The Journal of Immunology

The Human Endogenous Retrovirus Envelope Glycoprotein, Syncytin-1, Regulates Neuroinflammation and Its Receptor Expression in Multiple Sclerosis: A Role for Endoplasmic Reticulum Chaperones in Astrocytes

Joseph M. Antony, Kristofor K. Ellestad, Robert Hammond, Kazunori Imaizumi, Francois Mallet, Kenneth G. Warren and Christopher Power

J Immunol 2007; 179:1210-1224; ;
doi: 10.4049/jimmunol.179.2.1210
http://www.jimmunol.org/content/179/2/1210

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

The Human Endogenous Retrovirus Envelope Glycoprotein, Syncytin-1, Regulates Neuroinflammation and Its Receptor Expression in Multiple Sclerosis: A Role for Endoplasmic Reticulum Chaperones in Astrocytes[1]

Joseph M. Antony,* Kristofor K. Ellestad,¶ Robert Hammond,† Kazunori Imaizumi,‡ François Mallet,§ Kenneth G. Warren,¶ and Christopher Power[2]*¶

Retroviral envelopes are pathogenic glycoproteins which cause neuroinflammation, neurodegeneration, and endoplasmic reticulum stress responses. The human endogenous retrovirus (HERV-W) envelope protein, Syncytin-1, is highly expressed in CNS glia of individuals with multiple sclerosis (MS). In this study, we investigated the mechanisms by which Syncytin-1 mediated neuroimmune activation and oligodendrocytes damage. In brain tissue from individuals with MS, ASCT1, a receptor for Syncytin-1 and a neutral amino acid transporter, was selectively suppressed in astrocytes ($p < 0.05$). Syncytin-1 induced the expression of the endoplasmic reticulum stress sensor, old astrocyte specifically induced substance (OASIS), in cultured astrocytes, similar to findings in MS brains. Overexpression of OASIS in astrocytes increased inducible NO synthase expression but concurrently down-regulated ASCT1 ($p < 0.01$). Treatment of astrocytes with a NO donor enhanced expression of early growth response 1, with an ensuing reduction in ASCT1 expression ($p < 0.05$). Small-interfering RNA molecules targeting Syncytin-1 selectively down-regulated its expression, preventing the suppression of ASCT1 and the release of oligodendrocyte cytotoxins by astrocytes. A Syncytin-1-transgenic mouse expressing Syncytin-1 under the glial fibrillary acidic protein promoter demonstrated neuroinflammation, ASCT1 suppression, and diminished levels of myelin proteins in the corpus callosum, consistent with observations in CNS tissues from MS patients together with neurobehavioral abnormalities compared with wild-type littermates ($p < 0.05$). Thus, Syncytin-1 initiated an OASIS-mediated suppression of ASCT1 in astrocytes through the induction of inducible NO synthase with ensuing oligodendrocyte injury. These studies provide new insights into the role of HERV-mediated neuroinflammation and its contribution to an autoimmune disease. *The Journal of Immunology,* **2007, 179: 1210–1224.**

Multiple sclerosis (MS)[3] is a demyelinating disease of the CNS defined by inflammatory destruction of myelin and ensuing axonal damage (1). Although MS is widely assumed to be an adaptive T cell-mediated autoimmune disease, increasing evidence points to the pathogenic involvement of resident CNS cells including microglia/macrophages and astrocytes in MS pathogenesis, which participate in innate immune processes (2). Inflammatory responses mediated by glia-derived cytokines and chemokines in neurodegenerative diseases regulate levels of several amino acid transporters, which impact on the progression of disease (3). Interestingly, several transporters also function as receptors for different neurotropic retroviruses (4). The human endogenous retrovirus (HERV-W) envelope glycoprotein, Syncytin-1, binds to two receptors including the sodium-dependent transporters of polar neutral amino acids, alanine, serine, cysteine, and threonine (ASCT1 and ASCT2) (4), which are localized on both neurons and glia (5) and are known to modulate both neurotrophic (6) and neurotoxic (7) effects in the CNS.

Syncytin-1 is a complex human endogenous retroviral protein that is largely beneficial to the host in terms of facilitating placental development (8). However, in the brains of MS patients, Syncytin-1 modulates an inflammatory cascade when its expression is increased by various factors including exogenous viruses (9). Recent studies indicate that Syncytin-1 transcript levels are quantitatively increased in the brains of MS patients compared with other inflammatory neurological disease controls (10). Interestingly, TNF-α, an inflammatory molecule in the brains of MS patients, was found to enhance Syncytin-1 expression in astrocytes (11), which consequently results in production of free radicals and cytokines (12) that could affect expression of ASCT1 and ASCT2, given that free radicals influence expression of membrane proteins (13, 14). Free radicals are also produced when cells undergo

*Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada; †Department of Pathology, London Health Sciences Centre and University of Western Ontario, London, Ontario, Canada; ‡Department of Anatomy, Faculty of Medicine, University of Miyazaki, Japan; §Unité Mixte de Recherche, Centre National de la Recherche Scientifique-bioMerieux, l'Institut Fédératif de Recherche 128 BioSciences Lyon-Gerland, Ecole Normale Superieure de Lyon, Lyon, France; and ¶Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

Received for publication December 18, 2006. Accepted for publication May 1, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] J.M.A. was supported by a Studentship from the Alberta Heritage Foundation for Medical Research (AHFMR) and Multiple Sclerosis Society of Canada (MSSC). C.P. holds a Canada Research Chair (Tier 1) in Neurological Infection and Immunity and an AHFMR Senior Scholarship. The MSSC and Canadian Institutes for Health Research supported these studies.

[2] Address correspondence and reprint requests to Dr. Christopher Power, Departments of Medicine, Medical Microbiology, and Immunology, 611 Heritage Medical Research Center, University of Alberta, Edmonton, Alberta, T6G 2S2 Canada. E-mail address: chris.power@ualberta.ca

[3] Abbreviations used in this paper: MS, multiple sclerosis; ER, endoplasmic reticulum; MuLV, murine leukemia virus; MoMuLV, Moloney MuLV; iNOS, inducible NO synthase; BiP, IgH chain-binding protein; OASIS, old astrocyte specifically induced substance; Egr1, early growth response 1; Iba, ionized calcium-binding adaptor protein; GFAP, glial fibrillary acidic protein; siRNA, small-interfering RNA; CNP, 2′, 3′-cyclic nucleotide 3′-phosphodiesterase; Tg, transgenic; Wt, wild type; EGFP, enhanced GFP; MDM, monocyte-derived macrophage; YFP, yellow fluorescent protein; SNP, sodium nitroprusside; CGT, ceramide galactosyltransferase.

persistent endoplasmic reticulum (ER) stress (15), a constellation of host responses, which maintain cellular homeostasis, termed the unfolded protein response (16). Accumulation of misfolded proteins contribute to disruption of ER function, resulting in the unfolded protein response (17). Induction of ER stress molecules including activating transcription factor-4 has been previously demonstrated in MS lesions (18). Furthermore, accumulation of proteins such as MHC class I in the ER (15) or ER stress induction by IL-1β or NO impairs oligodendrocyte repair (19). Several retroviral envelope proteins induce ER stress, together with suppressing the cognate viral receptor (20). For example, infection by murine leukemia virus (MuLV) down-modulates expression of its cell surface receptor, mCAT-1 (21). Several lines of evidence implicate glia in retrovirus-induced ER stress and neuropathogenesis (22). Infection of astrocytes with Moloney MuLV (MoMuLV)-*ts*1, leads to an ER stress response defined by neuroinflammation and neurodegeneration. In fact, envelope proteins from both the retroviruses, MoMuLV-*ts*1 and FrCas^E, mediate ER stress in the brain (22–25) and directly affect oligodendrocyte viability by inducing the proapoptotic ER stress gene, *GADD153/CHOP* (26).

