Accepted Manuscript

Low amounts of bisecting glycans characterize cerebrospinal fluid-borne IgG

Jasmin Knopf, Iryna Magorivska, Juan M. Maler, Philipp Spitzer, Rostyslav Bilyy, Mona H.C. Biermann, Kateryna Hychka, Albert Bondt, Manfred Wuhrer, Rene E.M. Toes, Georg Schett, Martin Herrmann, Luis E. Muñoz

PII:	S0165-5728(18)30094-8
DOI:	doi:10.1016/j.jneuroim.2018.04.010
Reference:	JNI 476762
To appear in:	*Journal of Neuroimmunology*
Received date:	21 February 2018
Revised date:	13 April 2018
Accepted date:	13 April 2018

Please cite this article as: Jasmin Knopf, Iryna Magorivska, Juan M. Maler, Philipp Spitzer, Rostyslav Bilyy, Mona H.C. Biermann, Kateryna Hychka, Albert Bondt, Manfred Wuhrer, Rene E.M. Toes, Georg Schett, Martin Herrmann, Luis E. Muñoz , Low amounts of bisecting glycans characterize cerebrospinal fluid-borne IgG. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Jni(2018), doi:10.1016/j.jneuroim.2018.04.010

Low amounts of bisecting glycans characterize cerebrospinal fluid-borne IgG

Jasmin Knopf*[1], Iryna Magorivska*[1, 2], Juan M Maler*[3], Philipp Spitzer*[3], Rostyslav Bilyy[2], Mona HC Biermann[1], Kateryna Hychka[2], Albert Bondt[4, 5], Manfred Wuhrer[5], Rene EM Toes[4], Georg Schett[1], Martin Herrmann[1], Luis E Muñoz[1#]

* JK, IM, JMM, PS contributed equally to the work.

[1] Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Internal Medicine 3, Rheumatology and Immunology, Universitätsklinikum Erlangen, Erlangen, Germany

[2] Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

[3] Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, Erlangen, Germany

[4] Leiden University Medical Center, Department of Rheumatology, Leiden, the Netherlands

[5] Leiden University Medical Center, Center for Proteomics and Metabolomics, Leiden, the Netherlands

[#] Corresponding author:

Luis E. Munoz

luis.munoz@uk-erlangen.de

Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Internal Medicine 3 – Rheumatology and Immunology, Ulmenweg 18, 91054 Erlangen, Germany

Phone: +49 9131 85 43034

Fax: +49 9131 85 35776

Abstract

Immunoglobulin G (IgG) harbors a conserved N-glycosylation site which is important for its effector functions. Changes in glycosylation of IgG occur in many autoimmune diseases but also in physiological conditions. Therefore, the glycosylation pattern of serum IgG is well characterized. However, limited data is available on the glycosylation pattern of IgG in cerebrospinal fluid (CSF) compared to serum. Here, we report significantly reduced levels of bisected glycans in CSF IgG. Galactosylation and sialylation of IgG4 also differed significantly. Therefore, we propose a common mechanism mediating glycosylation changes of IgG at the transition from serum to CSF in steady state conditions.

Keywords: Glycosylation, Immunoglobulin G, Cerebrospinal fluid, Serum, Bisecting, Sialylation

1. Introduction

All immunoglobulin G (IgG) subclasses harbor a highly conserved N-glycosylation site at Asparagine^{297} (Huber et al. , 1976). Glycosylation of IgG is known to be important for mediating its effector functions *in vivo.* It was shown that genetic or enzymatic removal of the sugar moiety abolishes the activity of IgG (Bohm et al. , 2014). Glycosylation starts in the endoplasmic reticulum, then formed oligomannose structures are trimmed and addition of other sugars such as fucose, galactose and sialic acid happens in the Golgi apparatus (Stanley et al. , 2009). Around 11 mg/ml of IgG are found in the serum of healthy human individuals (Cassidy and Nordby, 1975), whereas in cerebrospinal fluid (CSF) the concentration is only around 0.02 mg/ml (Ganrot and Laurell, 1974). IgG in CSF of healthy individuals is derived from the blood plasma and gains access to the ventricular and subarachnoid space across the blood brain barrier (Keir G., 1986).

Since the publication of abnormal N-glycosylation patterns in context with rheumatoid arthritis over 30 years ago (Parekh et al. , 1985), glycosylation patterns of IgG in serum have been extensively researched in physiological and pathological conditions. It is now known that N-glycosylation patterns change during acute inflammation and aging but also during pregnancy (Arnold et al. , 2007, Kristic et al. , 2014, Parekh et al. , 1988, van de Geijn et al. , 2009). The first two conditions lead to a glycosylation pattern with less galactosylated IgG (G0) associated with increased inflammation, whereas in pregnancy galactosylated glycoforms (G1, G2) increase. From studies with intravenous IgG (IVIG) we know that antibodies with enhanced therapeutic activity show reduced affinity for most activating Fcγ receptors (FcγR) (Anthony et al. , 2008a, Anthony and Ravetch, 2010, Anthony et al. , 2008b). Studies of monoclonal therapeutic antibodies revealed that enhanced antibody-dependent cellular cytotoxicity (ADCC) results from an enhanced affinity for and better interaction with the activating human FcγRIIIA of antibodies lacking core fucose (Bruggeman et al. , 2017, Nimmerjahn and Ravetch, 2012, Sakae et al. , 2017). Subedi *et al.* not only found a decreased affinity for FcγRIIIA for fucosylated antibodies, but also for FcγRIIB, FcγRIIB and FcγRIIC (Subedi and Barb, 2016). Additionally, this enhanced ADCC can further be influenced by additional galactosylation of IgG (Subedi and Barb, 2016, Thomann et al. , 2016, Yamaguchi et al. , 2006).

