10 represent a general response of B cells and ASC to this cytokine, we next isolated $CD19^+$ B cells depleted of ASC and cultured these cells for 7 days in the presence or absence of IL-10 (figure 3A). As these B cells include naïve and memory B cells, it is necessary to provide stimulation in order to induce *in vitro* differentiation towards antibody secretion. Here, we chose to mimic a T-cell dependent stimulation (figure 3B) by adding irradiated CD40L-expressing cells to the cultures. This had, by itself, no influence on the galactosylation and sialylation contents of secreted IgG1, but may have enhanced GlcNAc levels (supplementary figure 2). Under the influence of IL-10, however, IgG1 secreted by stimulated $CD19^+$ cells showed a higher abundance of galactose and sialic acid residues (25% – 54% increase, respectively), while there was no clear trend in bisecting GlcNAc levels (figure 3C, D). Hence, the effects of IL-10 on the Fc glycosylation machinery of $CD19^+$ B cells, in the context of T-cell dependent stimulation, seem to oppose the effects of IL-10 on the Fc-glycosylation profile of IgG1 spontaneously secreted by ASC. All together, these results imply that IL-10 has contrasting effects on the Fc glycosylation profile of IgG1 secreted by *in vivo* differentiated ASC of by $CD19^+$ B cells that are *in vitro* driven to secrete IgG. This suggests that the effect of IL-10 modulation on IgG1 Fc glycosylation is dependent on the differentiation status of the B cell.

DISCUSSION

In this report, we show a distinct modulatory effect of IL-10 on the Fc glycosylation profile of IgG1 secreted by B cells in different differentiation stages. IgG1 molecules produced by $CD27^{++}CD38^{++}$ antibody secreting cells, which contain plasmablasts and plasma cells, showed a decreased galactosylation and sialylation content when ASCs were cultured with IL-10. The separate analysis of IgG1 secreted by either plasmablasts or plasma cells demonstrated that both populations show a similar response to IL-10. In contrast, IL-10 stimulation increased the Fc galactosylation and sialylation levels of IgG1 derived from stimulated, $CD19^+$ cells depleted of ASC, which mostly contain naïve and memory B cells. These results indicate that IL-10 has contrasting effects on the IgG Fc galactosylation and sialylation machinery of ASC and $CD19^+$ B cells depleted of ASC, despite the elevated IgG production in both populations.

We used an approach in which we first studied the spontaneous IgG production from *ex vivo* cultured ASC, thereby avoiding the addition of external stimuli (except for IL-10, the cytokine of interest). Compared to previous publications, this approach has the benefit that the IgG Fc glycosylation profile could not be influenced by factors required for the differentiation of B cells into plasma cells *in vitro*. To also analyse IgG from *in vitro*

