Biochimica et Biophysica Acta 1853 (2015) 2115–2121

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

The role of N-glycan in folding, trafficking and pathogenicity of myelin oligodendrocyte glycoprotein (MOG)☆

Joanna Jung, Elzbieta Dudek, Marek Michalak *

Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form 11 December 2014
Accepted 16 December 2014
Available online 23 December 2014

Keywords:
Myelin oligodendrocyte glycoprotein (MOG)
Endoplasmic reticulum
Molecular chaperones
Calnexin
Calreticulin
ERp57

ABSTRACT

Myelin oligodendrocyte glycoprotein (MOG) is a type I integral membrane protein that is expressed in the central nervous system. MOG has a single N-glycosylation site within its extracellular domain. MOG has been linked with pathogenesis of multiple sclerosis; anti-MOG antibodies have been detected in the sera of multiple sclerosis patients. N-glycosylation is an important post-translational modification of protein that might impact their folding, localization and function. However, the role of sugar in the biology of MOG is not well understood. In this study, we created a mutant MOG lacking N-linked glycan and tested its properties. We concluded that the lack of sugar did not impact on MOG abundance in the absence of endoplasmic reticulum molecular chaperone calnexin. We also show that the absence of N-glycan did not interfere with MOG's subcellular localization and it did not result in activation of endoplasmic reticulum stress. This article is part of a Special Issue entitled: 13th European Symposium on Calcium.

1. Introduction

Anti-myelin antibodies play a role in the pathogenesis of multiple sclerosis, a demyelinating disorder of the central nervous system (CNS) in a subset of patients [1]. Myelin oligodendrocyte glycoprotein (MOG) is a relatively minor component of the CNS, however, its localization to the outermost membrane of the myelin layer makes it an accessible target for pathogenic antibodies [2,3]. MOG has a single glycosylation site within its extracellular domain [4,5], exposed on the surface of the myelin layer, but the physiological and biochemical features of the MOG glycan remain largely unknown. Post-translational modifications, including glycosylation, have an important impact on the antigenic properties of proteins [6]. The role of MOG-glycosylation in autoantibody binding has been studied using various techniques [7–9]. Using cell-bound assay it has been shown that the absence of N-glycan had no significant effect on autoantibody binding [7]. However, in another studies, NMR analysis show that the presence of glycan on MOG peptide improves detection by autoantibodies present in the sera of multiple sclerosis patients, and anti-MOG antibody concentration corresponds to multiple sclerosis disease severity [10,11]. In the mouse model pathogenic IgGs, preferentially recognize glycosylated MOG [9]. Moreover, recently N-glycan on human MOG has been shown to play a role in immune homeostasis within the CNS [12]. We have previously shown that calnexin, a molecular chaperone, interacts with MOG within the lumen of the endoplasmic reticulum (ER) [13], where the maturation and post-translational modifications of the MOG take place. Calnexin and calreticulin are two lectin homolog chaperones that closely monitor protein folding and maturation in the lumen of ER; ERp57 is an oxidoreductase that catalysis disulfide bond formation and isomerization in glycoproteins (frequently calnexin/calreticulin substrates). Together, calnexin, calreticulin and ERp57 are essential players of the quality control machinery in the lumen of ER [14]. Here we investigated the effect of a single N-glycosylation site on MOG cell surface distribution, in a wild-type and chaperones deficient cells; MOG interaction with calnexin, induction of ER stress and encephalitogenic properties.

2. Materials and methods

2.1. Cell lines, cell culture, lentiviral transduction and immunofluorescence

Wild-type and calnexin-deficient mouse fibroblasts were isolated from newborn mice and immortalized [15]. Wild-type and calreticulin-deficient (K42) mouse embryonic fibroblasts were described previously [16]. ERp57-deficient mouse embryonic fibroblasts were isolated from *ERp57*$^{-/-}$ embryos and immortalized [17]. Lentiviral construct encoding mouse MOG [13] was used as template to create construct encoding MOG(N^{31}A)GFP (green fluorescent protein) using site directed

Abbreviations: CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; ER, endoplasmic reticulum; GAPDH, glyceraldehyde phosphate dehydrogenase; GFP, green fluorescent protein; MOG, myelin oligodendrocyte glycoprotein. MOG_{35-55} sequence: MetGluValGlyTrpTyrArgSerProPheSerArgValValHisLeuTyrArgAsnGlyLys

☆ This article is part of a Special Issue entitled: 13th European Symposium on Calcium.

* Corresponding author at: Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2S7. Tel.: +1 780 492 2256; fax: +1 780 492 0886.
E-mail address: marek.michalak@ualberta.ca (M. Michalak).

