Aberrant Immunoglobulin G glycosylation in Multiple Sclerosis

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ABSTRACT

A hallmark of the inflammatory response in multiple sclerosis (MS) is the presence of intrathecal Immunoglobulin G (IgG) antibodies and oligoclonal bands (OCBs). The biological activity of IgGs is modulated by changes in glycosylation. Using multiple immunoassays with common lectins for sialylation and galactosylation, we investigated levels of IgG glycosylation in 28 MS and 37 control sera as well as paired CSF and serum. We demonstrated the presence of significantly lower levels of IgG sialylation in MS CSF compared to paired serum. Further, we showed that in MS there was no correlation between sialylated IgG and total IgG antibodies, or between sialylated IgG in CSF and serum. ELISA with native IgG antibodies showed significantly higher levels of sialylated and galactosylated IgG in MS compared to other neurological disorders and normal healthy controls. We conclude that lower levels of sialylated intrathecal IgG and higher levels of serum IgG galactosylation in MS may play significant role in disease pathogenesis. The unique IgG glycosylation profiles suggest a complexed nature of the IgG antibodies which may influence its effector functions in MS.

INTRODUCTION

The inflammatory demyelinating disease Multiple Sclerosis (MS) is the most common cause of neurological disability in young adults in Northern temperate regions. A characteristic feature of the central nervous system (CNS) inflammatory response in MS is the intrathecal synthesis of Immunoglobulin G (IgG) and the presence of oligoclonal bands (OCBs) which occurs in over 90% of patients (1). There is mounting evidence that intrathecal IgG and OCBs are associated with increased levels of disease activity, disability, and cortical lesion load (2-5). OCBs have been assumed to target antigens relevant to MS pathogenesis. However, no specific targets have been found (6-10). Our recent studies showed that there is a complex relationship between OCBs and CSF IgG and the presence of higher levels of

serum IgG in MS (11) suggesting that a better understanding of not only the antigenic specificity (12,13) but the characteristics of the effector function of the IgG antibodies in MS would be of considerable importance.

IgG antibodies consist of two structural regions: a variable fragment (Fab) that mediates antigen binding and a constant fragment (Fc) that mediates effector functions via its interaction with Fc gamma receptors (FcγRs) on immune cells or with C1q, the recognition molecule of the complement system. All IgG subclasses contain a highly conserved asparagine-linked (N-)oligosaccharide located in the CH2 domain of the Fc region, and glycans are also present in about 15% of variable domains (14). Glycans on the immunoglobulin have a very strong influence on the binding to Fc receptors. Variations in IgG Fc N-glycosylation are associated with autoimmune disease activity by influencing binding to FcγRs on effector cells as well as immune mediators (15-18). Furthermore, it has been suggested that antibodies with decreased levels of terminal sugar residues might be more pathogenic (19-22). Relevant to this was the observation that IgG galactosylation was significantly altered in CSF but not in the serum of MS patients, and that this modification was correlated with an active progression of MS (23).

The extension of IgG glycans by the addition of sialic acid significantly changes the biological function of IgG, converting it from a pro-inflammatory into an anti-inflammatory agent (24). This relatively small fraction of sialylated IgG is believed to be responsible for the immunosuppressive activity of intravenously administered immunoglobulins and is associated with sialic acid in α 2,6 linkage to a terminal galactose on IgG (24,25). Further, sialylated autoantigen-reactive IgG antibodies have been shown to attenuate disease

development in autoimmune mouse models of lupus nephritis and rheumatoid arthritis (26). In contrast, asialylated IgG antibodies are proinflammatory and are induced by the combination of T cell-dependent protein antigens and pro-inflammatory co-stimulation (27). Further, low levels of sialyation and galactosylation in total IgG1, but not antigen-specific IgG, have been shown to predict disease reactivation in patients of granulomatosis with polyangiitis (28).