Up-regulation of HERVs has been observed in the context of neuroinflammation (27) and cytokine treatment (28). Earlier studies showed induction of both Syncytin-1 and inducible NO synthase (iNOS) in glial cells in the brains of MS patients, particularly in astrocytes (12, 29). Importantly, iNOS induced mitochondrial calcium flux thereby activating ATF-6 (30). The transcription factor, old astrocyte specifically induced substance (OASIS), is activated in response to ER stress by modulating expression of the Ig H chain-binding protein (BiP), thereby protecting astrocytes from ER stress (31). We hypothesized that in an inflammatory milieu mediated by increased Syncytin-1 levels in astrocytes, the expression of its receptors ASCT1 and ASCT2 might be modulated, possibly through ER stress-related mechanisms. The present studies revealed that Syncytin-1 induced several ER stress-associated molecules including OASIS and GADD153/CHOP, which in turn augmented iNOS expression in astrocytes. In this study, we describe a novel pathway for oligodendrocyte injury in which Syncytin-1 induced OASIS expression in astrocytes accompanied by production of NO and the transcription factor, early growth response 1 (Egr1), leading to the suppression of ASCT1 in astrocytes with ensuing adverse effects on oligodendrocyte proteins involved in myelin formation.

Materials and Methods

Human brain tissue and immunohistochemistry

Brain tissue (frontal white matter) was collected at autopsy as described previously (27). Control subjects included 19 patients: Alzheimer's disease ($n = 6$); HIV infection with encephalitis ($n = 4$) or gliosis ($n = 4$); cerebral arteriosclerosis ($n = 2$); anoxic encephalopathy ($n = 1$); normal brain pathology ($n = 2$). MS patients included 20 patients who had been classified as primary progressive ($n = 6$), secondary progressive ($n = 10$), and relapsing-remitting ($n = 4$) and had Estimated Disability Status Scale scores ranging from 7 to 9 before death. Frozen brain tissue from MS patients was obtained from the Multiple Sclerosis Patient Care and Research Clinic (Edmonton, Alberta, Canada) and the Neurovirology Laboratory Brain Bank (University of Calgary, Calgary, Alberta, Canada), as previously described (12, 32, 33). Histological sections of brain tissue with clear evidence of acute lesion formation and normal appearing white matter were used. Within limits of assessment, care was taken to isolate tissue that might have lesions. Paraffin-embedded sections from the above tissues were immunostained with Abs to ionized calcium-binding adaptor protein 1 (Iba-1; 1.0 μg/ml; Wako), glial fibrillary acidic protein (GFAP; 1/2000; DakoCytomation), Syncytin-1 (8) (6A2B2; 1/1000), ASCT1 and ASCT2 (1/40; U.S. Biologicals), mouse anti-myelin basic protein (MBP; 1/1000; Sternberger Monoclonals), 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNP) (1/500; Chemicon International), iNOS, Egr1, GADD153/CHOP, BiP, and ERp57 (1/100; Santa Cruz Biotechnology), as previously described (33–35). All slides were examined with a Zeiss Axioskop 2 upright microscope and the Spot system (Diagnostic Instruments) to provide digital images.

Cell culture and reagents

Cell cultures (U937, HFA, U373, HEK293T) were maintained, as described (12). Oligodendrocytes were harvested and prepared from adult Sprague-Dawley rats. Briefly, brains from 6-mo-old rats were harvested and pooled. Cells were dissociated by trypsin digestion and isolated by Percoll gradient centrifugation as described (36). Cell isolates, consisting of oligodendrocytes, astrocytes, and microglia, were plated onto uncoated 25-cm^2 flasks. In contrast to astrocytes and microglia, adult oligodendrocytes are poorly adherent on uncoated substrate; floating cells were collected the following day and, when subjected to another round of differential adhesion, resulted in oligodendrocytes cultures of >95% purity. Purified oligodendrocytes were then plated onto Lab-Tek 16-well chamber slides (Nunc) or glass coverslips coated with 10 μg/ml poly-L-ornithine (Sigma-Aldrich). Oligodendrocytes were cultured in MEM supplemented with 10% FBS, 20 μg/ml gentamicin, and 0.1% dextrose. Mouse bone marrow-derived macrophages were prepared as described (37). Cells were treated with human or murine TNF-α, IL-1β, IL-10 (R&D Systems), sodium nitroprusside (SNP; 100 nM; Axxora Life Science), benzylserine (Bachem), L-NAME (5.0 μM; Sigma-Aldrich), or 50 ng/ml PMA (Sigma-Aldrich).

Pseudotyped viral infections

Pseudotyped virions expressing Syncytin-1 were generated by cotransfecting 293T cells with plasmids expressing firefly luciferase within an envelope-inactivated HIV-1 clone (pNL-Luc-E$^-$R$^-$) and the expression vector containing the full-length Syncytin-1 sequence (pCDNA-Syncytin-1) or pCDNA3.1 alone (8, 38). As a control, HIV-JRFL envelope (38) was used for pseudotype formation. Transduction of target cells by pseudotyped virus led to expression of luciferase, which was quantified in cells, lysed 48 h following infection, using the Luciferase Assay kit (BD Pharmingen). For experiments described in the study, 100-μl supernatants were used for infection of target cells.

Small-interfering RNA (siRNA) and quantitative PCR

Human *Syncytin-1* gene (GenBank Accession ID: NM_014590) was amplified using the primers 5′-ATCTAAAGCTTGCCACCATGGCCCTCC CTTATC-3′ and 5′-TGAGTACCGCGGACTGCTTCCTGCTGAA-3′ and the PCR product was cloned into the *Hin*dIII and *Sac*II sites (underlined) of pYFP-N1 (BD Clontech) to obtain Syncytin-1-yellow fluorescent protein (YFP) fusion construct (2.5 μg), which was transfected into HEK293T cells with or without siRNA (200 pM). siRNA duplexes were synthesized by Invitrogen Life Technologies ("Stealth siRNA"). The sequences of the duplexes used were as follows: enhanced YFP sense, ACG GCA AGC UGA CCC UGA AGU UCA U; enhanced YFP antisense, AUG AAC UUC AGG GUC AGC UUG CCG U; Syncytin-1 sense, GCU AGG UGC ACU AGG UAC UGG CAU U; Syncytin-1 antisense, AAU GCC AGU ACC UAG UGC ACC UAG C. Semiquantitative real-time RT-PCR was performed by monitoring in real time the increase of fluorescence of the SYBR Green dye (Molecular Probes) on the iCycler (Bio-Rad) with normalization to GAPDH or β-actin (33) using primers described in Table I.