http://dx.doi.org/10.1016/j.bbamcr.2014.12.023
0167-4889/

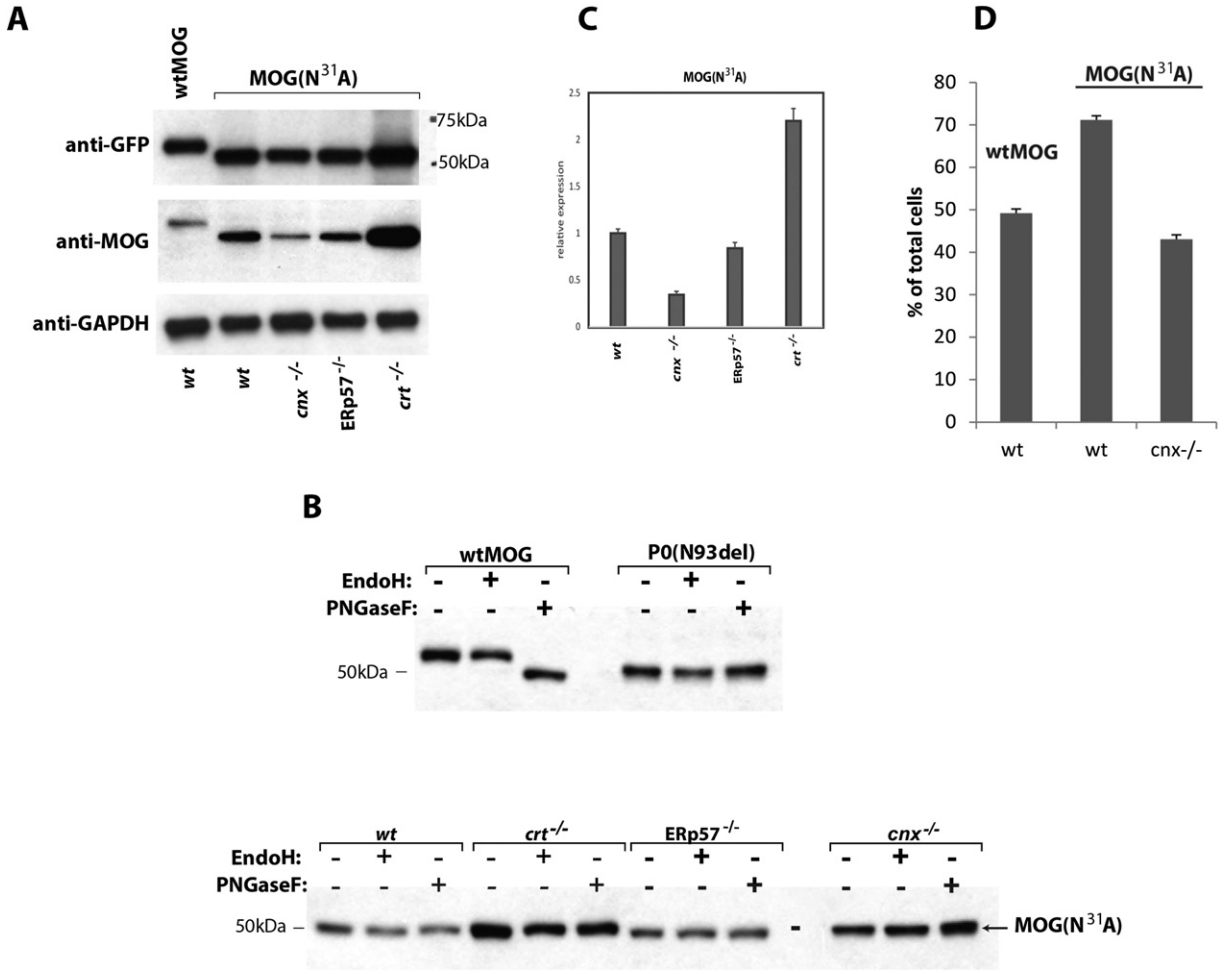

Fig. 1. Expression of MOG($N^{31}A$) mutant in wild-type and chaperone deficient cells. A. *wt*, $cnx^{-/-}$, $ERp57^{-/-}$ and $crt^{-/-}$ cells were transfected with expression vectors encoding MOG($N^{31}A$)GFP using a lentivirus system. Western blot analysis was carried out with anti-GFP, anti-MOG and anti-*GAPDH* antibodies. B. Endoglycosidase digestion of MOG–GFP and P0(N^{93}del)GFP (top panel) used as positive and negative controls, respectively. Lower panel shows endoglycosidase digestion of MOG($N^{31}A$)GFP expressed in wild-type (*wt*) or chaperone-deficient cell lines $cnx^{-/-}$, $crt^{-/-}$ or $ERp57^{-/-}$. Immunoblots of cell lysates are treated with EndoH or PNGaseF. C. Quantification of anti-MOG Western Blots using densitometry, n = 2. D. Flow cytometry analysis of MOG and MOG($N^{31}A$) expressing cells. Abbreviations: cnx, calnexin; GFP, green fluorescent protein; crt, calreticulin; GAPDH, glyceraldehyde phosphate dehydrogenase; MOG, myelin oligodendrocyte glycoprotein.

mutagenesis; primers: Forward 5′CTC TCC TGG GAA AGC TGC CAC GGG CA GGA3′; Reverse: 5′TCC ATG CCC GTG GCA GC TC CCA GGA GAG3′. Next, this construct was used to create wild-type, calnexin-deficient ($cnx^{-/-}$), calreticulin-deficient ($crt^{-/-}$) and ERp57-deficient cell lines stably expressing recombinant MOG($N^{31}A$)GFP protein. Cells were cultured in the presence of 7 μg/ml blasticidine for 14 days [13,18]. Expression of MOG($N^{31}A$)GFP protein was monitored by Western blot analysis using goat anti-GFP (Abcam) or rabbit anti-MOG (Santa-Cruz) antibodies, or by immunofluorescence, co-staining with ER marker TexasRed Concanavalin A conjugate using confocal microscopy. For immunofluorescence, cells expressing MOG–GFP were grown for 24 h after seeding in culture media on a glass coverslip and fixed in 4% paraformaldehyde for 12 min. Images were collected by spinning-disk microscopy (Guelph, Canada) set up on an Olympus IX-81 inverted stand (Markham, Canada). Images were acquired through a 63× objective (N.A. 1.42) with an Electron Multiplying Charge Coupled Device (EMCCD) camera (Hamamatsu, Japan).

2.2. Immunoprecipitation, Western blot and flow cytometry analyses

For immunoprecipitation, cells were grown to 80–90% confluency followed by the addition of 500 μl/10 cm dish of a lysis buffer containing 50 mM HEPES (pH 7.4), 200 mM NaCl, 2% CHAPS and protease inhibitors [19]. The samples were incubated on ice for 30 min and then spun at 11,600 ×*g* for 15 min to remove insoluble material. Supernatant was pre-cleared with a 1/15th of 10% protein A/G Sepharose (GE Healthcare Life Science) bead suspension in an HBS buffer containing 50 mM HEPES (pH 7.4) and 200 mM NaCl. Beads were centrifuged followed by addition of 2 μl of an appropriate antibody. The samples were incubated overnight at 4 °C with rotation, followed by addition of 100 μl of 10% protein A/G Sepharose in HBS buffer, and incubated for an additional 4 h. Beads were centrifuged, washed 3 times with HBS containing 1% CHAPS (Sigma), one time with HBS, followed by addition of 30 μl of SDS-PAGE sample buffer [19]. Proteins were separated by SDS-PAGE (10% acrylamide).