We hypothesized that MS CSF IgG have different levels and forms of glycosylation in the Fab and Fc regions compared to paired serum, and that IgG antibody in MS are differentially glycosylated. In this study, we investigated levels of IgG glycosylation in MS and controls using Western blots and ELISA with 3 common biotinylated lectins: Sambucus nigra agglutinin (SNA, for α2, 6 linked sialylation), Ricinus communis agglutinin (RCA, for terminal galactose, and Griffonia (Bandeiraea) Simplicifolia (GSA, for alpha- or beta-linked N-acetylglucosamine residues).

METHODS

Patients

The study protocol was approved by the University of Colorado Institutional Review Board (COMIRB # 00–688). CSF and sera from MS patients and controls were collected at University of Colorado Hospital. After blood collection, serum samples were treated using one of the following protocols. The sample was either allowed to coagulate, centrifuged at 1100 x g for 5 minutes at 4°C, and then the supernatant was removed and stored at -80°C until use; or the sample was mixed with an anticoagulant in its collection tube, centrifuged at 1500 x g for 20 minutes (no brake) at room temperature, and then the supernatant was

collected and stored at -80°C until use. CSFs were immediately centrifuged at 500 x g for 10 min, and the supernatant was collected. Both CSF and sera were stored at −80°C until use. The CSF of all MS patients contained oligoclonal bands. Patient diagnosis, age range, sex, and number of OCBs are detailed in Table 1.

CSF/Serum IgG purification

Dynabeads® Protein A (Invitrogen/Thermo Fisher) were prepared by using 35µl of the bead on a Dynal MPC©-S magnetic particle concentrator (Invitrogen). A 200 µl solution containing 7 µg of CSF or serum IgG in 0.05% PBS-Tween was added to the beads and incubated by rotation at room temperature for 40 minutes. The bead-antibody complex was then washed with 200 µl 0.05% PBS-Tween, and IgG was eluted by adding 45 µl of 0.2M glycine, pH 2.2, followed by addition of 5µl of 1M Tris-HCl pH 9.1 to neutralize. Pierce BCA Protein Assay Kit (Pierce Thermo Scientific/Thermo Fisher) was used for determining the concentration of purified CSF and serum IgG.

Lectin Western Blot

Mini-PROTEAN® TGX 4-15% Gels (BioRad) were used for SDS-PAGE analysis with 1× Tris/Glycine/SDS running buffer (BioRad). Purified CSF and serum IgG (1µg) in TBS were denatured and reduced by incubation with 1× lane marker reducing sample buffer containing dithiothreitol (Thermo Scientific) at 95°C for 10 min. Gels were electrophoresed for 40 min and electro-blotted onto a PVDF membranes (Bio-Rad) using Trans-Blot® Semi-Dry Cell (Bio-Rad). Membranes were blocked overnight in either 1× casein/TBS/0.05% Tween 20 (Vector Labs) for IgG blots or 1× carbo-free/TBS/0.05% Tween^{20} (Vector Labs) for lectin blots. IgG binding was detected with HRP-conjugated goat anti-human IgG (H+L) (Vector Labs) (1:5000) for 1 hour at room temperature, followed by incubation with SuperSignal®

West Pico substrate for detection (Pierce Thermo). Lectin binding was detected with 1µg/ml biotinylated elderberry bark lectin Sambucus nigra (SNA), 5µg/ml biotinylated griffonia simplicifolia lectin II (GSA) or 1µg/ml biotinylated ricinus communis agglutinin I (RCA) for 1 hour at room temperature. All lectin probes were from Vector Labs. Membranes were then incubated with Pierce® High Sensitivity NeutrAvidin®-HRP for 1 hour at room temperature at a dilution of 1:50,000 for SNA blots, 1:20,000 for GSA blots and 1:50,000 for RCA blots, followed by SuperSignal® West Pico substrate for chemiluminescent detection.

Quantifying Western blot band intensity

The FluorChem Q™ system (ProteinSimple) was used to detect the signal produced by chemiluminescent substrate. The images were analyzed quantitatively by AlphaView software for FluorChem™ systems.