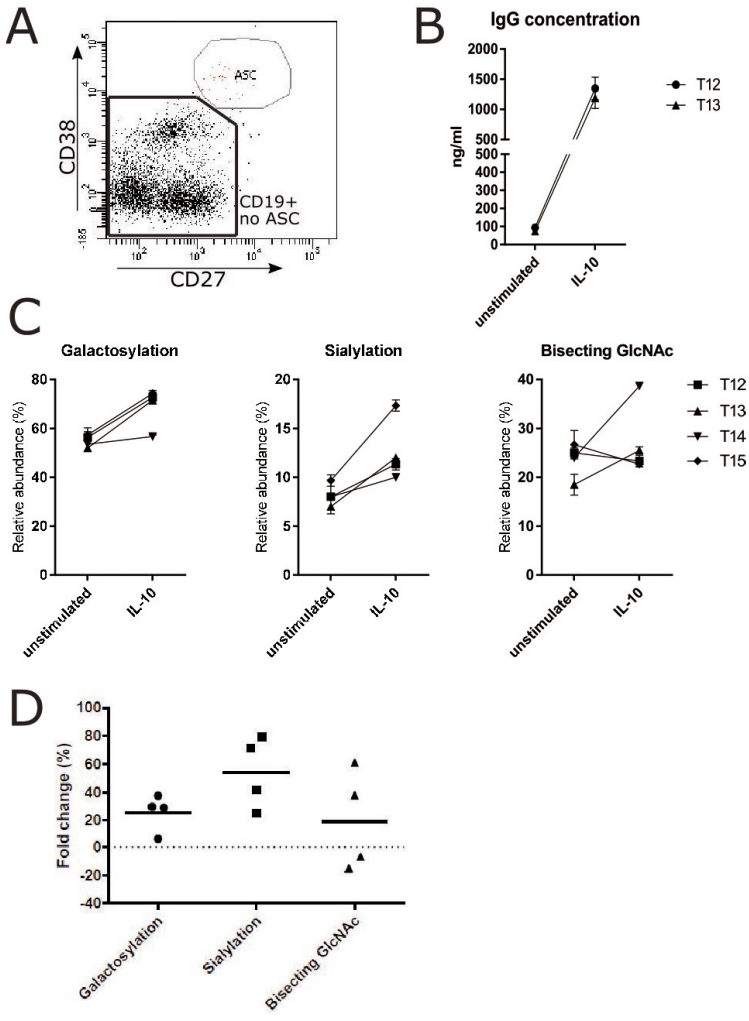


Figure 3. CD19⁺ cells depleted of ASC show opposite Fc glycosylation patterns upon IL-10 stimulation. (A) Next to ASC, CD19⁺ cells depleted of ASC were isolated by FACS, as indicated with the black box. A representative plot is shown. (B) Total IgG concentrations in culture supernatants of CD19⁺ cells cultured with or without IL-10 (n=2). IgG concentration was not assessed for T14 and T15. Lines indicate the standard deviation of 2 or 3 wells with the same conditions. (C) The relative abundance (in percentages) of IgG1 Fc galactosylation, sialylation and bisecting GlcNAc is shown for CD19⁺ B cells cultured with or without IL-10 (n=4). No statistical differences were observed using the Wilcoxon matched pairs signed rank test. Lines indicate the standard deviation of 2 or 3 wells with the same conditions. (D) The IgG1 Fc galactosylation, sialylation and bisecting GlcNAc expression profiles of IgG1 from CD19⁺ B cells cultured in the absence and presence of IL-10 were compared and calculated in a fold change (%) (n=4). Black line indicates the mean percentage of each Fc profile and the dotted line indicates where the fold change is zero.

differentiated, $CD19^+$ B cells depleted of ASC, we had to use irradiated CD40L cells to induce IgG production. The signal provided by CD40L itself or signals derived from the interaction between the B cells and the CD40L-expressing cells did not affect the IgG1 galactosylation and sialylation profiles, except for a slight increase in the bisecting GlcNAc levels of IgG1. Nonetheless, opposite changes in IgG Fc galactosylation and sialylation levels could be observed with IgG secreted from $CD19^+$ B cells depleted of ASC as compared to ASC upon IL-10 stimulation. This contrasting effect of IL-10 on the glycosylation profile of IgG secreted by naïve/memory B cells and ASC might be explained by the difference in maturity status. Due to B cell maturation, functionality such as the affinity for immune receptors, can differ between B cell populations which may require other glycosylation profiles.

The results from our study are in line with previous findings demonstrating that factors in the microenvironment of B cells can influence IgG Fc glycosylation patterns [13]. In addition, our data suggest that the Fc glycosylation profile of IgG secreted by differentiated plasma cells can still be modified. This is an important observation as terminally differentiated plasma cells are considered the end-stage of B cell differentiation, a stage in which diversification of the secreted antibodies has stopped. Such plasma cells do not divide and represent, when present as long-lived PC in specialized survival niches, a hallmark of protective but also pathogenic immunological memory. To date, it is still unclear whether such cells are 'functionally imprinted' during their differentiation towards PC. As long-lived PC are refractory to most therapeutic interventions and difficult to target [27, 28], ways to modulate the function of their secreted antibodies could be of potential therapeutic interest.

How IL-10 exactly influences IgG Fc glycosylation patterns in $CD19^+$ B cells and ASCs is not yet known. One likely explanation may be provided through the influence of IL-10 on the regulation of glycosyltransferases in B cells. Recently, it was demonstrated that IL-10 increased the expression of a glycosyltransferase in the Golgi apparatus responsible for the *N*-glycan branching of glycoproteins, in this case the T cell receptor (TCR). The increased *N*-glycan branching on the TCR served as a ligand to Galectin 3 which restricted the interaction of the TCR and co-receptor CD8, thereby preventing clustering of membrane proteins required for T cell activation and increasing the antigenic threshold for T cell activation during chronic infection [29]. As antibody-mediated effector functions greatly depend on their IgG Fc glycosylation profile, this IL-10 induced plasticity could have a great influence on biological functions of IgG, such as Fc gamma (FcγR) receptor binding, the activation of immune cells and activation of the complement system [30].