For Western blot analysis, cells expressing MOG–GFP or MOG($N^{31}A$)GFP were lysed on ice in RIPA buffer containing for 150 mM NaCl, 50 mM Tris–HCl (pH 7.4), 0.5% Nonidet P-40, 1% deoxycholate, 0.1% sodium dodecyl sulphate, and protease inhibitors for 15 min [15,20]. Brains from mice were isolated, crushed in liquid nitrogen, and lysed in RIPA buffer for 15 min on ice. The following antibodies were used: goat anti-GFP 1:10,000, rabbit anti-MOG 1:300 (Santa-Cruz, mouse monoclonal raised against C-terminus of MOG), rabbit anti-calnexin 1:1000 (Enzo Life Sciences), rabbit anti calreticulin 1:300, rabbit anti-ERp57 1:1000, and rabbit antiglyceraldehyde dehyrodenase (GAPDH) 1:1000. Sera from control or EAE mice were collected and used to probe Western blots at 1:300 dilution.

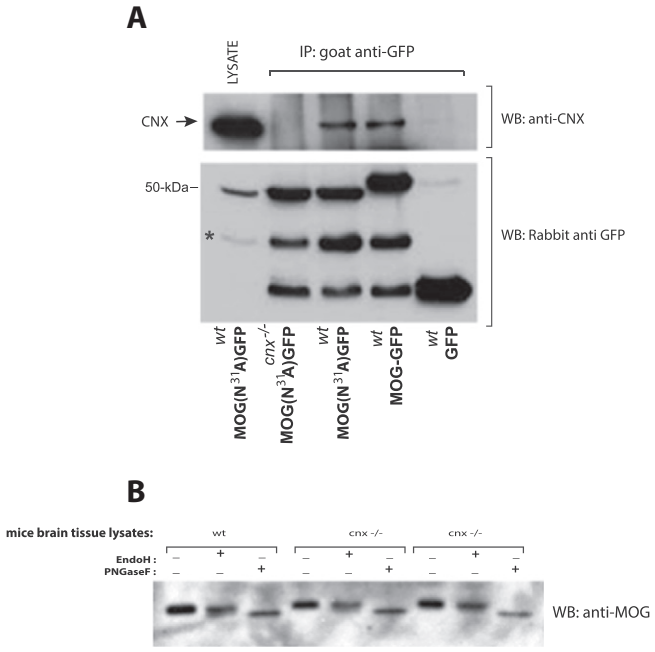

Fig. 2. Glycan independent interaction between calnexin and MOG. A. Cell lysates were collected from *wt* or *cnx*$^{-/-}$ cells stably expressing MOG–GFP, MOG(N^{31}A)GFP or GFP alone, followed by addition of the goat anti-GFP antibodies. Proteins were separated by SDS-PAGE followed by Western blot (WB) analysis with anti-calnexin (anti-*CNX*) antibodies. A representative Western blot of 3 independent experiments is shown. The arrow indicates the location of calnexin (CNX) and the asterisk marks unspecific band. IP, immunoprecipitation. B. Immunoblots of brain protein lysates obtained from *wt* or calnexin-deficient mice treated with EndoH or PNGaseF; Western blot with anti-MOG commercial antibodies.

For flow cytometry analysis, samples were analyzed on LSRFortessa SOPR (BD Bioscience) flow cytometer. Data were collected from 10,000 cells and analyzed by FlowJo software. Cells expressing wtMOG–GFP or MOG(N^{31}A)GFP were collected at confluence of 80–90% into 100 μl of 0.1% formaldehyde in PBS, and incubated for 30 min with the primary antibody or mice sera at a concentration of 1:50, for some analysis no antibodies was added. Cells were than washed 3 times with PBS containing 2% FBS followed by the addition of the secondary antibody (Alexa 647, Invitrogen). Staining with secondary antibodies alone was used as a negative control.

2.3. Endoglycosidase digestions and biotinylation

Cellular proteins were extracted with RIPA buffer containing protease inhibitors; and 20 μg of total protein was incubated with EndoH or PNGaseF at 37 °C for 3 h according to the manufacturer's protocols. Digested and undigested samples were separated by SDS-PAGE (10% acrylamide) followed by Western blot analysis with rabbit anti-GFP antibodies at 1:10,000.

For biotinylation of surface proteins, cells were placed on ice and washed three times with ice-cold PBS–Ca^{2+} Mg^{2+} buffer (PBS with 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$). Then, 1.25 mg/ml Sulfo-NHS-SS-biotin (Pierce) in biotinylation buffer (10 mM triethanolamine, 2 mM $CaCl_2$, 150 mM NaCl [pH 7.5]) was added at 4 °C and incubated for 30 min with gentle agitation. Later, the cells were washed and incubated with quenching buffer (PBS–Ca^{2+}–Mg^{2+} and 100 mM glycine) for 20 min with gentle agitation at 4 °C. The cells were then rinsed twice with PBS, scraped off in ice-cold PBS, and pelleted at 2000 rpm for 5 min at 4 °C. The resulting pellets were solubilized in 500 μl lysis buffer (10% glycerol, 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 50 mM Hepes (pH 7.4), with protease inhibitors) for 40–45 min, and the resulting lysates were centrifuged at 14,000 ×*g* for 10 min at 4 °C. The recovered supernatant was incubated overnight at 4 °C with gentle rotation with packed streptavidin-agarose beads (Invitrogen, Carlsbad, CA, USA), in order to separate the biotinylated proteins. The beads were then pelleted by centrifugation at 900 rpm for 2 min and aliquots of supernatants were taken to represent the unbound proteins. Subsequently, streptavidin-agarose beads were washed three times with washing buffer (10% glycerol, 0.1% Triton X-100, 150 mM NaCl, 20 mM HEPES [pH 7.4]). The biotinylated proteins (plasma membrane proteins) were recovered from the beads by heating them at 100 °C for 5 min in SDS-PAGE sample buffer. The intracellular protein fractions and plasma membrane protein fractions were then electrophoresed separately (but in parallel) and immunoblotted.