However, most of these studies have been conducted using either monoclonal therapeutic antibodies or IVIG isolated from the serum of thousands of healthy donors. So far, little is known about the glycosylation patterns of IgG in CSF. In one study released glycans were investigated, which were found to be dissimilar between serum and CSF (Karlsson et al. , 2017). Of note, the serum and CSF samples were not from the same donors. Another study was performed on IgG1 glycopeptides in paired CSF and serum samples from patients with

multiple sclerosis (MS) and healthy controls, showing altered IgG1 glycosylation patterns in CSF of MS patients with elevated bisecting N-acetylglucosamine (GlcNAc), afucosylation and reduced galactosylation that correlated with the degree of intrathecal IgG synthesis (Wuhrer et al. , 2015).

Taken together, there is only little data available on the glycosylation patterns of IgG subclasses in the CSF compared to serum. Therefore, we herein aim to identify differences in the glycosylation of CSF and serum IgG subclasses of the same individual.

2. Material and Methods

2.1. Cohort

Twelve patients were recruited at the department of Psychiatry at the University Hospital Erlangen. The patients referred to the clinic due to cognitive disturbances and received a diagnostic work-up comprising routine blood tests, cerebrospinal fluid (CSF) investigations including oligoclonal bands and neurochemical dementia diagnostics, brain-MRI, brain-perfusion SPECT and a neuropsychological investigation with the CERAD+ battery. Diagnoses were made according to the revised National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) criteria (Albert et al. , 2011, McKhann et al. , 2011). Only patients with mild cognitive impairment but without clinical and laboratory signs of Alzheimer's dementia were included. Most patients suffered from an affective disorder, two from suspected frontotemporal lobar degeneration and one from suspected vascular disease. Patients with malignomas, infections or a disturbed blood brain barrier were excluded from the study. Also, samples were assured to be free of a contamination with blood by an erythrocyte count. A short characterization of the patients is included in Table 1. The study protocol was approved by the ethical committee of the University Hospital Erlangen (No. 3987) and patients provided their informed written consent.

Table 1: Characterization of the patients included in the study

Patient ID	Age at lumbar puncture	Sex	Diagnosis
1	55	female	MCI (probable fronto temporal lobar degeneration)
2	60	female	MCI (probable depression)
3	85	female	MCI (probable depression)
4	75	male	MCI (probable vascular disease)
5	60	female	MCI (not Alzheimer´s disease)
6	54	female	MCI (probable fronto temporal lobar degeneration)
7	56	female	MCI (probable depression)

8	70	female	Depression
9	64	male	MCI (probable depression)
10	51	male	MCI (probable depression)
11	71	female	Bipolar disorder
12	58	male	MCI (probable depression)

Abbreviation: MCI, mild cognitive impairment

CSF and serum were centrifuged at 1600 g for 10 min within 2 h after sampling and stored in aliquots at -80°C until further use.

2.2. Immunoglobulin G purification from serum and cerebrospinal fluid

Immunoglobulin G (IgG) was purified using recombinant Protein G Agarose (ProteinMods, Madison, Wisconsin, USA). The column, containing 100 µl of Protein G Agarose was washed and equilibrated with 10 volumes of binding buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.5). The sample was diluted 1/1 with binding buffer, gently applied to the column (400 µl) and kept 30 min at room temperature (RT). All column washes/elutions were done by centrifugation at 3000 rpm. Flow-through fractions were collected and the column was washed with 2 ml of Tris-buffered saline (TBS) in 10 steps. IgG was eluted with 0.1 M glycine-HCl, pH 2.7, immediately neutralized by 1 M Tris-HCl, pH 9.0. Protein concentration was measured by the NanoDrop^{TM} 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.3. LC-ESI-MS analysis

For the analysis of Fc-glycosylation, the IgG eluates were subjected to tryptic digest by adding 600 ng tosyl-phenylalanyl-chloromethyl-ketone treated trypsin (Merck KGaA, Darmstadt, Germany) in 40 µl ammonium bicarbonate buffer followed by overnight incubation at 37°C. Digested IgG was separated and analyzed 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 (Falck et al. , 2017). Following extraction of tryptic glycopeptides by a C18 solid phase extraction trap column (Dionex Acclaim PepMap100), 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) after which 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 UHPLC 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.

Quality of mass spectra was evaluated based on total signal intensities per glycopeptide cluster. 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 (Jansen et al. , 2016). Included analytes and the calculation of galactosylation, sialylation and bisection are described in the following paragraphs (2.3.1 and 2.3.2).

2.3.1. Detected glycopeptide compositions

The glycosylation traits were calculated based on the glycopeptide composition in Table 2. H indicates the number of hexoses, N the number of N-acetylglucosamines, F the number of fucoses and S the number of sialic acid (N-acetylneuraminic acid).