Transgenic (Tg) mice and genotyping

(5′-AAGGAATAAAGCGGCCGCATGGCCCTCCCTTATCATATCTTT C-3′) and (5′-AAAAGGAAAAGCGGCCGCCTAACTGCTTCCTGCTG-3′) primers with *Not*I tags (underlined) and a silent mismatch (*C*, italicized) in the sense sequence were used to PCR amplify Syncytin-1 from phCMVph74 (8), resulting in a 1.6-kb PCR product, which along with a pFGH vector bearing the *GFAP* promoter (39) were digested with *Not*I. The PCR product was cloned into pFGH to obtain pFGH-Syncytin-1, which was digested with *Eco*R1 to produce a fragment of 5 kbp that was used for pronuclear microinjection. PCR genotyping from tail biopsies was performed to detect the transgene (267 bp) using Syncytin-1 primers 5′-ACCCATACCTCAAACCTCACCTG-3′ and 5′-CTTTTGTTGCGGGGCTTAGATA-3′. For the purposes of the present study, 12-wk-old F_5 Tg mice with wild-type (Wt) littermate controls ($n = 6$/group) were used. All studies and procedures adopted University of Calgary Animal Care Committee guidelines.

Western blot analysis

Ten micrograms of protein was separated by 10% SDS-polyacrylamide at 120 V for 2 h. Proteins were transferred overnight at 4°C onto nitrocellulose membranes, followed by blocking with 10% skimmed milk to prevent nonspecific binding. Western blot analysis was performed using the mAb 6A2B2, which detects Syncytin-1 (8, 12), OASIS (31), actin-HRP, and iNOS (1/100; Santa Cruz Biotechnology); GADD153/CHOP and BiP were detected by Abs, mentioned above.

Table I. *List of oligonucleotide PCR primers used in the study*

Primer	Sequence
PLP	5′-CTTCCCTGGTGGCCACTGGATTGT-3′ and
	5′-CCGCAGATGGTGGTCTTGTAGTCG-3
MOG	5′-CCTCTCCCTTCTCCTCCTTC-3′ and
	5′-AGAGTCAGCACACCGGGGTT-3′
CNPase	5′-CTACCCTCCACGAGTGCAAGACGCT-3′ and
	5′-AGTCTAGTCGCCACGCTGTCTTGGG-3′
OASIS	5′-CAACGCACCCCACTCACAGACACC-3′ and
	5′-GGAGCAGCAAAGCCCGCACTAACT
GADD153	5′-AACCAGCAGAGGTCACAAGC-3′ and
	5′-AGCCGTTCATTCTCTTCAGC-3′
CGT	5′-TTATCGGAAATTCACAAGGAT-3′ and
	5′-TGGCGAAGAATGTAGTCTATC-3′
IFN-α	5′-GTGATCTCCCTGAGACCCAC-3′ and
	5′-GGTAGAGTTCGGTGCAGAAT
PERK	5′-AAGTAGATGACTGCAATTACGCTATCAA-3′ and
	5′-TTTAACTTCCCGCATTACCTTCTC-3′
ERp57	5′-TCAAGGGTTTTCCTACCATCTACTTC-3′ and
	5′-TTAATTCACGGCCACCTTCAT-3′
BiP	5′-TCATCGGACGCACTTGGAA-3′ and
	5′-CAACCACCTTGAATGGCAAGA-3′
hASCT1	5′-TCCCCATAGGCACTGAGATAGAAG-3′ and
	5′-CAAGGAACATGATGCCCACAGGTA-3′
hASCT2	5′-CCTGCTGGGGGTGCTCTTTGGACA-3′ and
	5′-TTGAGTTGGGGACATGAGTGAGAA-3′
mASCT1	5′-CCTGGCTTGATGATGAACGC-3′
	5′-CTGGTGCTGCTCACCGTGTC-3′
mASCT2	5′-CCATCGGCGCCACGGTCAACAT-3′
	5′-GTGGCGAGGGGCAGTGGATTCAGA-3′
Egr1	5′-AGCAGCACCTTCAACCCTCA-3′ and
	5′-CAGCACCTTCTCGTTGTTCAGA-3′
Egr3	5′-TTGGGAAAGTTCGCCTTCG-3 and
	5′-ATGATGTTGTCCTGGCACCA-3′
Egr4	5′-CCCCGCTGGATGCCCCTTTTC-3′ and
	5′-ACTCTCCGCCGTCGCCGCTACTCC-3′
Syncytin-1	5′-TGCCCCATCGTATAGGAGTCT-3′ and
	5′-CATGTACCCGGGTGAGTTGG-3′
iNOS	5′-CAAAGGCTGTGAGTCCTGCAC-3′
	5′-ACTTTGATCAGAAGCTGTCCC-3′

Transfection

Five micrograms of the constructs OASIS-FLAG (31) and pVGW427 (40) were transfected into HFA or HEK293T cells using Transfectin Lipid reagent (Bio-Rad) or Lipofectamine 2000 (Invitrogen Life Technologies), respectively.

Syncytin-1 overexpression and microarray analysis

Astrocytes were infected with a Sindbis virus-derived vector expressing Syncytin-1 (SINrep5-Syncytin-1), enhanced GFP (EGFP; SINrep5-EGFP), or mock infected as described (12) and immunostained for Syncytin-1 expression using 6A2B2. Total cellular RNA from infected astrocytes was hybridized using Affymetrix Human Genome U133 Plus 2 arrays. Expression values were calculated using GeneChip Operating Software (Affymetrix). The experimental approach and data acquisition were performed in accordance with Minimum Information about a Microarray Experiment requirements.

Quantitative immunofluorescence

After fixation in 4% paraformaldehyde, cells were permeabilized by PBS containing 0.1% Triton X-100. Cells were then blocked with Li-Cor Odyssey blocking buffer and primary Abs were added at a concentration of 1/500 and incubated overnight at 4°C. Following washes with PBS containing 0.1% Tween 20, secondary Abs (IRDye 800 conjugated or Alexa Fluor conjugated) were added at a concentration of 1/200 and incubated 1 h at room temperature, after which cells were washed. The plate was then scanned using the Odyssey Infrared Imaging System (600 and 700 nm, 169 μm resolution, 2 mm offset, and intensity setting of 5 for both channels). Label intensity was measured by densitometric analysis of the wells.

Cell counts

Fluorescently (Alexa 488; Molecular Probes/Invitrogen Life Technologies)-labeled cells were counted (10 fields) from a total area of 23,760 μm^2 using a ×10 objective on a Zeiss Axioskop microscope and expressed as a percentage.