2.4. EAE

Nine-week old C57BL/6J mice were randomly selected to be immunized with 100 μg $MOG_{35–55}$ peptide emulsified (1:1) in complete Freund's adjuvant. Pertussis toxin (300 ng, List Laboratories, Campbell, CA, USA) was injected intraperitoneal on the day of $MOG_{35–55}$ immunization and two days later. The remaining three sham mice underwent the same immunization procedure with incomplete Freund's adjuvant (without $MOG_{35–55}$). The mice were assessed daily for neurological disabilities using a standard clinical score. All animal experiments were carried out according to the University of Alberta Animal Policy and Welfare Committee and the Canadian Council on Animal Care Guidelines. The approval for use of animals in research was granted by the Animal Care and Use Committee for Health Sciences, a University of Alberta ethics review committee.

2.5. Unfolded protein response

Unfolded protein responses were measure in wild-type and chaperone deficient cells expressing wild-type MOG (wtMOG) or MOG(N^{31}A). The luciferase reporter gene assay was utilized to measure ER stress. The assay utilized an internal Renilla control, the nucleotide sequence of Xbp1 followed by the firefly luciferase gene [15].

3. Result and discussion

3.1. N-glycosylation mutant of MOG

MOG contains a single N-linked glycosylation site at Asn^{31} and it is a powerful antigen for the induction of EAE in mice [21]. Immunization of mice with $MOG_{35–55}$ peptide results in the development of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis [22,23]. The role of MOG's carbohydrate moiety and its contribution to the pathogenesis of MS are unclear. To further investigate the role of sugar in the biology of MOG, we created a MOG mutant with substitution of Asn^{31} for Ala, and expressed this non-glycosylated MOG in wild-type and chaperone-deficient cells (Fig. 1A).

We further confirmed the absence of N-glycan on MOG(N^{31}A)GFP with a digestion experiment using the specific endoglycosidases EndoH or PNGaseF (Fig. 1B). Digestion with EndoH removes high mannose N-linked carbohydrates, a characteristic of ER localized glycoproteins. Digestion with PNGaseF removes both high mannose and complex carbohydrates found on Golgi and plasma membrane localized proteins. The upper panel of Fig. 1B shows digestion of a positive (glycosylated) control (wtMOG–GFP), and a negative control (P0(N^{93}del) GFP), fully resistant to digestion by glycosidases. As predicted, MOG(N^{31}A)GFP expressed in wild-type and chaperone-deficient cells were strongly resistant to both EndoH and PNGaseF digestion, indicating the lack of glycan on the mutated protein (Fig. 1B).

Wild-type, *cnx*$^{-/-}$, *crt*$^{-/-}$ and *ERp57*$^{-/-}$ cells were transfected with an expression vector encoding MOG(N^{31}A)GFP followed by Western blot analysis with anti-GFP and anti-MOG antibodies. Fig. 1A shows that the MOG(N^{31}A)GFP protein has a faster mobility in SDS-PAGE when compared to the wild-type wtMOG–GFP protein, due to the