Capture Enzyme-Linked Immuno-Sorbent Assay (ELISA)

ELISA plates (Thermo Fisher) were coated with 50 µg/ml (50µl/well) unconjugated goat anti-Human IgG (Vector Labs) as capture antibody overnight at 4ºC. CSF and serum at 0.25µg IgG/well (50 µl /well) were added and incubated for 1 hour at room temperature. After washing with 0.05% TBST, wells were blocked with either Carbofree blocking buffer (for detections of lectin binding, Vector Labs) or with 5% milk (for detection of IgG) for 1 hour at room temperature. We used biotinylated goat anti-human IgG Antibody (1:5000, Vector Labs) for total IgG quantification and biotinylated SNA and RCA (5ug/ml) for determination of glycosylated IgG. The plates were washed 10 times with 0.05% TBST. For secondary antibody/reagent incubation, 1:500 dilution of Neutr-Avidin HRP (Pierce) was added. Color detection was achieved by addition of ABTS (VWR) and OD reading was at 405 nm.

Statistical analysis

For paired CSF and serum ELISA. Distributions were checked and summary statistics were prepared for each outcome. The MS group was compared to the control group using two-sample Satterthwaite T-tests. The magnitudes of the differences were expressed as percent changes relative to the control group. The family-wise type I error rate was controlled with the Bonferroni correction. The boxplots were generated in R. Whiskers extend the entire length of the data. The box showed the interquartile range (25^{th} percentile to 75^{th} percentile). The bar is the median (50^{th} percentile), and the mean is indicated by the diamond symbol.

For all other analyses: One-way ANOVA followed by Tukey's analysis for multiple comparisons were used to examine differences between groups. Pearson's correlation was used for statistical correlation analysis. The differences between groups were considered significant if the *p*-value was less than 0.05 (two-tailed). All statistical analyses were performed and graphs generated using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

We applied three common biotinylated lectins, Sambucus nigra agglutinin (SNA, for α2, 6-linked sialylation), Ricinus communis agglutinin (RCA, for terminal galactose), and Griffonia (Bandeiraea) Simplicifolia (GSA, for alpha- or beta-linked N-acetylglucosamine residues) to investigate levels of glycosylation in MS IgG antibodies.

Significantly lower levels IgG sialylation were detected in MS CSF by lectin-probed Western blots

We first carried out Western blots using native unpurified MS CSF and paired serum and probed with biotinylated lectins SNA, RCA, and GSA. We showed that there were different lectin binding patterns between CSF and serum in all three lectins tested. Fig. 1A shows a representative blot. SNA lectin blot showed more extensive binding in both CSF and serum compared to RCA and GSA blots. To further characterize IgG glycosylation of MS IgG antibodies, we purified total IgG using Protein A Magnetic Beads from 12 paired MS CSF and sera as well as nine inflammatory control CSF and sera. Fig. 1B shows a representative Western blot comparing original CSF/serum to Protein A purified CSF/serum probed with goat anti-human IgG antibody (H+L). Notice that Protein A purified IgG show a different pattern than original samples. We performed Western blots of the purified MS and IC IgG antibodies and detected levels of glycosylation with SNA, RCA, and GSA probes, respectively. Fig. 1C, top panel shows a representative MS IgG blot probed with SNA lectin which detects α2,6-linked sialylation. The Protein A purified MS CSF IgG showed a less intensely stained heavy chain band (50 kDa) without a 75 kDa heavy chain band compared to paired serum IgG. However, the duplicate blot detecting total IgG (with goat anti-human IgG antibodies as positive control) did not reveal such a difference, showing approximately equal amounts of IgG heavy chain (50 kDa) in both CSF and paired serum, and no extra 75 kDa band in the serum (Fig. 1C, middle panel). Coomassie blue stained gel shown in the lower panel of Fig. 1C confirmed the equal loading of purified IgG antibodies and the missing extra 75 kDa bands in CSF. These results suggest that only with the SNA lectin probe that the presence of lower levels of sialic acid in MS CSF IgG can be detected. For the detection of galactosylation and N-acetylglucosamine, similar Western blots were carried out with corresponding lectin probes RCA and GSA, and data are summarized below.