Neurobehavioral studies

Behavioral tests were conducted as described (41). The horizontal bar test involved a test of coordination and forelimb strength using a horizontal bar that was 0.2 cm thick, 38 cm long, held 49 cm above a bench (41). The static rod test involved a test of coordination using five rods each 60 cm long and of varying thickness (diameter) (rod 1: 35 mm; rod 2: 28 mm; rod 3: 22 mm; rod 4: 15 mm; and rod 5: 9 mm). These rods were bolted to the edge of a bench such that the rods horizontally protruded their full 60-cm length into space. A mouse was placed at the exposed end of the widest rod and the time taken to orient 180 degrees from the starting position and the time taken to travel to the other end were noted (41). A test of muscle strength and seeking behavior were determined using the inverted screen test. The inverted screen was a 43-cm square inch of wire mesh consisting of 12-mm squares of 1-mm diameter wire and surrounded by a 4-cm deep wooden beading (41). We modified the inverted screen test by placing the mouse at a point that was equidistant from the edges of the screen and the stop clock was started once the screen was inverted and measured the time taken to reach the edge of the inverted screen, as a measure of curiosity and seeking behavior.

Statistical analyses

Statistical tests were performed using GraphPad InStat version 3.01 software including the Mann-Whitney *U* or unpaired *t* tests and, when multiple treatments were used, a one-way ANOVA with the Tukey-Kramer multiple comparisons test.

Results

ASCT1 expression is selectively diminished in MS brain tissue

Loss of amino acid transporter expression from astrocytes in neurodegenerative diseases results in glutamate-mediated excitotoxic damage and death of neurons (42). Indeed, decreased levels of glutamate transporters have also been described in MS lesions (5). To investigate the expression of other amino acid transporter proteins serving as Syncytin-1 receptors in the nervous system, we examined ASCT1 and ASCT2 expression. We analyzed tissue sections from MS and non-MS control brains. Demyelinated (D) lesions from MS patient brains (Fig. 1*Aii*) stained less intensely with Luxol fast blue and H&E compared with a non-MS control brain section (Fig. 1*Ai*). Serial sections of demyelinated regions in the brains of MS patients demonstrated a marked increase in Syncytin-1 immunoreactivity (Fig. 1*Aiv*), particularly in astrocytes expressing GFAP (Fig. 1*Aiii*; Syncytin-1: blue; astrocytes: brown; *inset*, GFAP-positive astrocyte). Similar sections revealed intense ASCT1 immunoreactivity in the white matter of non-MS brain sections (Fig. 1*Av*) on glial cells including both astrocytic (Fig. 1*A*, *inset*, arrow) and monocytoid cells (data not shown) but conversely, ASCT1 expression was reduced in MS sections (Fig. 1*Avi*, arrow indicates an ASCT1-positive cell). Expression of ASCT2, however, revealed no differences in immunoreactivity between the white matter of non-MS (Fig. 1*Avii*) and MS patients (Fig. 1*Aviii*). ASCT2 expression was predominantly expressed on activated microglia (Fig. 1*Aviii*, arrow). Corroborating these findings was the observation that ASCT1 transcript levels were significantly diminished in MS brain white matter relative to non-MS brains (Fig. 1*B*). Conversely, ASCT2 transcript levels did not differ in white matter between clinical groups. ASCT1 transcript levels did not differ in (frontal) cortex between non-MS and MS patients, while ASCT2 was not detected in the cortex (Fig. 1*B*). Thus, ASCT1 was selectively down-regulated in astrocytes of white matter tissue from MS patients in conjunction with increased expression of Syncytin-1.

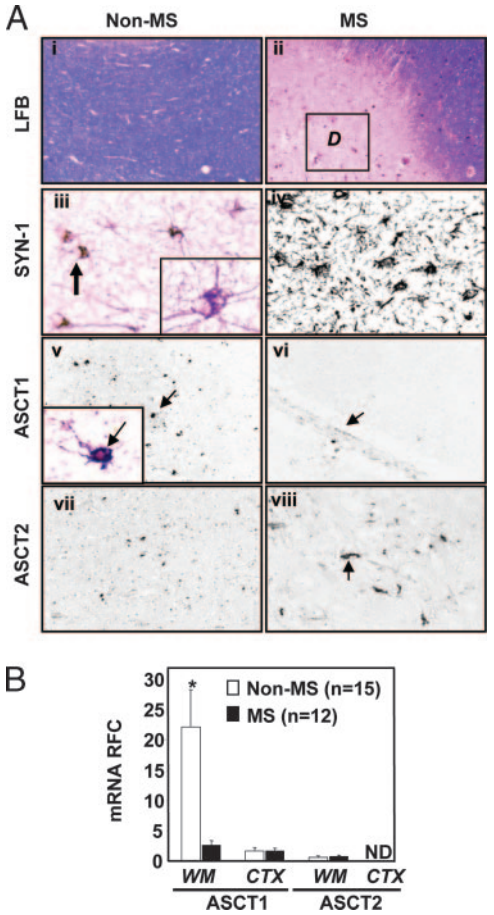

FIGURE 1. ASCT1 expression is suppressed in MS. *A*, Demyelinated (D) lesions from chronic MS patient brains (*ii*) stained less intensely with Luxol fast blue, H&E compared with non-MS controls (*i*). MS brains showed marked increase in Syncytin-1 immunoreactivity (*iv*), particularly in GFAP-positive astrocytes (*iii*, *inset*). ASCT1 expression was detected in non-MS brains (*v*), particularly in astrocytes (*v*, *inset*) compared with minimal immunoreactivity in acute MS white matter (*vi*). ASCT2 expression did not differ between MS (*viii*) and non-MS controls (*vii*). MS brains showed diminished ASCT1 mRNA in white matter (WM) but not in the frontal cortex (CTX) compared with non-MS controls. ASCT2 mRNA was undetectable in the CTX and did not differ between groups in the WM (*B*). (Original magnification, ×50 (*A*, *i* and *ii*); ×400 (*A*, *iii–viii*) (*inset*, ×1000)) (***, $p < 0.001$; *, $p < 0.05$).

Inflammation increases Syncytin-1 expression while Syncytin-1 diminishes ASCT1 levels in astrocytes

The envelope proteins of the HERV-W family induce several proinflammatory molecules (12, 43). We have also demonstrated previously that Syncytin-1 expression in brain-derived cells is induced by different mitogens (12). Because *HERV-R* gene expression is induced by TNF-α and IL-1β (28), we determined whether inflammation might induce Syncytin-1. Examination of Syncytin-1 mRNA in astrocytes treated with proinflammatory molecules revealed its induction by TNF-α in a concentration-dependent manner (Fig. 2*A*). Because ASCT1 down-regulation was observed in astrocytes of MS lesions, which also exhibited enhanced Syncytin-1 expression, we confirmed this in vivo result in an ex vivo system and determined the mechanism by which Syncytin-1-mediated suppression of its receptors in astrocytes. Cell cultures of purified brain-derived astrocytes, neurons, and monocyte-derived macrophages (MDM) were established. Expression of ASCT2 has been previously demonstrated in human fetal astrocytes (44) and placenta (45). However, the evidence for ASCT1 and ASCT2 protein expression in the brain-derived cells is sparse. Indeed, immunocytochemical detection of ASCT1 and ASCT2 revealed abundant expression in cultured human fetal astrocytes (Astro, Fig. 2*B*, *ii* and *vi*), MDM (Fig. 2*B*, *iii* and *vii*), and neurons (Fig. 2*B*, *iv* and *viii*) compared with the controls (Fig. 2*B*, *i* and *v*) suggesting ubiquitous expression in these brain-derived cells. To investigate the effects of mitogen/cellular differentiation on individual receptor expression, we examined transcript levels of each gene in primary human fetal astrocytes, MDMs, and neuronal (LAN-2) cells. PMA treatment disclosed significantly increased ASCT1 mRNA expression in MDMs (Fig. 2*C*), whereas no changes were seen with respect to ASCT1 and ASCT2 in other cell types (Fig. 2*C* and data not shown).