Table 2: Glycopeptide composition used for calculating the glycosylation traits

IgG1	IgG2/3	IgG4
H3N4F1	H3N4F1	H3N4F1
H4N4F1	H4N4F1	H4N4F1
H5N4F1	H5N4F1	H5N4F1
H3N5F1	H3N5F1	H3N5F1
H4N5F1	H4N5F1	
H5N5F1	H5N5F1	
H4N4F1S1	H4N4F1S1	
H5N4F1S1	H5N4F1S1	H5N4F1S1
H4N5F1S1		
H5N5F1S1		
H5N4F1S2		
H3N4		
H4N4		
H5N4		
H4N5		
H5N5		

2.3.2. Calculations

Fucosylation: SUMIF(IgG*x**F*)/SUMIF(IgG*x**), where *x* is the subclass identifier, asterisks are wildcards, and F indicates fucosylation.

Galactosylation: (0.5 × SUMIF(IgG*x**H4*) + SUMIF(IgG*x**H5*))/SUMIF(IgG*x**), where *x* is the subclass identifier, 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(IgG*x**N5*)/SUMIF(IgG*x**), where *x* is the subclass identifier, asterisks are wildcards, and N5 indicates the presence of a bisecting GlcNAc.

Sialylation: (0.5 × SUMIF(IgG*x**S1) + SUMIF(IgG*x**S2))/SUMIF(IgG*x**), where *x* is the subclass identifier, asterisks are wildcards, S1 indicates the presence of one sialic acid, and H5 indicates the presence of two sialic acids.

2.4. Lectin-ELISA

To detect the exposure of sialic acid on IgG from serum and CSF, we conducted Lectin-ELISA as described previously (Stumer et al. , 2017). Briefly, 96-wells MaxiSorp™ microtitre plates (442404, Nunc, Roskilde, Denmark) were coated with 2 µg/ml $F(ab')_2$-fragment of goat anti-human IgG (109-006-003, Jackson ImmunoResearch Europe Ltd, Ely, United Kingdom) overnight at +4°C in coating buffer (0.1 M Na_2CO_3/$NaHCO_3$, pH 9.6). After each incubation, the plate was washed three times with phosphate buffered saline (PBS) containing 0.05% Tween-20 (PBS-T) (8.22184.1000, Merck, Darmstadt, Germany). As blocking step, we used a special gelatin blocking buffer containing 3% deglycosylated gelatin, 0.1% $CaCl_2$ and 0.1% $MgCl_2$ for 2 hours at RT. Serum was diluted 1:5000 and CSF 1:300 in PBS-T and applied for 1 hour at RT to the plates. After another washing step, we employed horseradish peroxidase (HRP) labelled goat anti-human IgG (2048-05, Southern Biotech, Birmingham, Alabama, USA) or IgM antibodies (2020-05, Southern Biotech) or biotinylated *Sambucus nigra* lectin (SNA) (B1305, Vector Laboratories, Burlingame, California, USA) to detect exposure of α2,6-linked sialic acid. Pierce™ High Sensitivity Streptavidin-HRP (21130, Thermo Fisher Scientific) was used to detect the biotinylated SNA. Optical density was read with the Sunrise (Tecan, Männedorf, Switzerland) at 450 nm with a reference at 620 nm.

2.5. Statistics

To describe the significance of differences in one glycan between serum and corresponding CSF, we used paired Wilcoxon-signed rank test as normal distribution of values was not fulfilled. To describe the correlation of sialic acid exposure with IgG and IgM, we used Spearman-Rho. Significant differences in the ratio of SNA to IgG in CSF and serum was calculated using Wilcoxon-signed rank test. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Statistics were calculated using the SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) and GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com).

3. Results

3.1. LC-ESI-MS analysis

The Fc part of immunoglobulin G harbors a conserved N-glycosylation site at Asparagine[297] (Figure 1). We analyzed the glycosylation of IgG in the serum and corresponding CSF of twelve individuals without any diagnosed neuro-inflammatory disorder by liquid

chromatography electrospray ionization mass spectrometry (LC-ESI-MS) and calculated the relative ratio of Bisection, Galactosylation, Sialylation and Fucosylation using the glycopeptides depicted in Figure 1.