To quantify the levels of glycosylation between CSF and paired serum, and between MS and IC patients, we measured the 50 kDa IgG heavy chain band intensity and calculated the ratio

of CSF to paired patients' sera. The data summary is presented in Fig. 1D-1F. We show that Western blots with protein A purified IgG demonstrated significantly lower levels of sialylated IgG in MS CSF compared to total CSF IgG of the same patients (p<0.0001, n=12), but no such difference was observed in patients with inflammatory CNS diseases (n=9). Further, the ratio of sialylated IgG between MS CSF and sera was significantly lower compared to that of IC patients (p<0.0001, Fig. 1D). For RCA Western blots, there was no difference between levels of RCA binding of IgG compared to total IgG, indicating that galactosylation of MS CSF IgG are the same compared to total CSF IgG. Similarly, no such differences were detected in IC patients (n=7) (Fig. 1E). GSA Western blots detecting levels of N-acetylglucosamine did not reveal any differences for GSA binding in the MS CSF/serum ratio (n=12) compared to that found in total IgG and GSA binding in IC patients (n=5) (Fig. 1F).

Capture ELISA of paired native CSF and serum IgG antibodies demonstrated that there were no differences in SNA lectin binding between MS and other inflammatory CNS disorders

We next carried out dose-response capture ELISA of paired MS CSF and serum to determine levels of sialic acid in native IgG antibodies (Fab and Fc) in MS patients compared to patients with other inflammatory CNS diseases as well as optic neuritis (ON). A total of 14 paired MS CSF/serum, six pairs of IC CSF/serum, and seven pairs of ON CSF/serum were studied. Biofluid IgGs were captured on ELISA wells coated with capture antibodies (goat anti-human IgG, H+L chains). In contrast to the results of Western blots of Protein A purified IgG, the capture ELISA results showed that there was no difference in the amount of SNA binding between MS CSF IgG and control CSF IgG, nor between MS serum and control

serum (Fig. 2A, B). We did, however, observed a significant difference in the total IgG levels between MS CSF compared to ON CSF (p=0.007) and between MS serum and ON serum (p=0.024) (Fig. 2C, D). Notice that there are similar binding profiles for sialylated IgG and total IgG between CSF and serum in MS and inflammatory controls.

Capture ELISA of native IgG reveal that there was no correlation between sialylated IgG and total IgG, and between sialylated CSF and serum IgG in MS

To determine the relationship between sialylated IgG and total IgG in paired MS CSF and serum, we analyzed the results of native IgG capture ELISA probed with SNA and goat anti-human IgG (H+L) using the Pearson correlation coefficient. We showed that there was no correlation between SNA binding (sialylated IgG) and total IgG in both MS CSF (r = -0.06; p=0.82) and serum (r=0.36; p=0.19) (Fig. 3, A, B). However, no such correlation was observed between CSF sialylated IgG and total IgG of IC and ON patients (Fig. 3C, E), although strong relations were found between sialylated IgG and total IgG in the sera of IC patients (r=0.86; p=0.017) and ON patients (r=-0.74, p=0.07) (Fig. 3, D, F).

To further highlight the distinctive characters of MS IgG antibodies, we showed that there was no correlation in MS between sialylated IgG in CSF and that of paired serum (r=0.46; p=0.085, Fig. 4A). By contrast, there was a strong correlation detected between sialylated IgG in CSF and that of paired serum in IC patients (r=0.87; p=0.053) and patients with ON (r=0.88; p=0.0032) (Fig. 4B, C). Interestingly, significant correlations between total IgG in CSF and paired serum were observed in MS (r=0.97; p<0.0001) (Fig. 4D), consistent with

our previous findings (11). Similarly, strong correlations were found in IC patients (r=0.87; p=0.013) and patients with ON (r=0.82, p<0.0001) (Fig. 4E, F).