Syncytin-1 is able to form pseudotyped virus particles (4, 46, 47), but its cell tropism in the nervous system was unknown. We investigated whether Syncytin-1 was functional in the above cell types by using a pseudotyped virus assay. Supernatants from cells transfected with only pCDNA-Syncytin-1 or cotransfected with pNL-Luc-E$^-$R$^-$ exhibited Syncytin-1 immunoreactivity suggesting that Syncytin-1-pseudotyped virions were released into the culture medium (Fig. 2*D*). Infection with the pseudotyped viruses revealed that human MDMs and astrocytes but not human neurons were permissive to infection (Fig. 2*E*). Moreover, a feline lymphocyte line (MYA-1) was not permissive to the pseudotyped viruses (data not shown). Because Syncytin-1 was functional in these latter studies, we overexpressed Syncytin-1 in human fetal astrocytes by transduction of a Syncytin-1-expressing neurotropic Sindbis virus (12), which revealed syncytia formation in astrocytes (Fig. 2*F*), confirmed by glial fibrillary acidic protein (GFAP) positivity (Fig. 2*G*, *inset*). cDNA microarray analysis was subsequently performed of astrocytic cells transduced with the Sindbis virus-derived vector expressing Syncytin-1. The cDNA expression profile revealed several genes that were also affected in acute lesions from brains of MS patients in an earlier study (48) (Table II). Importantly, the two receptors for Syncytin-1, ASCT1 and ASCT2, were markedly suppressed in astrocytes overexpressing Syncytin-1 (Table III). Moreover, several genes involved in MS, particularly an MS lesion-specific transcript and a disintegrin and metalloproteinase (ADAM)-10 in addition to multiple genes involved in ER stress, myelin synthesis, and immune response, previously uncharacterized in MS lesions, were modulated in astrocytes expressing Syncytin-1 (Table III). These results suggested that TNF-α induces Syncytin-1 expression in astrocytes, whose enhanced levels contribute to diminished expression of its receptors in astrocytes. Nonetheless, the mechanism(s) by which Syncytin-1 contributed to suppression of its receptors remained uncertain.

ER chaperones are up-regulated in astrocytes overexpressing Syncytin-1

As several retroviral proteins cause neuropathogenic effects through aberrant protein expression (24, 26, 49), we investigated whether the increased levels of Syncytin-1 in the brains of MS patients (12) contributed to an integrated response leading to cellular pathology. Human fetal astrocytes, which overexpressed Syncytin-1, demonstrated significantly increased transcript levels of GADD153/CHOP, BiP, PERK, ERp57, and OASIS (Fig. 3*A*), but not in astrocytes transduced by an EGFP-expressing virus (control) ($p < 0.05$). However, ER chaperone genes were not induced in MDMs overexpressing Syncytin-1 relative to EGFP-expressing MDMs (Fig. 3*B*). Indeed, astrocytes transduced with an HIV-1 envelope-expressing construct also did not show induction of the same ER chaperone genes (data not shown). To determine the