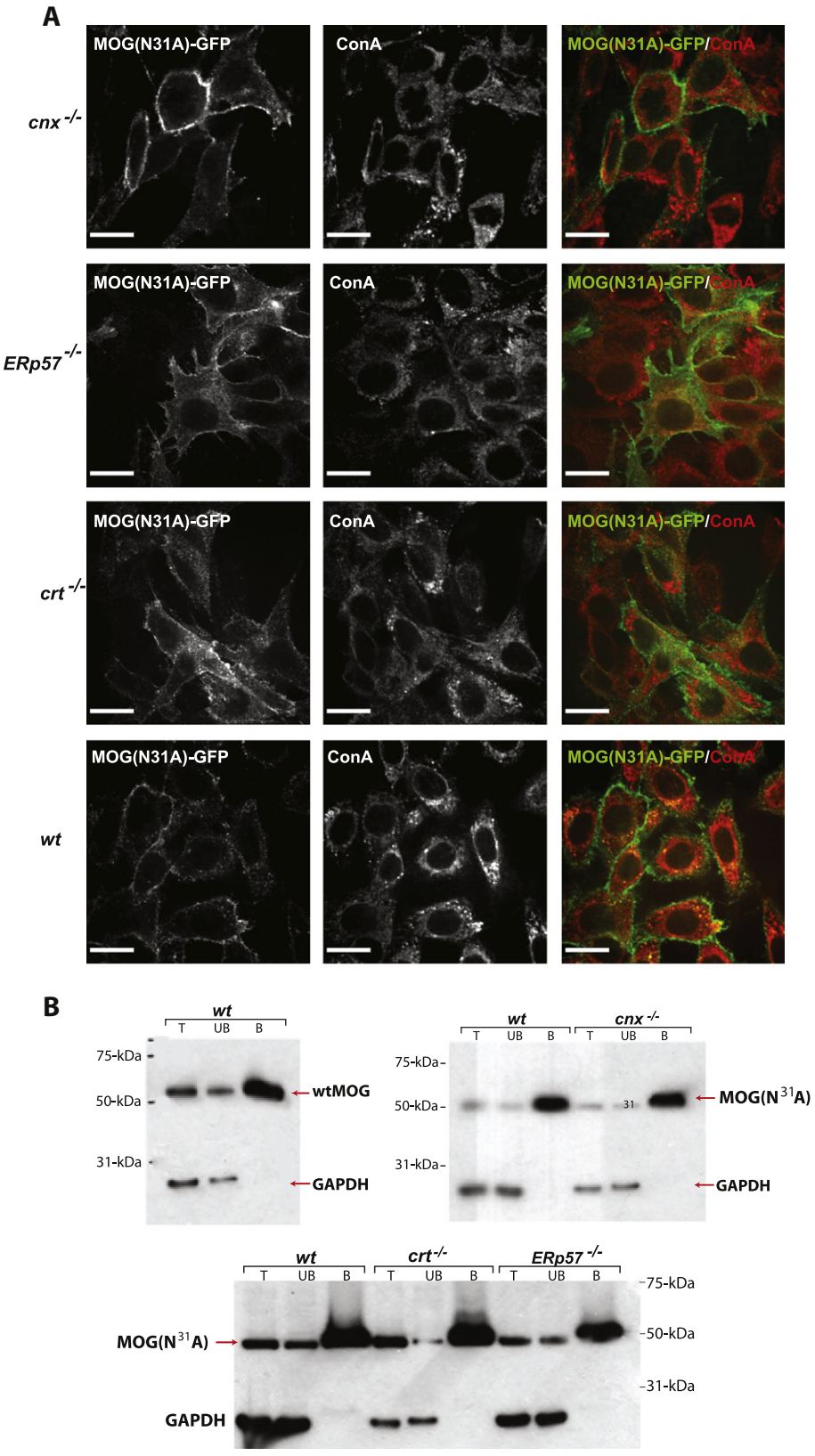

Fig. 3. Cell surface targeting of MOG($N^{31}A$)GFP in wild-type and chaperone-deficient cells. A. Confocal images of cell surface expression of MOG($N^{31}A$)GFP (left panels) in calnexin-deficient (*cnx*$^{-/-}$), ERp57-deficient (*ERp57*$^{-/-}$), calreticulin-deficient (*crt*$^{-/-}$) and wild-type (*wt*) cells. Concanavalin-A (middle panel) was used as an ER marker. Right panel shows merging of GFP (green) and Concanavalin-A (red) imaging. Scale bar = 17 μm. B. Cell surface biotinylation of MOG–GFP, MOG($N^{31}A$)GFP expressed in wild-type (*wt*), calreticulin (*crt*$^{-/-}$), calnexin- (*cnx*$^{-/-}$) and ERp57-deficient (*ERp57*$^{-/-}$) cell lines. Western blots were probed with anti-GFP or anti-GAPDH antibodies. *T* lanes, total cellular extracts; *UB* lanes, material not bound to the streptavidin affinity beads; *B* lanes, streptavidin bound, biotinylated fractions. Abbreviations: GFP, green fluorescent protein; GAPDH, glyceraldehyde phosphate dehydrogenase.

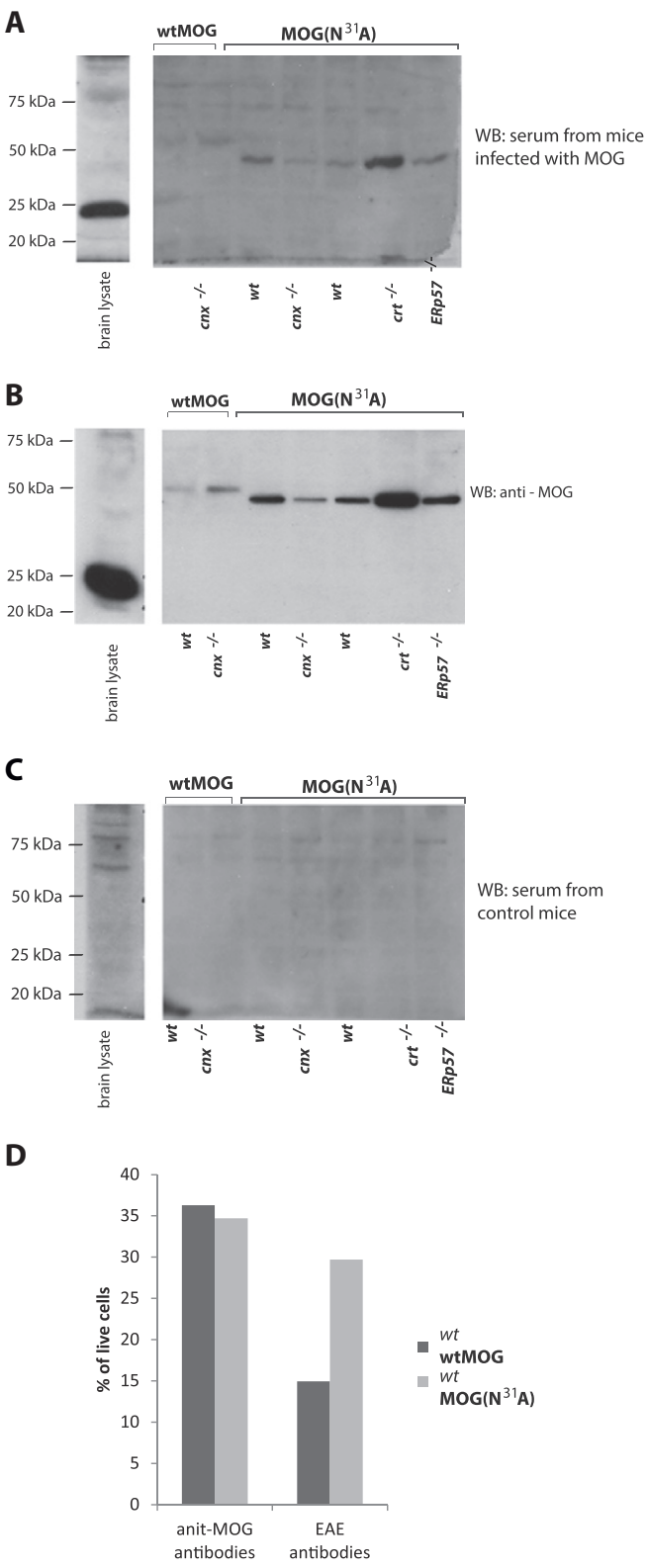

Fig. 4. Autoantibodies binding to cell MOG(N^{31}A)GFP. Western blot (WB) analysis of lysates from mouse brain or cells expressing wtMOG–GFP or MOG(N^{31}A)GFP serum form EAE mice (A), using commercial anti-MOG antibody (Santa-Cruz) (B) or serum for control mice (C). D. Flow cytometry analysis of cell surface fraction (plasma membrane localized) of wtMOG–GFP or MOG(N^{31}A)GFP in *wt* cells with commercial anti-MOG antibody or serum form EAE mice.