Our study has several limitations. First, the populations of ASCs and PB/PC were defined based on the expression of surface markers only. Second, statistical analysis of the data shown in this publication did not show statistical significance in IgG Fc-glycosylation for unstimulated versus IL-10 stimulated ASC or $CD19^+$ B cells. This is most likely due to the lack of power (n=4), as a clear and consistent effect was observed in all samples to a comparable extent. This limitation can be overcome with the inclusion of more samples in future experiments. Furthermore, we used tonsils as cellular source and did not study ASCs from other sources such as bone marrow. B cells from different tissues have different maturity and can be functional distinct. Secondary lymphoid organs such as tonsils are generally considered a source of early plasma cells, whereas plasma cells released in the (blood) circulation are considered to be in transitional phase and the bone marrow is considered the final target organ for long-lived antibody secreting plasma cells [31]. In addition, depending on the tissue location, B cells may differ in Ig secretion kinetics and the ability to undergo apoptosis [31, 32]. Therefore, it is possible that the alterations in IgG Fc glycosylation we now observed with tonsillar B cells are different in comparison to B cells from the circulation or bone marrow. Further research is therefore needed to determine if our results can be translated to IgG secreted by B cells and ASC from other compartments.

Finally, not all glycoforms present on the IgG Fc tail could be included in the analysis of glycan abundance. In fact, fucosylation could not be analysed properly in our experiments. Normal fucosylation levels of IgG reach around 90-95% occupancy. In the samples of the current study, however, sometimes only a few tens of nanograms were available per sample, which made it difficult to detect and quantify the remaining 10% of afucosylated species. Therefore, we excluded this glycan species from the analysis. Although the same applies, although to a lesser extent, for bisecting GlcNAc residues, we here could measure detectable levels of bisection on the IgG Fc. We therefore cannot exclude the possibility that IL-10 may have additional effects on IgG fucosylation levels and indirectly impacts on antibody effector functions such as FcγRIII binding [33, 34].

To summarize, we here demonstrated that IL-10 has different modulatory effects on IgG Fc-glycosylation profiles depending on the differentiation status of the B cell. As IgG Fc-glycosylation directly impacts on antibody effector functions, altering Fc glycosylation by cytokines/micro-environmental factors may therefore be an interesting approach in the immuno-modulation of pathogenic antibody responses.