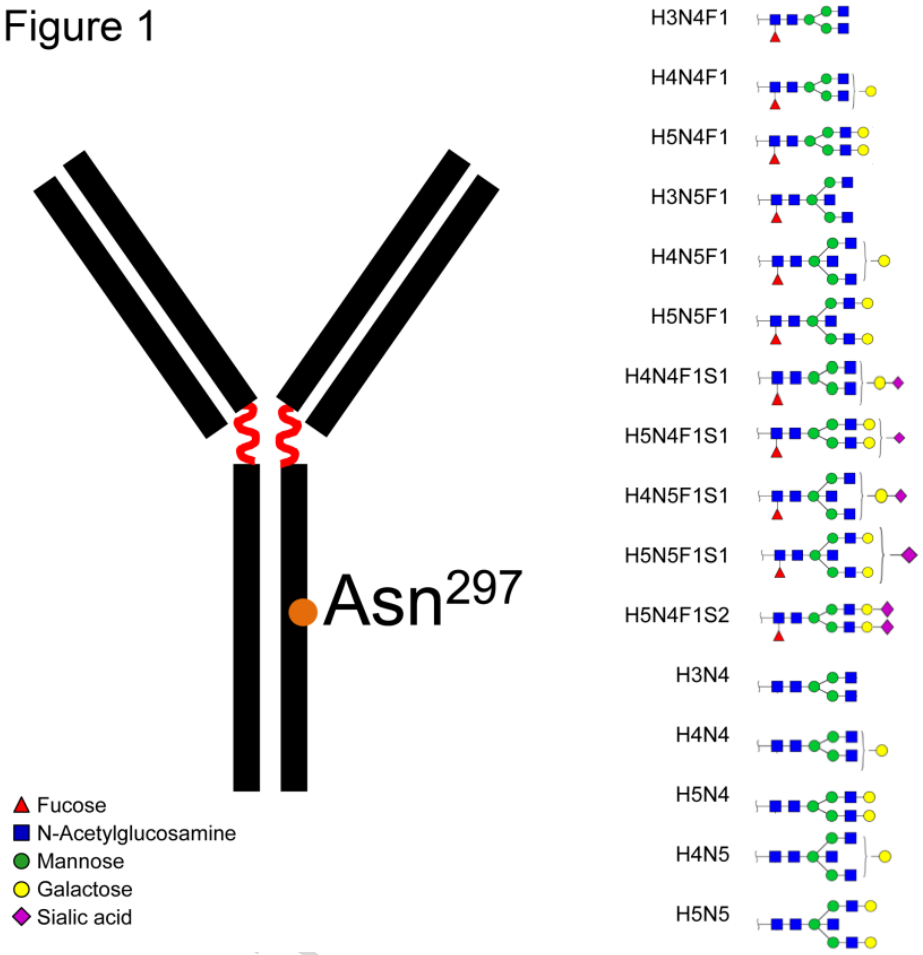


Figure 1 N-glycosylation of Asparagine[297] in the Fc part of immunoglobulin G with the glycopeptides depicted that were used for calculating the glycosylation traits. Oligomannose is shown as a green circle. N5 means bisecting (5 N-acetylglucosamine, blue square). H3 is used for no galactose, H4 for 1 galactose, H5 for 2 galactose (yellow circle), respectively. F1 means fucose (red triangle). S1 and S2 is used for 1 or 2 sialic acid (purple rhombus), respectively.

For all subclasses of IgG analyzed, the level of bisection was significantly lower in CSF compared to serum (Figure 2). Galactosylation, sialylation and fucosylation did not differ significantly in CSF IgG compared to serum IgG for IgG1 and IgG2/3. However, for IgG4 galactosylation and sialylation as well as the amount of sialic acids per galactose (SA/Gal) were significantly lower in CSF compared to serum IgG of the same individual. Interestingly, our individuals can be divided into two groups depending on total sialylation of IgG1 and IgG2/3. One group shows a higher sialylation in CSF compared to corresponding serum and the second group lower CSF sialylation. However, only one individual showed higher

sialylation of both IgG1 and IgG2/3, whereas for the other individuals in CSF IgG1 sialylation was higher as compared to serum, and IgG2/3 sialylation was lower.

Figure 2

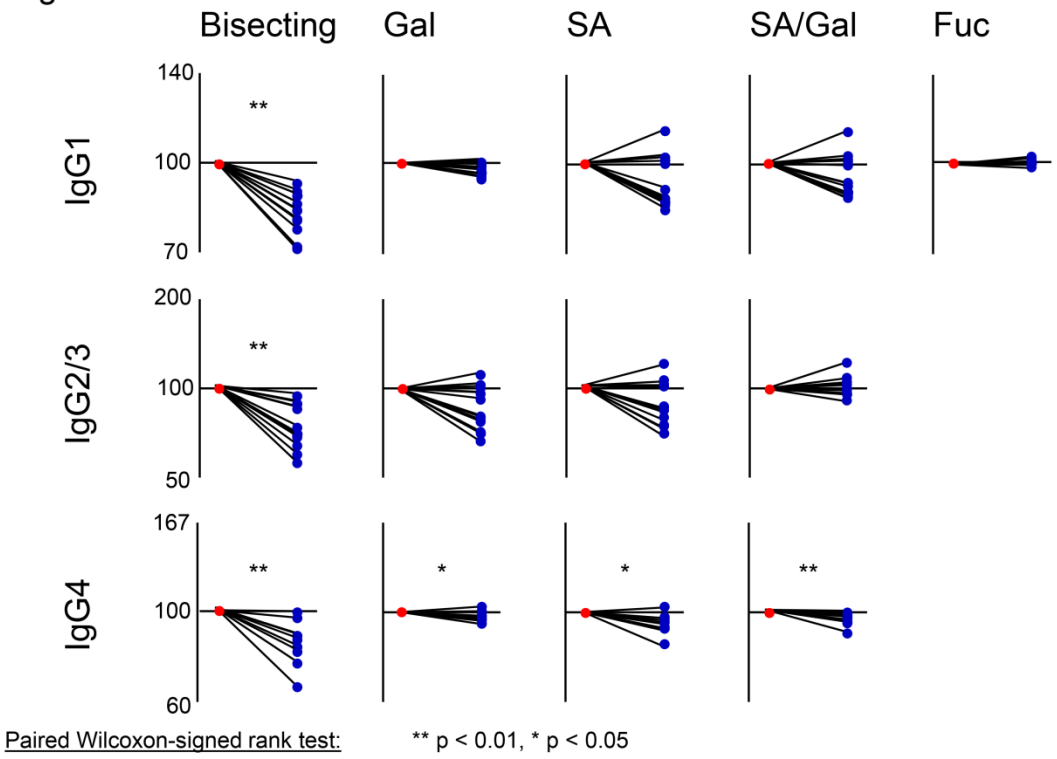


Figure 2 IgG glycosylation traits differ in serum vs. corresponding CSF for IgG1, IgG2/3 and IgG4. IgG glycosylation was quantified using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) in paired serum/CSF samples from 12 individuals. Relative abundance of each CSF glycosylation trait compared to corresponding serum was calculated. Lines indicate corresponding serum/CSF pairs. CSF samples are depicted as blue dots, serum samples as red dots. Gal is used as abbreviation for Galactosylation, SA for Sialylation and SA/Gal for sialic acid per galactose. Statistical significance was determined using Wilcoxon-signed rank test for paired samples. ** $p < 0.01$; * $p < 0.05$.