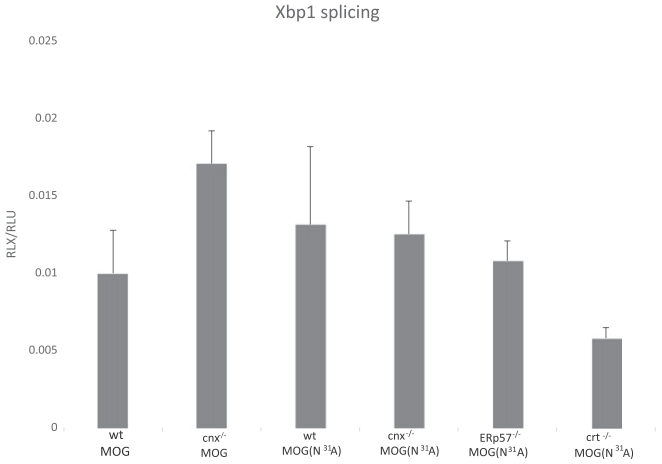

Fig. 5. ER stress induced splicing of XBP1 mRNA. To measure the unfolded protein response triggered by MOG(N^{31}A), *wt*, *cnx*$^{-/-}$, *crt*$^{-/-}$ or *ERp57*$^{-/-}$ cells stably expressing MOG(N^{31}A)GFP were transfected with pRL-XFL vector encoding Renilla luciferase and firefly luciferase reporter genes. Cells expressing wtMOG–GFP were used as a negative control for ER stress. Cell extracts were collected and assayed for luciferase activity with the Dual-Luciferase Assay Kit (Promega) using a luminometer (Berthold-Lumat LB 9501) [10,14]. Relative light units (RLU) were normalized to internal control. Average values ± standard deviation (SD) (n = 3).

absence of the glycan. Unlike expression of wtMOG–GFP which was at comparable levels in chaperone-deficient and wild-type cells [24], expression of the mutant protein lacking the glycan was reduced the absence of the calnexin (Fig. 1A, C). To further examine expression of MOG(N^{31}A)GFP we performed flow cytometry analysis (Fig. 1D). Again we saw reduced expression of mutant MOG(N^{31}A)GFP in the absence of calnexin. Although not clear at present, the reduced expression of MOG(N^{31}A)GFP in calnexin-deficient cells might be due to enhanced proteasomal degradation (ERAD) in the absence of calnexin [15]. Interestingly, MOG(N^{31}A)GFP was more abundant in *crt*$^{-/-}$ cells when compared to the levels in the wild-type cells (Fig. 1A, C), indicating that expression of MOG(N^{31}A)GFP was sensitive to the presence of calreticulin.

3.2. MOG interacts with calnexin in a glycosylation independent manner

We have previously shown that calnexin interacts with wtMOG [13]. Here we examined if calnexin forms complexes with MOG lacking glycosylation at N^{31} [MOG(N^{31}A)GFP]. Fig. 2A shows that calnexin formed immunoprecipitable complexes with MOG(N^{31}A)GFP, indicating that interaction between calnexin and MOG was glycan independent. Our results confirm the observation that calnexin can act as a chaperone in a glycan-independent way [25]. Moreover, since MOG(N^{31}A)GFP protein is not retained (shown later) in the ER, immunoprecipitation results of MOG(N^{31}A)GFP and calnexin were not just an effect of accumulation of misfolded protein in the ER.

Calnexin deficiency has no effect on MOG expression in the mouse central nervous system [18]. Here we used EndoH and PNGaseF endoglycosidases to test the glycosylation state of MOG in brain samples collected from calnexin-deficient mice (Fig. 2B). N-glycosylation of MOG in the brain samples from calnexin-deficient mice was unaltered when compared to its wild-type littermate (Fig. 2B). Furthermore, sugar might not be critical for MOG folding but may affect the efficiency of folding hence lower level of MOG(N^{31}A) expression in the calnexin-deficient cells (Fig. 1A, B) while the expression levels of wtMOG in wild-type vs *cnx*$^{-/-}$ cells was at comparable levels [13].

3.3. Subcellular localization of the N-glycosylation MOG mutant

Next, we used confocal microscopy to investigate the intracellular localization of MOG(N^{31}A)GFP in cells deficient in ER quality control chaperones. The bulk of MOG(N^{31}A)GFP was localized to the plasma

membrane in the wild-type, and in calnexin-, calreticulin- or ERp57-deficient cells (Fig. 3A) suggesting that the N-glycosylation of MOG was not essential for cell surface targeting and that the absence of calnexin, calreticulin and ERp57 did not affect cell surface targeting of MOG($N^{31}A$)GFP.

Cell surface localization of MOG($N^{31}A$)GFP was further investigated using a membrane-impermeant biotin that labels surface proteins (Fig. 3B). Wild-type and chaperone-deficient cell lines expressing MOG($N^{31}A$)GFP were labeled with sulfo-NHS-biotin followed by isolation of the biotinylated proteins using streptavidin-affinity chromatography [26]. Total cellular proteins (T), unbound and streptavidin bound proteins were separated by SDS-PAGE followed by Western blot analysis with anti-GFP antibodies to identify in which fraction MOG($N^{31}A$)GFP was present. Western blot analysis of GAPDH, a cytoplasmic protein, was used as an internal control indicating the cell surface biotinylating protocol identified cell surface proteins only. Fig. 3B shows that biotinylated MOG($N^{31}A$)GFP was correctly localized to plasma membrane in wild-type and chaperone-deficient cells was relatively similar to the cell surface localized wtMOG–GFP.