Capture ELISA of MS sera showed significantly higher levels of sialylated and galactosylated IgG antibodies

We expanded the IgG glycosylation study with larger number of MS sera and various control sera and carried out both SNA and RCA lectin ELISA using captured native serum IgG from unpurified sera. Although we detected no differences between MS (n=28) and IC patients (n=11) in SNA binding of serum IgG, significantly higher levels of sialylated IgG were present in MS sera compared to other controls which included 10 non-inflammatory neurological controls (NIC), 8 ON, and 8 healthy controls (p=0.0072, Fig. 5A). Similarly, no differences were detected between MS (n=23) and IC patients (n=7) for RCA binding in serum IgG, but significantly higher levels of galactosylated IgG were found in MS sera compared to non-inflammatory neurological controls (n=10) (Fig. 5B). Tukey's multiple comparisons test was used for p values in both lectin ELISA.

DISCUSSION

We applied three biotinylated lectins for Western blots and sandwich ELISA to determine the degree of IgG glycosylation in the CSF and paired serum of patients with MS and controls. The key findings were: (i) Protein A-purified IgG lectin Western blots (under reduced and denatured conditions) showed significantly lower levels of sialylated IgG in MS CSF compared to total IgG of the same patients as well as to sialylated CSF IgG in patients with other inflammatory CNS diseases; (ii) capture ELISA of native serum IgG revealed higher

levels of SNA and RCA binding, suggesting the presence of higher levels of IgG antibodies with both sialylation and galactosylation; (iii) there are no correlations between sialylated IgG and total IgG antibodies in both CSF and serum, or between sialylated CSF IgG and sialylated serum IgG in MS patients. Our data suggest the presence of unique glycosylation patterns of Fc and Fab IgG antibodies and an altered complexed nature of these antibodies in patients with MS.

Our study further demonstrates how the exposure of glycans in native complexed IgG in MS can allow the detection of particular glycosylation and its changes by IgG purification methods. We found marked differences in glycosylation, and particular sialylation, that were detected in reduced and denatured Protein A purified MS CSF IgG but not in native MS IgG, and CSF rather than serum of MS patients. This demonstrates the value of analysing IgG antibodies in both CSF and (paired) serum of MS patients, and also that different data sets can be obtained depending on the particular IgG purification method and immunoassays performed, as well as specific lectin probes used. The importance of comparing such findings with those obtained from disease and healthy control CSF and serum was also evident in this analysis.

The different glycosylation patterns of Fc and Fab antibodies in MS are derived based on the contrasting data that lower levels of SNA binding in Protein A-purified IgG Western blots (Fc binding) and higher levels of SNA and RCA binding in capture (sandwich) ELISA of native IgG antibodies (detecting both Fab and Fc glycosylation). Our findings are consistent with those reported by Decker et al. (23) which demonstrated altered IgG glycosylation in the CSF but not the serum of MS patients with the modification being correlated with progressive disease. Our findings that MS sera have higher levels of IgG galactosylation (detected by

RCA lectin binding) are significant and indicate that serum IgG antibodies could play an important role in disease pathogenesis as suggested by our recent papers (11,29). Higher Fc-galactosylation of IgG has been shown to improve C1q binding and enhances complement-dependent cytotoxicity (30).

The higher levels of RCA binding in MS sera are supported by the findings that Fc-galactosylation of IgG improves C1q binding and enhances complement-dependent cytotoxicity (30). It has also been noted above that IgG glycosylation is associated with several other neurological diseases with an impact on disease activity (17). Antibody effector functions relevant to immunity are influenced by different IgG glycosylation profiles following influenza and tetanus vaccination (31). In addition, it has been shown that IgG in diabetic patients is non-enzymatically glycosylated, an immunological modification that might be associated with a functional alteration relevant to disease (32). Further, Sjowall et al (33) reported that alteration of glycosylation of complexed native IgG was associated with disease activity in the autoimmune disease systemic lupus erythematosus. The actual mechanism of this IgG modification is unclear but it is of relevance that Wang et al (34) showed that different stimuli of B cells during their activation and differentiation can modulate the Fc-linked glycosylation of secreted IgG without affecting the general glycosylation machinery.