3.2. Lectin-ELISA results

Next, we wanted to know if the observed differences for overall sialylation of IgG4 correlate with the actual exposure of sialic acid on IgG in native conditions. Therefore, we performed Lectin-ELISA with *Sambucus nigra* lectin (SNA) that binds specifically to sialic acid α2,6-linked to galactose if the sugar is present and accessible for binding. In both, serum and CSF, the binding of SNA to α2,6-linked sialic acid correlates positively and significantly with the detected level of IgG (Figure 3a; $p = 0.00313$ and 0.00015, respectively). The correlation between lectin binding and IgG content resulted stronger in CSF compared to serum (Figure 3a; Spearman-Rho = 0.761 and 0.641, respectively). The presence of α2,6-linked sialic acid associated with IgG was significantly higher in the serum compared to CSF (Figure 3b, paired t test, $p < 0.001$). This higher binding of SNA in serum is not due to contaminating IgM. IgM detection correlates with IgG only in serum (Figure 3c, Spearman-Rho, $p = 0.00002$) and not with SNA binding.

Figure 3

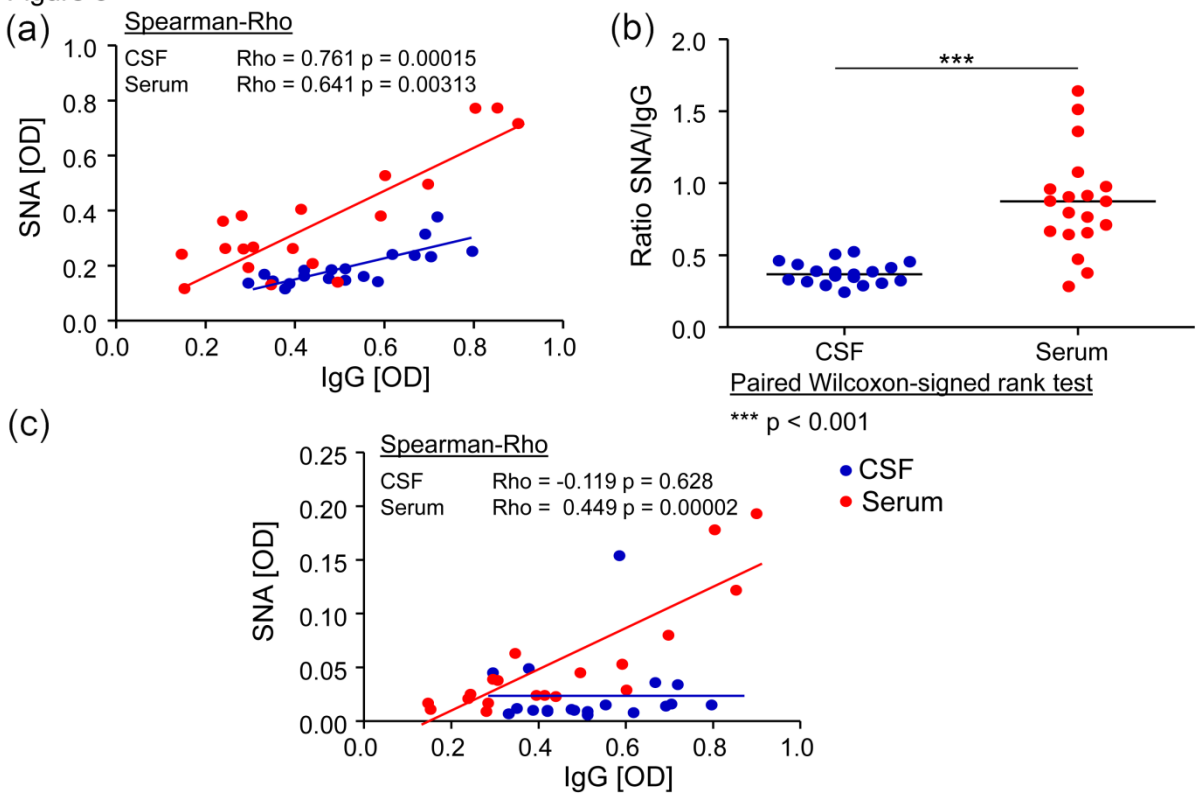


Figure 3 Exposure of sialic acid is increased in serum compared to CSF independent of contaminating IgM. (a) Exposure of α2,6-linked sialic acid correlates with IgG detection in serum and corresponding CSF samples of 19 individuals as detected by Lectin-ELISA. Optical densities for Sambucus nigra lectin (SNA) and IgG are depicted. CSF samples are depicted in blue circles, serum in red circles. Correlation was calculated using Spearman-Rho and trend lines are depicted in blue for CSF and red for serum. (b) SNA bound to sialic acid is higher in serum compared to CSF as detected by Lectin-ELISA. The ratio of SNA/IgG in serum and CSF is depicted. CSF samples are depicted in blue circles, serum in red circles. Significance was determined using a paired t-test. *** p< 0.001. (c) Detection of IgM correlates with IgG only in serum of 19 individuals as detected by ELISA. Optical densities for Sambucus nigra lectin (SNA) and IgG are depicted. CSF samples are depicted in blue circles, serum in red circles. Correlation was calculated using Spearman-Rho and trend lines are depicted in blue for CSF and red for serum.