3.4. Autoantibodies binding to glycosylated vs unglycosylated MOG

MOG plays role in the pathology of MS, a chronic inflammatory disease of the CNS. Interestingly, MOG was first identified as the antigen responsible for the demyelination observed in animals injected with whole CNS homogenate [27]. Anti-MOG antibodies have been found in sera of subset of patients with MS, and their presence is directly associated with myelin damage [1,28]. Anti-MOG antibodies are also part of pathogenesis of EAE an animal model that resembles many pathological features of human MS [29]. We used sera from mice immunized with $MOG_{35–55}$ peptide — (EAE), and tested for recognition in lysates from cells deficient in ER chaperones expressing wtMOG–GFP or MOG($N^{31}A$)GFP. Western blot analysis using EAE sera (Fig. 4A) were consistent with results obtained with the commercial anti-MOG antibodies (Fig. 4B), sera from the control mice were used as a control (Fig. 4C). However, Western blot analysis provides a measure of total protein but does not allow to distinguish between antibodies that preferentially recognize intact, correctly folded MOG. Next we utilize cell based assay (flow cytometry) to address wheatear pathogenic antibody from EAE mice sera show specificity to glycan on plasma membrane localized MOG (Fig. 4D). Interestingly, antibodies from the EAE mice preferentially recognized MOG($N^{31}A$)GFP to wild-type glycosylated variant of MOG. It is possible that the N-glycan on the MOG reduces the interaction with the pathogenic antibodies that were developed against non-glycosylated peptide. Our finding is in line with data results of Mayer et al., which show that antibodies form human MS sera can in some cases preferentially recognize unglycosylated human MOG [7].

3.5. Endoplasmic reticulum stress in cell expressing mutant MOG

ER stress is usually triggered in response to build up of misfolded proteins in the lumen of ER [30]. The reason for accumulation of unfolded proteins can range from protein mutations to changes in the environment of the lumen of ER [31]. Recently, ER stress has been linked with the pathology of multiple sclerosis [32]. Consequently, we tested if the expression of non-glycosylated MOG would induce ER stress. Surprisingly, expression of glycan deficient MOG did not induce Xbp-1 splicing (Fig. 5), a marker of unfolded protein response, indicating that the absence of glycan did not interfere did not result in the ER retention of the potentially misfolded protein.

Acknowledgements

We thank Chris Power, University of Alberta, for the invaluable help with EAE experiments. This work was supported by grants to M.M. from the Canadian Institutes of Health Research (CIHR) (MOP-53050, MOP-15415, MOP-15291) and Alberta Innovates Health Solutions (AI-HS). J.J. was a recipient of a studentship award from AI-HS and E.D. was a recipient of a postdoctoral fellowship from AI-HS.