Our observation that lower levels of sialylated IgG antibodies were detected in the CSF of MS patients is likely to be pathologically relevant since sialylated IgG is known to have anti-inflammatory activity (24,25). It is logical to assume that a lower level of sialylated CSF IgG in MS would lead to the CSF antibodies having a more pro-inflammatory function, which may be the driver of disease, reflecting the role of intrathecal IgG in MS. A clue was provided by Hess et al (27) who found in experimental mice that T-cell independent immune responses induced suppressive sialylated IgGs, in contrast to T-cell dependent pro-

inflammatory Th1 and Th17 immune response which induced galactosylated and asialylated IgG. Moreover, the transfer of low amounts of antigen-specific sialylated IgG antibodies was sufficient to inhibit B cell activation and pathogenic immune reactions (27).

Our ELISA data that no correlations were observed between sialylated IgG and total IgG in both CSF and serum, or between sialylated CSF and serum IgG in MS patients suggest that MS IgG antibodies may be present in conformationally complexed forms which prevent direct bindings by lectins, further support our previous findings of a complicated relationship between oligoclonal bands and IgG in MS (11). This unique characteristic of MS IgG also provides clues suggesting why discovery of a common antigen has been elusive (10).

Our studies have three intrinsic limitations. First, the number of CSF and serum samples analysed was relatively small. Second, we are unable to assume that the particular IgG glycosylation and sialyation modifications pertain to all patients with MS, a neurological disease with marked heterogeneity. Third, we are unable to exclude with complete confidence the very small possibility that our observations represent epiphenomena that are in reality unrelated to MS disease pathogenesis. However, the abundant evidence that these two types of IgG glycosylation are causative rather than irrelevant to autoimmune and other inflammatory diseases is very much in favour of our observations being relevant to disease causation and/or activity. Our studies may provide therapeutic strategies.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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FIGURE LEGENDS

Fig. 1. Western blot analysis of MS CSF/serum and their purified IgG antibodies with lectin and IgG (H+L) probes.

A. Western blots of original CSF (C) and paired serum (S) from one MS patient probed with SNA, RCA, and GSA lectins, respectively. Notice that there are different glycosylation patterns between CSF and serum. B. Western blots of original CSF/serum and Protein A purified CSF/serum IgG probed with goat anti-human IgG antibody (H+L). Notice that Protein A purified IgG show a different pattern than original samples. C. Representative Western blots and Coomassie blue stained gel of Protein A purified IgG from 6 paired MS CSF and sera probed with SNA lectin and goat anti-human IgG. Top panel: SNA lectin detects α2,6 linked sialylation in IgG molecules. Protein A purified MS CSF IgG show a lighter heavy chain band (50 kDa) and no 75 kDa band compared to paired serum IgG. Middle panel: Duplicate blot probed with goat-antihuman IgG antibody demonstrating equal loading of purified IgG. There is no difference in IgG heavy chain band intensity (50 kDa) between CSF and serum. Bottom panel: Equal amount of Protein A purified CSF and serum IgG (based on BCA assay) loading was demonstrated by Coomassie blue staining.

We measured the band intensity of the IgG heavy chain (50 kDa) of lectin Western blots and calculated the ratio for CSF to paired serum in MS and IC patients, and summarized the data as shown in D-F. D. SNA lectin blots summary. There are significantly lower levels of sialylated IgG in MS CSF compared to total CSF IgG of the same patients ($p<0.0001$, $n=12$), and compared to sialylated CSF IgG in patients with inflammatory diseases (IC, $n=9$). E. Summary of RCA Western blots showing no difference for galactosylation of IgG in MS CSF/serum ratio ($n=12$) compared to total IgG and galactosylation of IgG in IC patients

(n=7). F. Summary of GSA lectin blots showing no difference for GSA binding to purified IgG in MS CSF/serum ratio compared to total IgG and GSA binding in IC patients (n=5).

Fig. 2. Capture ELISA of native MS CSF and paired serum IgG demonstrated that there are no differences in SNA lectin binding between MS and patients with other inflammatory disorders, and optic neuritis.