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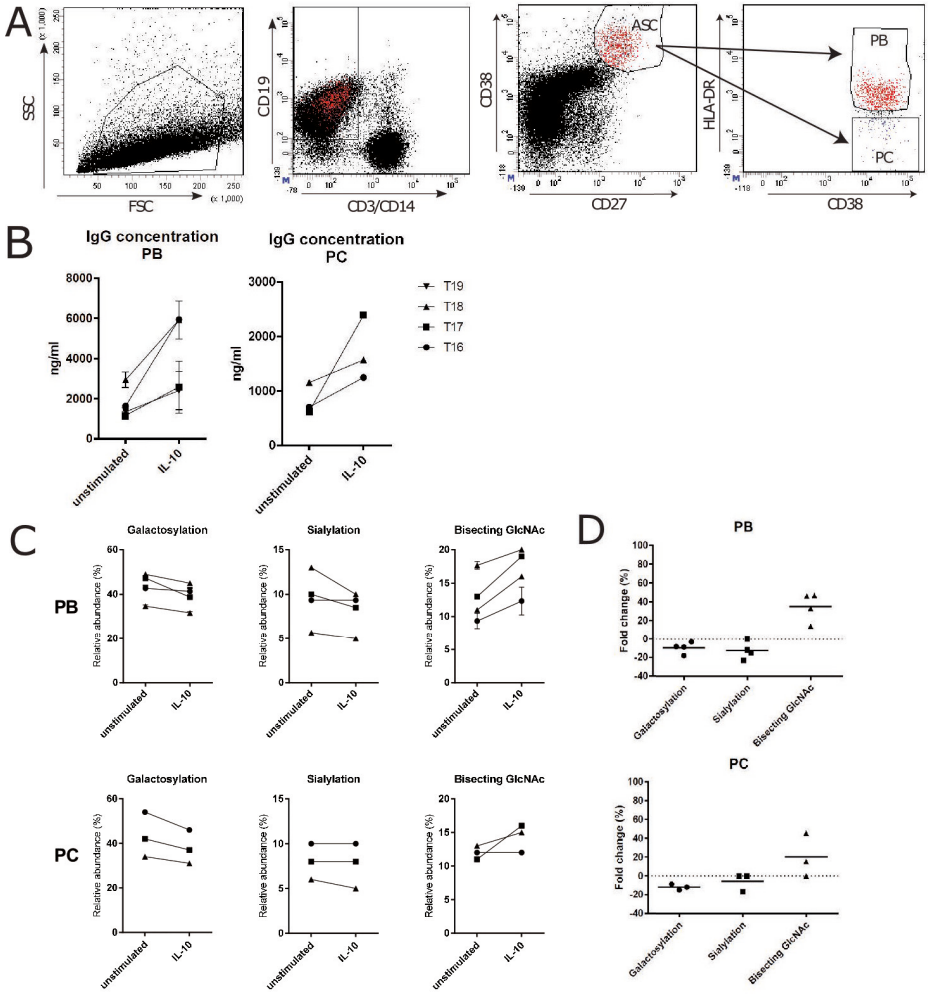
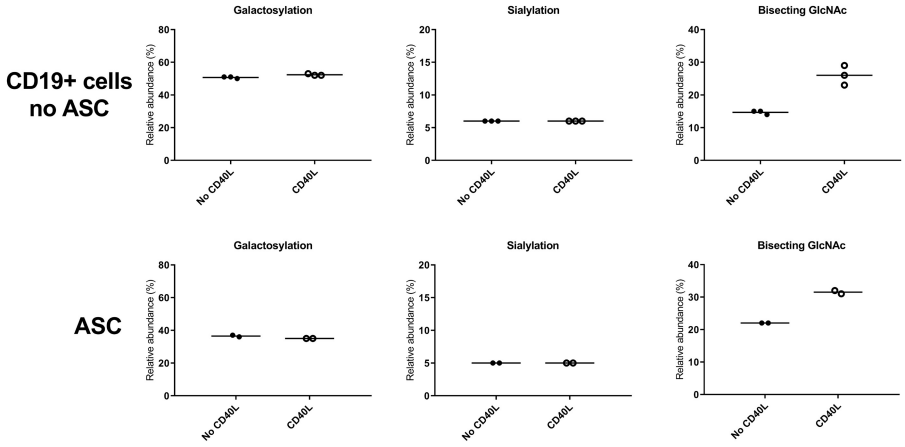
SUPPLEMENTARY FIGURES



Supplementary figure 1. IL-10 modulates the Fc glycosylation profile of IgG1 secreted by plasma blasts as well as IgG1 from plasma cells. (A) Representative plot showing how plasmablasts and plasma cells were isolated from ASC based on the expression of HLA-DR. (B) Total IgG concentration in ng/ml was measured for IgG produced by plasmablasts (n=4) and plasma cells (n=3) with and without the presence of IL-10. (C) Fc galactosylation, sialylation and bisecting GlcNAc levels from IgG1 produced by plasmablasts (n=4) and plasma cells (n=3) cultured in the absence or presence of IL-10, expressed in relative abundance (%). No statistical differences were observed using the Wilcoxon matched pairs signed rank test. (D) The IgG1 Fc galactosylation, sialylation and bisecting GlcNAc expression profile of IgG1 from PB (n=4) and PC (n=3) cultured in the presence of IL-10 was compared to IgG1 from PB and PC cultured in the absence of IL-10 and calculated to a fold change (%). Black line indicates the mean percentage of each Fc glycosylation profile.



Supplementary figure 2. Irradiated, CD40L-expressing stromal cells have minimal effects on IgG1 Fc glycosylation profiles in the cultures. CD19⁺ cells and ASC were cultured with (white bullets) and without (black bullets) CD40L cells in the absence or presence of IL-10. Bullets indicate one well (n=1 tonsil donor). Fc galactosylation, sialylation and bisecting GlcNAc levels were measured and expressed in relative abundance (%).