References

[1] M.C. Mayer, E. Meinl, Glycoproteins as targets of autoantibodies in CNS inflammation: MOG and more, Ther. Adv. Neurol. Disord. 5 (2012) 147–159.
[2] C.S. Clements, H.H. Reid, T. Beddoe, F.E. Tynan, M.A. Perugini, T.G. Johns, C.C. Bernard, J. Rossjohn, The crystal structure of myelin oligodendrocyte glycoprotein, a key autoantigen in multiple sclerosis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 11059–11064.
[3] C. Brunner, H. Lassmann, T.V. Waehneldt, J.M. Matthieu, C. Linington, Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2′,3′-cyclic nucleotide 3′-phosphodiesterase in the CNS of adult rats, J. Neurochem. 52 (1989) 296–304.
[4] C. Breithaupt, A. Schubart, H. Zander, A. Skerra, R. Huber, C. Linington, U. Jacob, Structural insights into the antigenicity of myelin oligodendrocyte glycoprotein, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9446–9451.
[5] M.V. Gardinier, P. Amiguet, C. Linington, J.M. Matthieu, Myelin/oligodendrocyte glycoprotein is a unique member of the immunoglobulin superfamily, J. Neurosci. Res. 33 (1992) 177–187.
[6] A.N. Burska, L. Hunt, M. Boissinot, R. Strollo, B.J. Ryan, E. Vital, A. Nissim, P.G. Winyard, P. Emery, F. Ponchel, Autoantibodies to posttranslational modifications in rheumatoid arthritis, Mediat. Inflamm. 2014 (2014) 492873.
[7] M.C. Mayer, C. Breithaupt, M. Reindl, K. Schanda, K. Rostasy, T. Berger, R.C. Dale, F. Brilot, T. Olsson, D. Jenne, A.K. Probstel, K. Dornmair, H. Wekerle, R. Hohlfeld, B. Banwell, A. Bar-Or, E. Meinl, Distinction and temporal stability of conformational epitopes on myelin oligodendrocyte glycoprotein recognized by patients with different inflammatory central nervous system diseases, J. Immunol. 191 (2013) 3594–3604.
[8] V. Lampasona, D. Franciotta, R. Furlan, S. Zanaboni, R. Fazio, E. Bonifacio, G. Comi, G. Martino, Similar low frequency of anti-MOG IgG and IgM in MS patients and healthy subjects, Neurology 62 (2004) 2092–2094.
[9] C.B. Marta, A.R. Oliver, R.A. Sweet, S.E. Pfeiffer, N.H. Ruddle, Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13992–13997.
[10] A. Carotenuto, A.M. D'Ursi, E. Nardi, A.M. Papini, P. Rovero, Conformational analysis of a glycosylated human myelin oligodendrocyte glycoprotein peptide epitope able to detect antibody response in multiple sclerosis, J. Med. Chem. 44 (2001) 2378–2381.
[11] S. Mazzucco, S. Mata, M. Vergelli, R. Fioresi, E. Nardi, B. Mazzanti, M. Chelli, F. Lolli, M. Ginanneschi, F. Pinto, L. Massacesi, A.M. Papini, A synthetic glycopeptide of human myelin oligodendrocyte glycoprotein to detect antibody responses in multiple sclerosis and other neurological diseases, Bioorg. Med. Chem. Lett. 9 (1999) 167–172.
[12] J.J. Garcia-Vallejo, J.M. Ilarregui, H. Kalay, S. Chamorro, N. Koning, W.W. Unger, M. Ambrosini, V. Montserrat, R.J. Fernandes, S.C. Bruijns, J.R. van Weering, N.J. Paauw, T. O'Toole, J. van Horssen, P. van der Valk, K. Nazmi, J.G. Bolscher, J. Bajramovic, C.D. Dijkstra, B.A. t Hart, Y. van Kooyk, CNS myelin induces regulatory functions of DC-SIGN-expressing, antigen-presenting cells via cognate interaction with MOG, J. Exp. Med. 211 (2014) 1465–1483.
[13] J. Jung, M. Michalak, Cell surface targeting of myelin oligodendrocyte glycoprotein (MOG) in the absence of endoplasmic reticulum molecular chaperones, Biochim. Biophys. Acta 1813 (2011) 1105–1110.
[14] K. Bedard, E. Szabo, M. Michalak, M. Opas, Cellular functions of endoplasmic reticulum chaperones calreticulin, calnexin, and ERp57, Int. Rev. Cytol. 245 (2005) 91–121.
[15] H. Coe, K. Bedard, J. Groenendyk, J. Jung, M. Michalak, Endoplasmic reticulum stress in the absence of calnexin, Cell Stress Chaperones 13 (2008) 497–507.
[16] K. Nakamura, A. Zuppini, S. Arnaudeau, J. Lynch, I. Ahsan, R. Krause, S. Papp, H. De Smedt, J.B. Parys, W. Müller-Esterl, D.P. Lew, K.-H. Krause, N. Demaurex, M. Opas, M. Michalak, Functional specialization of calreticulin domains, J. Cell Biol. 154 (2001) 961–972.
[17] H. Coe, J. Jung, J. Groenendyk, D. Prins, M. Michalak, ERp57 modulates STAT3 signaling from the lumen of the endoplasmic reticulum, J. Biol. Chem. 285 (2010) 6725–6738.
[18] J. Jung, H. Coe, M. Michalak, Specialization of endoplasmic reticulum chaperones for the folding and function of myelin glycoproteins P0 and PMP22, FASEB J. 25 (2011) 3929–3937.
[19] A. Zuppini, J. Groenendyk, L.A. Cormack, G. Shore, M. Opas, R.C. Bleackley, M. Michalak, Calnexin deficiency and endoplasmic reticulum stress-induced apoptosis, Biochemistry 41 (2002) 2850–2858.
[20] N. Calloway, M. Vig, J.P. Kinet, D. Holowka, B. Baird, Molecular clustering of STIM1 with Orai1/CRACM1 at the plasma membrane depends dynamically on depletion of Ca2+ stores and on electrostatic interactions, Mol. Biol. Cell 20 (2009) 389–399.
[21] P.A. Smith, N. Heijmans, B. Ouwerling, E.C. Breij, N. Evans, J.M. van Noort, A.C. Plomp, C. Delarasse, B. t Hart, D. Pham-Dinh, S. Amor, Native myelin oligodendrocyte glycoprotein promotes severe chronic neurological disease and demyelination in Biozzi ABH mice, Eur. J. Immunol. 35 (2005) 1311–1319.
[22] S.S. Zamvil, L. Steinman, The T lymphocyte in experimental allergic encephalomyelitis, Annu. Rev. Immunol. 8 (1990) 579–621.
[23] I.M. Stromnes, J.M. Goverman, Active induction of experimental allergic encephalomyelitis, Nat. Protoc. 1 (2006) 1810–1819.

[24] H. Coe, J. Jung, J. Groenendyk, D. Prins, M. Michalak, ERp57 modulates STAT3 signaling from the lumen of the endoplasmic reticulum, The Journal of Biological Chemistry 285 (2010) 6725–6738.
[25] U.G. Danilczyk, D.B. Williams, The lectin chaperone calnexin utilizes polypeptide-based interactions to associate with many of its substrates in vivo, J. Biol. Chem. 276 (2001) 25532–25540.
[26] T. Keskanokwong, H.J. Shandro, D.E. Johnson, S. Kittanakom, G.L. Vilas, P. Thorner, R.A. Reithmeier, V. Akkarapatumwong, P.T. Yenchitsomanus, J.R. Casey, Interaction of integrin-linked kinase with the kidney chloride/bicarbonate exchanger, kAE1, J. Biol. Chem. 282 (2007) 23205–23218.
[27] R. Lebar, C. Lubetzki, C. Vincent, P. Lombrail, J.M. Boutry, The M2 autoantigen of central nervous system myelin, a glycoprotein present in oligodendrocyte membrane, Clin. Exp. Immunol. 66 (1986) 423–434.
[28] N. Kerlero de Rosbo, P. Honegger, H. Lassmann, J.M. Matthieu, Demyelination induced in aggregating brain cell cultures by a monoclonal antibody against myelin/oligodendrocyte glycoprotein, J. Neurochem. 55 (1990) 583–587.
[29] P.H. Lalive, N. Molnarfi, M. Benkhoucha, M.S. Weber, M.L. Santiago-Raber, Antibody response in MOG(35–55) induced EAE, J. Neuroimmunol. 240–241 (2011) 28–33.
[30] Y. Kimata, K. Kohno, Endoplasmic reticulum stress-sensing mechanisms in yeast and mammalian cells, Curr. Opin. Cell Biol. 23 (2011) 135–142.
[31] C. Hetz, The unfolded protein response: controlling cell fate decisions under ER stress and beyond, Nat. Rev. Mol. Cell Biol. 13 (2012) 89–102.
[32] A.N. Mhaille, S. McQuaid, A. Windebank, P. Cunnea, J. McMahon, A. Samali, U. FitzGerald, Increased expression of endoplasmic reticulum stress-related signaling pathway molecules in multiple sclerosis lesions, J. Neuropathol. Exp. Neurol. 67 (2008) 200–211.