Paired MS CSF and serum (n=14) and controls (IC=6; ON=7) at 0.25 µg of IgG equivalent were added to wells precoated with capture antibody unconjugated goat anti-human IgG (H+L). The captured IgG were probed with biotinylated lectin SNA (5 µg/ml) followed by incubation with NeutrAvidin-HRP (1:500). Duplicate ELISA plates with captured MS IgG antibodies were detected with biotinylated goat anti-Human IgG (1:5000) followed by the addition of NeutrAvidin-HRP (1:500). After washing, ABTS was added for color development, and OD was measured at 405 nm. No differences were found in SNA binding between MS CSF and control CSF (A), nor between MS serum and control serum (B). However, a significant difference in total IgG levels were found in MS CSF compared to ON CSF (p=0.007) (C) and between MS serum and ON serum (p=0.024) (D).

Fig. 3. ELISA of native MS IgG show that there was no correlation between sialylated IgG and total IgG antibodies in both CSF and serum.

We further analyzed the native IgG ELISA data to determine the relationship between SNA lectin binding (detecting sialylated IgG) and total IgG in CSF and paired serum in MS, IC, and ON patients. Native MS IgG captured in ELISA plates were detected by SNA lectin and goat anti-human IgG respectively. We show that there is no correlation between sialylated IgG and total IgG in both MS CSF (A, r=-0.06; p=0.82) and serum (B, r=0.36; p=0.19). Although there is no correlation between sialylated IgG and total IgG in CSF of IC (C) and

ON patients (E), a correlation between sialylated IgG and total IgG was detected in sera of IC patients (D, r=0.86; p=0.017) and ON patients (F, r=-0.74, p=0.07).

Fig. 4. ELISA of native MS IgG data further demonstrate that there is no relationship between sialylated IgG in MS CSF and paired serum.

We further analyzed the native IgG ELISA data to determine the relationship between SNA lectin binding (detecting sialylated IgG) in CSF and paired serum in MS. We show that there is no correlation between sialylated IgG in MS CSF and paired serum (A, r=0.46; p=0.085). In contrast, strong correlations were observed between sialylated IgG in CSF and paired serum in IC (B, r=0.87; p=0.053) and ON patients (C, r=0.88; p=0.0032). For total IgG, significant correlations between IgG in CSF and serum were observed in MS (D, r=0.97; p<0.0001), IC (E, r=0.87; p=0.013), and ON (F, r=0.82, p<0.0001).

Fig. 5. Serum SNA and RCA lectin ELISA with captured native IgG show that there are no differences between MS and IC, but significantly higher levels of sialylated IgG and galactosylated IgG in MS compared to other neurological disorders and healthy donors.

A. SNA ELISA detecting sialylated IgG in MS and controls. Although no differences were detected between MS (n=28) and IC patients (n=11) in SNA binding, significantly higher levels of sialylated IgG were present in MS sera compared to other controls (ON=8, NIC=10, HC=8; p=0.0072). p value was calculated based on Tukey's multiple comparisons test. B. RCA ELISA detecting galactosylated IgG in MS and controls. Although no differences were detected between MS (n=23) and IC patients(n=7) in RCA binding, significantly higher levels of galactosylated IgG were observed in MS sera compared to non-inflammatory neurological controls (n=10). p values were calculated based on Tukey's multiple comparisons test.