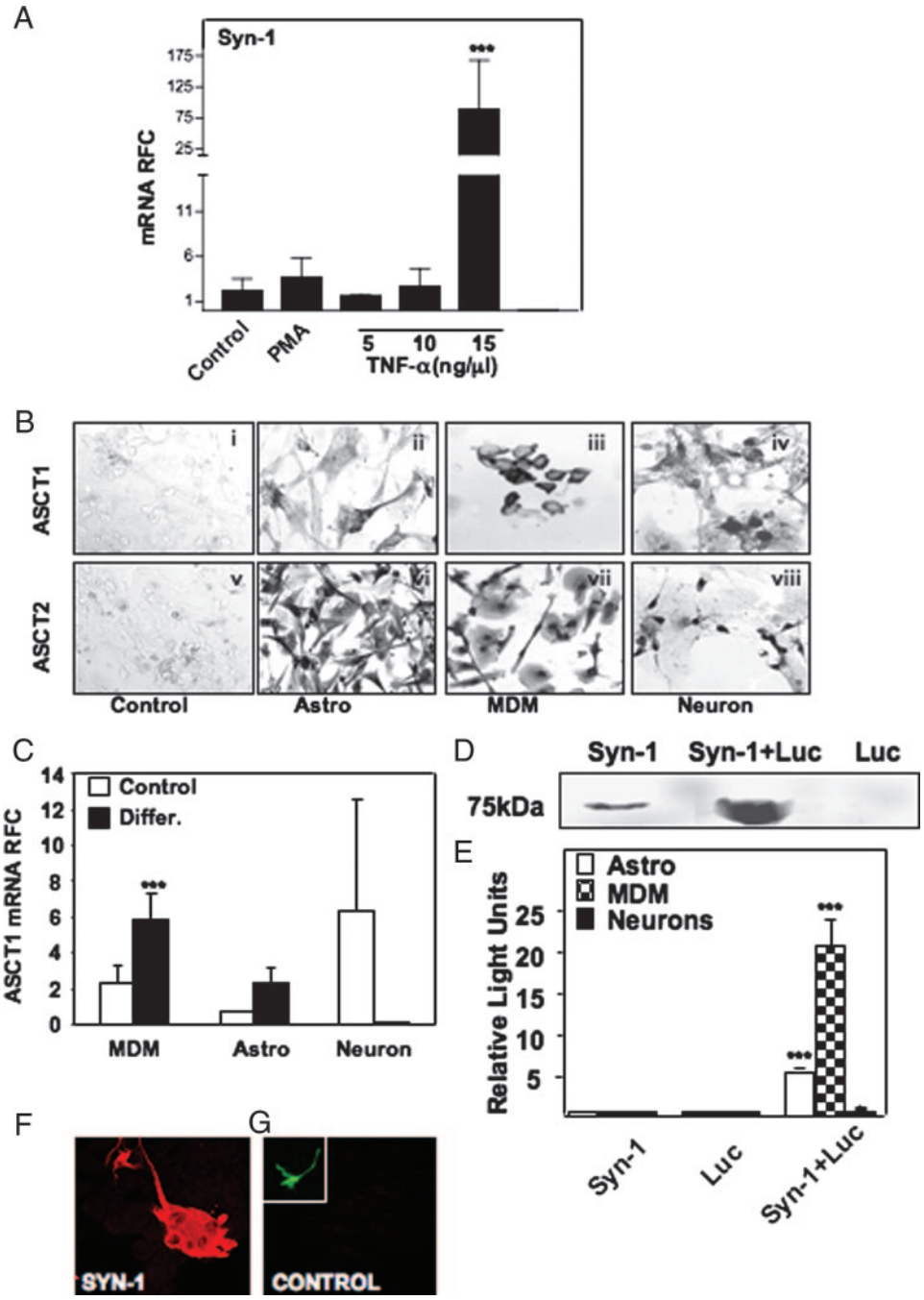

FIGURE 2. Inflammation drives Syncytin-1 expression. Syncytin-1 expression was significantly induced in astrocytes treated with 15 ng/ml TNF-α compared with untreated control (*A*). *B*, Immunostaining of human fetal astrocytes (*ii* and *vi*), macrophages (*iii* and *vii*), and primary neurons (*iv* and *viii*) revealed the expression of ASCT1 and ASCT2, compared with control cells for which the primary Abs were omitted (*i* and *v*). Relative quantification of ASCT1 and ASCT2 was performed using cDNA prepared from MDM, astrocytes (U373), and neurons (LAN-2). ASCT1 mRNA expression was significantly increased in differentiated MDMs (*C*). However, there were no significant differences in the levels of ASCT2 expression among the cell lines examined. Syncytin-1 immunoreactivity in supernatants from HEK293T cells transfected with pCDNA-Syncytin-1 (Syn-1) was enhanced by cotransfection with pNL-Luc-E$^-$R$^-$ (Luc) compared with pNL-Luc-E$^-$R$^-$ alone (*D*). MDMs and astrocytes but not neurons were permissive to infection by the pseudotyped virus (*E*). Syncytin-1 (SYN-1) expressing human fetal astrocytes (*F*) but not controls (*G*) exhibit syncytia formation ((*inset*, GFAP-positive astrocytes) (original magnification, $\times$200 (*B*, *F* and *G*)) (***, $p < 0.001$).

comparative transcript levels of ER chaperone genes in Syncytin-1-expressing astrocytes and brains of MS patients, we examined ER chaperone gene induction in brain white matter from MS ($n = 15$) and non-MS controls ($n = 12$). The expression profile of ER chaperones observed in Syncytin-1-expressing astrocytes closely resembled transcript abundance in brain white matter tissue of MS patients relative to non-MS controls (Fig. 3*C*). In particular, the induction of OASIS was highly significant ($p < 0.001$) in MS brains compared with non-MS controls. To determine the cell types expressing ER chaperones, we analyzed tissue sections from MS and non-MS control brain sections. Serial sections from the same lesions showed increased GADD153/CHOP (Fig. 3*Dii*) and BiP (Fig. 3*Div*) expression compared with non-MS control brain (Fig. 3*D*, *i* and *iii*, respectively). GADD153/CHOP expression was increased in several cell types, but most prominently in astrocytes, colocalized with GFAP immunoreactivity (Fig. 3*Dii*, *inset*) (GADD153/CHOP: blue; GFAP: brown) in brains of MS patients. BiP expression was found in several cell types including astrocytes (Fig. 3*Div*, arrow) and macrophages (Fig. 3*Div*, arrowhead) in the white matter of MS demyelinating lesions compared with non-MS controls (Fig. 3*Diii*), whereas ERp57 (data not shown) was minimally expressed. However, PERK, a key component of the ER stress pathway, was not detected by immunohistochemistry, which might be a limitation of the Ab used in this study, rather than its absence, as transcript levels were detected (Fig. 3*C*). Considering the recent evidence showing ER stress in oligodendrocytes (15) and induction of GADD153/CHOP by retroviral infections leading to oligodendropathy (26), we expected to see ER stress proteins in these myelin-producing cells. However, the acute demyelinating lesions examined in the present study did not reveal any GADD153/CHOP-immunopositive

Table II. *Genes increased and decreased in Syncytin-1-expressing astrocytes, showing a similar profile in lesions from MS patients*

	Gene Name	Function	Fold Change	GenBank ID
Increased	*CEBPG*	CCAAT/enhancer-binding protein (C/EBP), γ	2.75	BE622659
	TNFRSF6	Tumor necrosis factor receptor superfamily, member 6	2.433333	NM_000043
	RCN1	Reticulocalbin 1, EF-hand calcium-binding domain	1.607143	NM_002901
Decreased	*GAD2*	Glutamate decarboxylase 2 (pancreatic islets and brain, 65 kDa)	0.961538	BQ128302
	SPOCK2	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2	0.955752	NM_014767
	SST	Somatostatin	0.913462	NM_001048
	NEUROD2	Neurogenic differentiation 2	0.875	AB021742
	PVALB	Parvalbumin	0.869565	NM_002854
	CCNI	Cyclin I	0.854701	BG530368
	SCD	Stearoyl-CoA desaturase (δ-9-desaturase)	0.842105	BC005807
	RPS4Y1	Ribosomal protein S4, Y-linked 1	0.827586	NM_001008
	RAB5B	RAB5B, member RAS oncogene family	0.820896	AF267863
	PEG3	Paternally expressed 3	0.817308	AF208967
	HTR7	5-Hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled)	0.706767	NM_019859
	ACAT2	Acetyl-coenzyme A acetyltransferase 2 (acetoacetyl coenzyme A thiolase)	0.704403	BC000408
	HSPA6	Heat shock 70-kDa protein 6 (HSP70B′)	0.7	NM_002155
	AQP11	Aquaporin 11	0.680272	AI886656
	MAP1D	Methionine aminopeptidase 1D	0.675676	AA779679
	KIF5A	Kinesin family member 5A	0.666667	AF063608
	CNP	2′,3′-Cyclic nucleotide 3′ phosphodiesterase	0.619792	AK098048
	EXTL3	Exostoses (multiple)-like3	0.619355	BC006363

oligodendrocytes. Thus, multiple ER chaperones including OASIS and GADD153/CHOP were overexpressed in brain glia of MS patients, suggesting a role for ER stress in the pathogenesis of MS.