Taken together, sialylated IgG4 is not only increasingly detected in the serum compared to CSF but also the binding of SNA to sialic acid on IgG is higher in the serum. Additionally, IgG4 sialylation and galactosylation in CSF and corresponding CSF behaves differently and most importantly, bisecting IgG is less common in CSF IgG independent of the subclass. Since the most common causes of intrathecal production of IgG in our individuals were excluded at the start of the study, this observation might raise the question for a different mechanism of IgG glycan tree modification at the transition from serum to CSF.

4. Discussion

In this study, we detected significant differences for various glycosylation traits between serum and CSF of the same individual, most prominently for IgG4. IgG glycosylation traits calculated from individual glycans detected in serum (see 2.3.1) are in line with previous publications (Clerc et al. , 2016, Karlsson, Ndreu, 2017, Kristic, Vuckovic, 2014) and glycans

detected in CSF in our individuals are similar to previous reports (Karlsson, Ndreu, 2017, Wuhrer, Selman, 2015).

Wuhrer *et al.* described in their study a more pro-inflammatory pattern of IgG glycosylation with elevated levels of bisecting N-acetylglucosamin (GlcNAc), afucosylation and reduced galactosylation for IgG1 in the CSF of patients with multiple sclerosis (MS) (Wuhrer, Selman, 2015). We only detected a significantly reduced galactosylation of IgG in the CSF compared to corresponding serum in our donors for IgG4. However, for all IgG subclasses analyzed the levels of bisecting GlcNAc were reduced in the CSF, whereas fucosylation did not differ significantly. Additionally, the relative amount of sialic acid was reduced in CSF IgG4 compared to serum in our cohort.

More complex glycosylation patterns of CSF proteins compared to serum were already described in the 1990s and the term “brain-type glycosylation” was coined for low amounts of terminal sialic acid, elevated levels of bisecting GlcNAc and terminal fucosylation (Hoffmann, 1996, Hoffmann et al. , 1995). However, this “brain-type glycosylation” is not in concordance with the glycosylation patterns of IgG detected in the CSF in this study. For all IgG subclasses, we detected lower levels of bisecting GlcNAc and no difference in fucosylation of IgG1. With the methods used here, we are not able to distinguish between core (a1,6-fucosylation) and terminal (a1,2-galactose or a1,3/4-GlcNAc) fucosylation, however, to our knowledge, terminal fucosylation for IgG N-glycans was so far not described, indicating that the pathways used to synthesize glycans of IgG appearing in serum and CSF are sharing common glycosyltransferases.

In general, high amounts of sialic acid and galactose are considered rather anti-inflammatory as reviewed in (Maverakis et al. , 2015). This speaks for a more pro-inflammatory effector type of IgG in CSF of our donors as we detected significantly reduced galactosylation and sialylation of IgG at least for IgG4 in the CSF. Additionally, the binding of the SNA lectin, specific for α2,6-linked sialic acid, was significantly reduced in IgG derived from CSF in our cohort. Whether this hyposialylation of CSF IgG has relevance for its effector functions needs to be further investigated. From the functional point of view, one of the main drivers for enhanced antibody-dependent cellular cytoxicity (ADCC) is afucosylation (Bruggeman, Dekkers, 2017, Nimmerjahn and Ravetch, 2012, Sakae, Satoh, 2017, Subedi and Barb, 2016). Bisecting was also published to enhance ADCC (Umana et al. , 1999); however, it is not clear if the effect is due to bisecting GlcNAc alone or an accompanying afucosylation (Shinkawa et al. , 2003). We did not detected significantly reduced levels of total fucoslyation for IgG1 in the CSF compared to corresponding serum in our individuals. Additionally, to unleash more pro-inflammatory IgG effector functions, complement and cells that mediate ADCC are needed. Wuhrer *et al.* explained the more pro-inflammatory glycosylation pattern

of IgG in the CFS of patients with MS as a result of intrathecal IgG production (Wuhrer, Selman, 2015). The inflammatory milieu of the central nervous system in MS patients shifts IgG1 glycosylation towards enhanced effector functions. However, individuals with intrathecal IgG production were excluded at the start from our study. From the twelve subjects included in this study six had only a subjective cognitive impairment probably due to depression. The other six suffered from an objective mild cognitive impairment but had no diagnosis of Alzheimer's disease or other dementia. Therefore, the intrathecally produced IgG can, in our case, not be responsible for the differences in glycosylation of CSF compared to serum.

Possible explanations for the observed differences are (I) distinct IgG glycoforms are transported preferentially into the central nervous system (CNS), (II) clearance of CSF IgG is dependent on different glycosylation compared to serum IgG or (III) glycosylation of IgG is modified at the transition from serum to CSF. As bisected IgG was significantly less detected in CSF compared to serum independent of the IgG subclass, one could argue that bisection somehow prevents the IgG from transport into the CNS. However, for sialylation our cohort was divided into two groups. The first group showed a higher sialyation of IgG1 or IgG2/3 in CSF, the second a lower sialylation of IgG1 or IgG2/3 in CSF. Therefore, not only the glycan seems to influence which IgG is transported into the CNS but maybe also the subclass. Regarding the second possible explanation, one should mention that clearance of IgG in the serum is dependent on the neonatal Fc receptor (FcnR). This receptor is also expressed at the brain microvasculature and choroid plexus epithelium as demonstrated in rats (Schlachetzki et al. , 2002). However, clearance of IgG by the FcnR is independent of IgG Fc glycosylation and happens in a pH dependent manner (Datta-Mannan et al. , 2007, Roopenian and Akilesh, 2007). Unless another Fc receptor is responsible for the clearance of IgG from the CSF this explanation seems unlikely.