IC *	Diagnosis	Age	Sex	OCB	WB
IC-1 [1,2]	Lymphoma	30-40	F	0	X
IC-2 [1]	Meningitis	50-60	M	3	X
IC-3 [2]	Meningitis	40-50	F	0	X
IC-4 [1, 2]	C. Meningitis	50-60	M	2	
IC-5 [2]	SSPE	20-30	M	17	X
IC-6	SSPE	30-40	F	4	X
IC-7 [1,2]	Neoplastic S.	70-80	M	21	
IC-8 [1,2]	M.E.	40-50	M	7	
IC-9 [2]	Crohn's disease	40-50	F	2	
IC-10 [1,2]	VZV	70-80	F	0	
IC-11 [1,2]	Retrobulbar ON	50-60	F	8	
IC-12 [2]	Meningitis	50-60	M	3	
IC-13 [1,2]	Papilledema	40-50	F	0	
IC-14 [1,2]	ADEM	40-50	M	0	
ON-1 [*1]	NMO Sero+	30-40	F	0	
ON-2	NMO sero+	30-40	M	0	
ON-3	NMO sero+	50-60	F	0	
ON-4	NMO sero-	20-30	M	15	
ON-5	NMO sero+	20-30	F	0	
ON-6	NMO sero+	50-60	F	5	
ON-7	NMO sero-	20-30	F	0	
ON-8	NMO sero-	20-30	F	2	
NIC-1 [*2]	Headache	50-60	F	N/A	
NIC-2	Headache	50-60	F	N/A	
NIC-3	Headache	60-70	F	N/A	
NIC-4	Headache	40-50	F	N/A	
NIC-5	Headache	20-30	M	N/A	
NIC-6	Headache	30-40	F	N/A	
NIC-7	Headache	50-60	M	N/A	
NIC-8	Headache	40-50	F	N/A	
NIC-9	Headache	50-60	M	N/A	
NIC-10	Headache	50-60	M	N/A	
HC-1 [*2]	N/A	30-40	M	N/A	
HC-2	N/A	20-30	F	N/A	
HC-3	N/A	30-40	F	N/A	
HC-4	N/A	20-30	M	N/A	
HC-5	N/A	50-60	F	N/A	
HC-6	N/A	20-30	F	N/A	
HC-7	N/A	50-60	F	N/A	
HC-8	N/A	20-30	F	N/A	

MS *	Age	Sex	OCB	WB
MS-1 [2]	30-40	F	5	X
MS-2 [1,2]	20-30	F	3	X
MS-3 [1,2]	20-30	F	2	
MS-4 [1,2]	50-60	F	4	
MS-5 [1]	40-50	F	0	
MS-6 [2]	40-50	M	0	
MS-7	30-40	F	1	X
MS-8 [1,2]	20-30	F	6	
MS-9 [2]	40-50	F	3	X
MS-10 [2]	40-50	M	0	X
MS-11 [1,2]	20-30	F	4	
MS-12 [2]	50-60	F	6	X
MS-13	40-50	F	3	X
MS-14 [1,2]	30-40	F	8	X
MS-15 [2]	40-50	F	5	X
MS-16 [1,2]	40-50	F	7	
MS-17	40-50	F	5	X
MS-18 [2]	40-50	F	4	
MS-19 [1]	20-30	F	5	X
MS-20 [1,2]	50-60	F	2	
MS-21 [2]	30-40	M	2	
MS-22 [2]	50-60	F	1	
MS-23 [2]	20-30	F	2	
MS-24 [1,2]	40-50	M	0	
MS-25 [1]	50-60	F	19	X
MS-26 [2]	30-40	F	21	
MS-27	50-60	F	22	X
MS-28 [1,2]	50-60	M	16	
MS-29 [1,2]	60-70	M	14	
MS-30	40-50	M	8	X
MS-31	40-50	F	19	X
MS-32 [1,2]	40-50	F	18	
MS-33 [1,2]	60-70	F	28	X
MS-34 [2]	30-40	F	12	
MS-35 [2]	20-30	F	14	
MS-36 [2]	30-40	M	3	
MS-37	50-60	F	19	X
MS-38	40-50	M	19	X

Table 1. Characteristic of patients studied.

*=IC and MS patients. Diagnosis, age, sex, and number of OCB are listed. WB=Western blot. Patients are indicated where WB were performed. [1] ELISA 1 (CSF and serum paired ELISA) were performed. [2] ELISA 2: serum ELISA were performed. ON [*1]: only ELISA 1 (CSF and serum paired ELISA) were performed on these patients. NIC [*2] and HC [*2]: ELISA 2 (serum ELISA) were performed).