OASIS contributes to inflammation and diminished ASCT1 expression in astrocytes

As several retroviral proteins, including Syncytin-1, induce free radicals in glia (12), we hypothesized that Syncytin-1 might influence expression and function of its putative receptors, ASCT1 and ASCT2, in the brain (50), perhaps through regulation by free radical production and removal, similar to the related transporter, xCT (13, 51, 52), whose expression in microglia potentiates neuronal injury in Alzheimer's disease (53). Corresponding to increased transcript levels of OASIS in MS brain lesions (Fig. 3*C*), Western blot analysis (Fig. 4*A*, *inset*) of non-MS ($n = 6$) and MS ($n = 6$) patient brain lysates revealed a significant increase in OASIS immunoreactivity in the brain (Fig. 4*A*). Because OASIS induces the transcription of target genes by acting on the ER stress responsive element and cAMP responsive element (54), we hypothesized that OASIS might trigger iNOS, perhaps through the cAMP responsive element within the iNOS

Table III. *Gene profile in Syncytin-1-expressing astrocytes showing up-regulation and down-regulation of immune response, myelin-related, and ER stress genes*

Gene Name	Function	Fold Change	GenBank ID
ER stress response			
STCH	Stress 70 protein chaperone, microsome-associated, 60 kDa	8.3	AI718418
HSPH1	Heat shock 105-kDa/110-kDa protein 1	5.285714	NM_006644
LONP	Peroxisomal lon protease	3.32	AV700132
FLJ23560	Hypothetical protein FLJ23560	2.564103	NM_024685
FLJ14281	Hypothetical protein FLJ14281	2.375	NM_024920
HSPD1	Heat shock 60-kDa protein 1 (chaperonin)	2.236842	NM_002156
SERP1	Stress-associated endoplasmic reticulum protein 1	2.261905	AL136807
HERPUD1	Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	0.735294	BC015447
HSPA5BP1	Heat shock 70-kDa protein 5 (glucose-regulated protein, 78 kDa) binding protein 1	0.710692	AB046803
GADD45B	Growth arrest and DNA-damage-inducible, β	0.702899	AV658684
Syncytin-1	HERV-W envelope glycoprotein	3.114286	AC000064
SLC1A4 (ASCT1)	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	0.677632	W72527
SLC1A5 (ASCT2)	Solute carrier family 1 (neutral amino acid transporter), member 5	0.693431	AF105230
MS related			
ADAM10	A disintegrin and metalloproteinase domain 10	8.25	N51370
MS lesion	MS lesion transcript	6.818182	N73682
CMT4B2	Charcot-Marie-Tooth neuropathy 4B2 (autosomal recessive, with myelin outfolding)	4.478261	AK022478
MBP	Myelin basic protein	0.734266	NM_002385
MAG	Myelin-associated glycoprotein	0.685039	X98405
Immune response			
SOCS5	Suppressor of cytokine signaling 5	5.375	NM_014011
STAT1	Signal transducer and activator of transcription 1, 91 kDa	2.121951	NM_007315
TLR4	Toll-like receptor 4	2.05	NM_003266
TLR9	Toll-like receptor 9	0.731707	AB045180
IL23A	Interleukin 23, α subunit p19	0.724138	NM_016584
OLIG1	Oligodendrocyte transcription factor 1	0.722222	AL355743
TLR8	Toll-like receptor 8	0.606838	NM_016610
OAS1	2′,5′-oligoadenylate synthetase 1, 40/46 kDa	0.413408	NM_002534

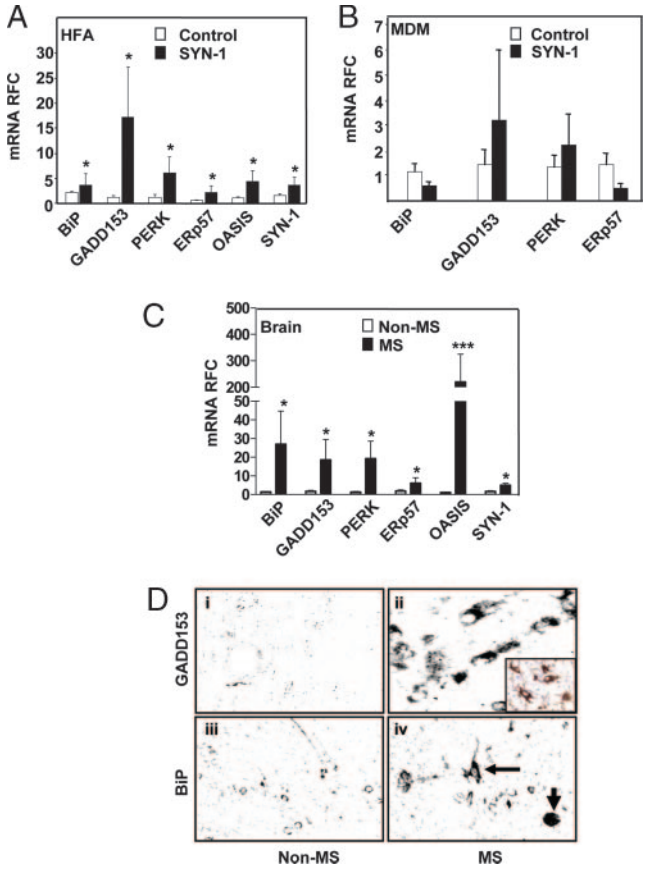

FIGURE 3. ER chaperones are up-regulated in astrocytes overexpressing Syncytin-1. Syncytin-1 (SYN-1) expressing human fetal astrocytes (HFA) but not controls exhibit increase in mRNA relative fold change (RFC) in mRNA of Syncytin-1 and ER chaperone genes, BiP, GADD153/CHOP, PERK, ERp57, and OASIS compared with controls (*A*). MDMs expressing Syncytin-1 did not show any increase in the transcript levels of ER chaperone genes (*B*). Increased transcript levels of Syncytin-1, BiP, GADD153/CHOP, PERK, ERp57, and OASIS were evident in the white matter tissue of MS ($n = 12$) brains relative to non-MS controls ($n = 11$) (*C*). *D*, MS brains showed marked increase in GADD153/CHOP immunoreactivity (*ii*), particularly in GFAP-positive astrocytes (*ii*, *inset*) and BiP (*iv*) expression compared with non-MS controls (*i* and *iii*). (Original magnification, ×400 (*i–iv*); *inset*, ×1000) (***, $p < 0.001$; *, $p < 0.05$).

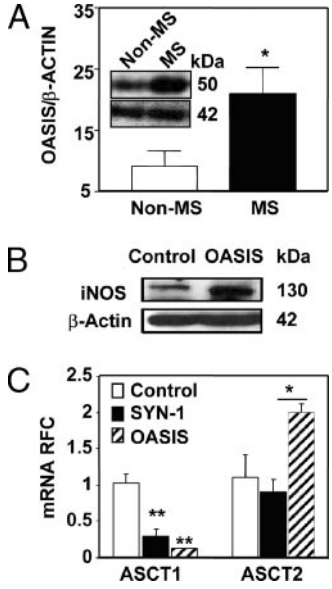

FIGURE 4. OASIS contributes to inflammation and diminished ASCT1 expression in astrocytes. OASIS immunoreactivity (50 kDa) was increased in MS brains compared with non-MS controls (*A*, *inset*). Graphic analysis of OASIS immunoreactivity relative to β-actin revealed a significant increase in MS brains (*B*). iNOS immunoreactivity was increased in astrocytes overexpressing OASIS relative to the empty vector (Control) (*C*). Astrocytes overexpressing OASIS or Syncytin-1 (SYN-1) down-regulated ASCT1, but not ASCT2 compared with empty pcDNA 3.1 vector (Control) (C) (**, $p < 0.01$; *, $p < 0.05$).

promoter (55). Indeed, increased expression of iNOS in OASIS-transfected astrocytes was evident compared with the control (empty vector) (Fig. 4*B*). Based on these results, we transfected astrocytes with an OASIS expression vector. Overexpression of OASIS resulted in down-regulation of Syncytin-1 receptor, ASCT1, but not ASCT2, with the latter gene demonstrating increased expression (Fig. 4*C*). Interestingly, soluble Syncytin-1 also induced OASIS transcription in astrocytes, which was inhibited by a mAb to Syncytin-1 (data not shown). Thus, Syncytin-1-induced OASIS expression, resulting in iNOS induction, which was associated with down-regulation of ASCT1 expression, but not ASCT2, thereby recapitulating the present observations in MS brain lesions.