Therefore, we propose that modification of IgG glycosylation at the transition of serum to CSF is at least partly responsible for the observed differences. These modifications would result in a lower galactosylation of IgG in CSF at least for IgG4 and maybe some of IgG1 and IgG2/3. However, the exact mechanism of these modifications and why not all subclasses are affected in the same way needs further investigations.

Acknowledgements

This work was funded partially by the Volkswagen Foundation project 90361, the Thyssen-Stiftung, the German Research Foundation (DFG, CRC1181-C03, KFO257, GK1660) and the EU H2020-MSCE-RISE-2015 project no. 690836 PANG, and a grant from Ministry of Healthcare of Ukraine.

The authors also acknowledge support by the funding program Open Access Publishing of the Deutsche Forschungsgemeinschaft and the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU).

Author Contributions

J. K., R. B., I. M, and M. H. C. B planned and performed experiments, conducted data analysis and wrote the manuscript. K. H., J. M. M. and P. S. collected serum, cerebrospinal fluid samples and clinical data, performed routine laboratory analysis and wrote the manuscript. A. B., M. W. and R. E. M. T. performed LC-ESI-MS and data analysis and wrote the manuscript. G. S., M. H. and L. E. M. supervised the project, planned experiments, performed data analysis and wrote the manuscript. All authors read and approved the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7:270-9.

Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science. 2008a;320:373-6.

Anthony RM, Ravetch JV. A novel role for the IgG Fc glycan: the anti-inflammatory activity of sialylated IgG Fcs. Journal of clinical immunology. 2010;30 Suppl 1:S9-14.

Anthony RM, Wermeling F, Karlsson MC, Ravetch JV. Identification of a receptor required for the anti-inflammatory activity of IVIG. Proceedings of the National Academy of Sciences of the United States of America. 2008b;105:19571-8.

Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annual review of immunology. 2007;25:21-50.

Bohm S, Kao D, Nimmerjahn F. Sweet and sour: the role of glycosylation for the anti-inflammatory activity of immunoglobulin G. Current topics in microbiology and immunology. 2014;382:393-417.

Bruggeman CW, Dekkers G, Bentlage AEH, Treffers LW, Nagelkerke SQ, Lissenberg-Thunnissen S, et al. Enhanced Effector Functions Due to Antibody Defucosylation Depend on the Effector Cell Fcgamma Receptor Profile. Journal of immunology. 2017;199:204-11.

Cassidy JT, Nordby GL. Human serum immunoglobulin concentrations: prevalence of immunoglobulin deficiencies. The Journal of allergy and clinical immunology. 1975;55:35-48.

Clerc F, Reiding KR, Jansen BC, Kammeijer GS, Bondt A, Wuhrer M. Human plasma protein N-glycosylation. Glycoconjugate journal. 2016;33:309-43.

Datta-Mannan A, Witcher DR, Tang Y, Watkins J, Wroblewski VJ. Monoclonal antibody clearance. Impact of modulating the interaction of IgG with the neonatal Fc receptor. The Journal of biological chemistry. 2007;282:1709-17.

Falck D, Jansen BC, de Haan N, Wuhrer M. High-Throughput Analysis of IgG Fc Glycopeptides by LC-MS. Methods in molecular biology. 2017;1503:31-47.

Ganrot K, Laurell CB. Measurement of IgG and albumin content of cerebrospinal fluid, and its interpretation. Clinical chemistry. 1974;20:571-3.

Hoffmann A. “Brain-Tissue-Specific” Versus “Serum-Specific” Posttranslational Modification of Human Cerebrospinal Fluid Polypeptides with N-Linked Carbohydrates. In: Couraud P-O, Scherman, Daniel, editor. Biology and Physiology of the Blood-Brain Barrier - Transport, Cellular Interactions, and Brain Pathologies1996. p. 103-7.

Hoffmann A, Nimtz M, Getzlaff R, Conradt HS. 'Brain-type' N-glycosylation of asialo-transferrin from human cerebrospinal fluid. FEBS letters. 1995;359:164-8.

Huber R, Deisenhofer J, Colman PM, Matsushima M, Palm W. Crystallographic structure studies of an IgG molecule and an Fc fragment. Nature. 1976;264:415-20.

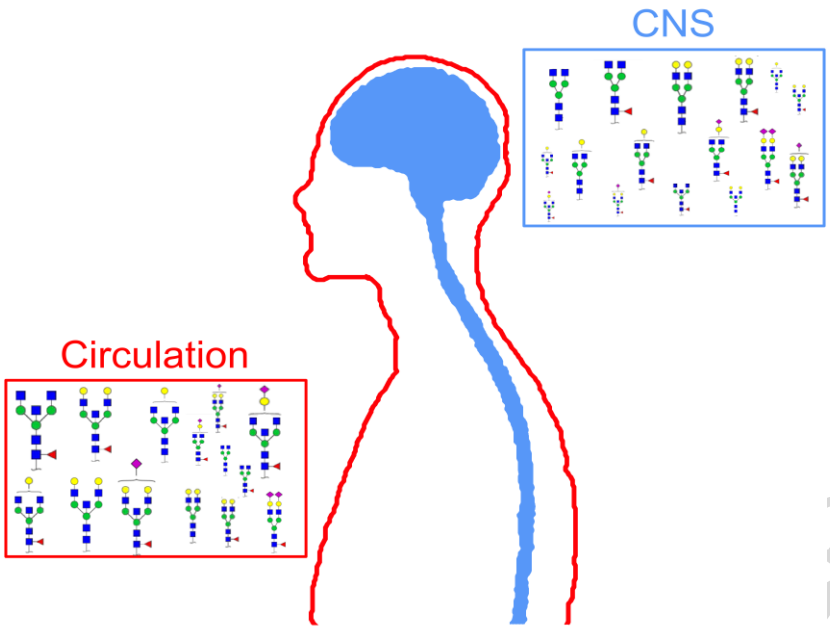
Jansen BC, Falck D, de Haan N, Hipgrave Ederveen AL, Razdorov G, Lauc G, et al. LaCyTools: A Targeted Liquid Chromatography-Mass Spectrometry Data Processing Package for Relative Quantitation of Glycopeptides. Journal of proteome research. 2016;15:2198-210.

Karlsson I, Ndreu L, Quaranta A, Thorsen G. Glycosylation patterns of selected proteins in individual serum and cerebrospinal fluid samples. Journal of pharmaceutical and biomedical analysis. 2017;145:431-9.

Keir G. TEJ. Immunoglobulins in CSF. In: French MAH, editor. Immunoglobulins in Health and Disease. Dordrecht: Springer; 1986. p. 173-87.

Kristic J, Vuckovic F, Menni C, Klaric L, Keser T, Beceheli I, et al. Glycans are a novel biomarker of chronological and biological ages. The journals of gerontology Series A, Biological sciences and medical sciences. 2014;69:779-89.

Maverakis E, Kim K, Shimoda M, Gershwin ME, Patel F, Wilken R, et al. Glycans in the immune system and The Altered Glycan Theory of Autoimmunity: a critical review. Journal of autoimmunity. 2015;57:1-13.
McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7:263-9.
Nimmerjahn F, Ravetch JV. Translating basic mechanisms of IgG effector activity into next generation cancer therapies. Cancer immunity. 2012;12:13.
Parekh RB, Dwek RA, Sutton BJ, Fernandes DL, Leung A, Stanworth D, et al. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature. 1985;316:452-7.
Parekh RB, Roitt IM, Isenberg DA, Dwek RA, Ansell BM, Rademacher TW. Galactosylation of IgG associated oligosaccharides: reduction in patients with adult and juvenile onset rheumatoid arthritis and relation to disease activity. Lancet. 1988;1:966-9.
Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nature reviews Immunology. 2007;7:715-25.
Sakae Y, Satoh T, Yagi H, Yanaka S, Yamaguchi T, Isoda Y, et al. Conformational effects of N-glycan core fucosylation of immunoglobulin G Fc region on its interaction with Fcgamma receptor IIIa. Scientific reports. 2017;7:13780.
Schlachetzki F, Zhu C, Pardridge WM. Expression of the neonatal Fc receptor (FcRn) at the blood-brain barrier. Journal of neurochemistry. 2002;81:203-6.
Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, et al. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. The Journal of biological chemistry. 2003;278:3466-73.
Stanley P, Schachter H, Taniguchi N. N-Glycans. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, et al., editors. Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY)2009.
Stumer J, Biermann MHC, Knopf J, Magorivska I, Kastbom A, Svard A, et al. Altered glycan accessibility on native immunoglobulin G complexes in early rheumatoid arthritis and its changes during therapy. Clinical and experimental immunology. 2017;189:372-82.
Subedi GP, Barb AW. The immunoglobulin G1 N-glycan composition affects binding to each low affinity Fc gamma receptor. mAbs. 2016;8:1512-24.
Thomann M, Reckermann K, Reusch D, Prasser J, Tejada ML. Fc-galactosylation modulates antibody-dependent cellular cytotoxicity of therapeutic antibodies. Molecular immunology. 2016;73:69-75.
Umana P, Jean-Mairet J, Moudry R, Amstutz H, Bailey JE. Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. Nature biotechnology. 1999;17:176-80.
van de Geijn FE, Wuhrer M, Selman MH, Willemsen SP, de Man YA, Deelder AM, et al. Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study. Arthritis research & therapy. 2009;11:R193.
Wuhrer M, Selman MH, McDonnell LA, Kumpfel T, Derfuss T, Khademi M, et al. Pro-inflammatory pattern of IgG1 Fc glycosylation in multiple sclerosis cerebrospinal fluid. Journal of neuroinflammation. 2015;12:235.
Yamaguchi Y, Nishimura M, Nagano M, Yagi H, Sasakawa H, Uchida K, et al. Glycoform-dependent conformational alteration of the Fc region of human immunoglobulin G1 as revealed by NMR spectroscopy. Biochimica et biophysica acta. 2006;1760:693-700.



Graphical abstract

Highlights

- Immunoglobulin G (IgG) in cerebrospinal fluid differs significantly from serum IgG
- Cerebrospinal fluid IgG is characterized by significantly less bisected glycans
- IgG4 derived from serum is more galactosylated and sialylated
- Sialic acid specific *Sambucus nigra* lectin preferentially binds to serum IgG