Syncytin-1 directly contributes to ASCT1 suppression

Retroviral envelope glycoproteins including Syncytin-1 mediate immune responses (12, 43) and receptor expression (45). To examine whether the down-regulation of ASCT1 in our model was directly due to enhanced Syncytin-1 expression, we constructed a vector that expressed Syncytin-1-YFP fusion protein. Syncytin-1 was cloned into the pYFP vector for subsequent expression in various target cells. Transfected cells expressed the fusion protein with minimal cell death for at least 72 h (Fig. 5*A*, *i* and *ii*). Human fetal astrocytes, which expressed Syncytin-1-YFP (Fig. 5*Ai*), were transfected with siRNAs directed to the surface unit of Syncytin-1 (Fig. 5*Aiii*) and expression of YFP was monitored as an indication of Syncytin-1 knockdown. Cells transfected with Syncytin-1-YFP, with (Fig. 5*A*, *iii* and *iv*) or without (Fig. 5*A*, *i* and *ii*) siRNA treatment, were viable but siRNA treatment abrogated Syncytin-1 expression, indicated by absence of YFP expression (Fig. 5*Aiii*). Having confirmed knockdown of Syncytin-1 by monitoring expression of YFP by confocal microscopy of live cells (Fig. 5*A*, *i* and *iii*) and preserved cellular viability by bright field microscopy (Fig. 5*A*, *ii* and *iv*) for 72 h, we proceeded to confirm whether Syncytin-1 transcripts were similarly inhibited by siRNA against Syncytin-1. Syncytin-1-YFP expression was correlated with high transcript levels of Syncytin-1 in astrocytes relative to mock-transfected control cells (Fig. 5*B*). siRNA directed to Syncytin-1 suppressed transcript levels of Syncytin-1 in HFA (Fig. 5*B*) and HEK293T cells (data not shown). Because siRNA-mediated knockdown of Syncytin-1 was optimized, we tested whether Syncytin-1 influenced the expression of its cognate receptors. Indeed, astrocytes that expressed Syncytin-1 displayed significantly lower levels of ASCT1 but not that of another amino acid transporter, EAAT1 (Fig. 5*C*). However, when these cells were subsequently treated with siRNA to block Syncytin-1, the transcript level of ASCT1 was restored (Fig. 5*C*). These results indicated that the down-regulation of ASCT1 in astrocytes is a consequence of enhanced Syncytin-1 expression, which was consistent with observations made in the white matter lesions of MS patients.

Syncytin-1 impairs expression of myelin proteins

Oligodendrocyte injury and death might represent an early neuropathological feature of MS, in advance of infiltrating inflammatory

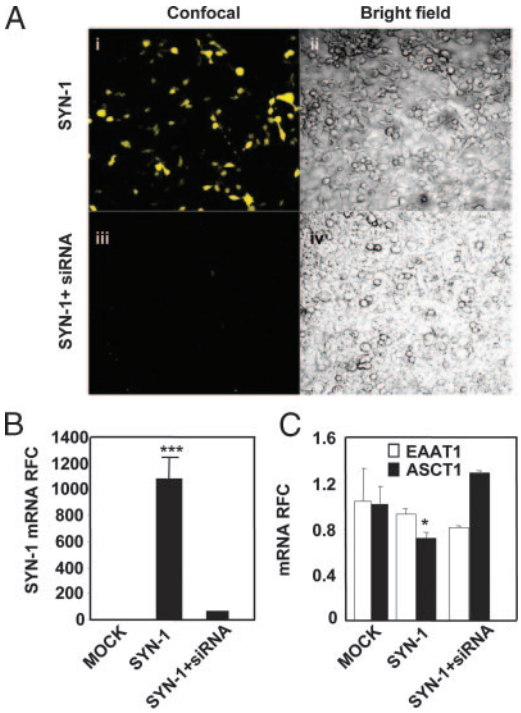

FIGURE 5. Syncytin-1 down-regulates ASCT1. *A*, Confocal (*i* and *iii*) and bright-field microscopy (*ii* and *iv*) images of cells expressing Syncytin-1 (SYN-1) (*i*) together with siRNA against Syncytin-1 (SYN-1 + siRNA) showed reduced expression of Syncytin-1 (*iii*). RT-PCR confirmed Syncytin-1 knockdown by Syncytin-1-directed siRNA (*B*). Syncytin-1 expression reduced the ASCT1 transcript level, which was significantly reversed by siRNA-mediated knockdown of Syncytin-1 (*C*). Under similar conditions, expression of another amino acid transporter, EAAT1 was not altered (*C*) (***, $p < 0.001$; *, $p < 0.05$).

cells (56), but it is also a cardinal feature of established MS lesions (57). In our earlier study, we used other oligodendrocyte markers such as adenomatous polyposis coli and GSTpi, revealing that Syncytin-1 mediated cell death through an uncertain mechanism (12). To determine whether a direct cause-and-effect relationship existed between expression of Syncytin-1 in astrocytes and expression of myelin proteins, we examined the effects of supernatants from astrocytes overexpressing Syncytin-1 on an early myelination marker, CNP, and a late myelination marker, MBP, both of which are present in the present oligodendrocyte cultures. The cell culture protocol was a modification of procedures adopted to isolate mouse oligodendrocytes and we have been successful in using this culture system in earlier studies (12, 58). Supernatants from Syncytin-1-transfected astrocytes reduced the number of CNP-positive oligodendrocytes (Fig. 6*Aii*). However, siRNA-mediated knockdown of Syncytin-1 in astrocytes markedly reversed these adverse effects, restoring CNP immunoreactivity (Fig. 6*Aiii*) to control levels (Fig. 6*Ai*). siRNA-mediated inhibition of Syncytin-1 expression restored the total number of CNP-positive oligodendrocytes, which was reduced by Syncytin-1 expression in astrocytes (Fig. 6*B*). Immunofluorescence levels of CNP-expressing oligodendrocytes (Fig. 6*C*), which were reduced by Syncytin-1-mediated toxicity, were also rescued by siRNA-mediated inhibition of Syncytin-1 expression in astrocytes but there was no effect of Syncytin-1 on MBP immunofluorescence (Fig. 6*C*). Because our observations indicated a reduction in expression of ASCT1 in MS brain white matter (Fig. 1) and in astrocytes overexpressing Syncytin-1 (Fig. 4 and Table III), we hypothesized that inhibition of ASCT transporters may adversely affect astrocyte function. Supernatants from astrocytes treated with benzylserine, an inhibitor of ASCT transporters (59), were found to activate caspase-3 in rat oligodendrocytes in a concentration-dependent manner (Fig. 6*D*). Indeed, similar

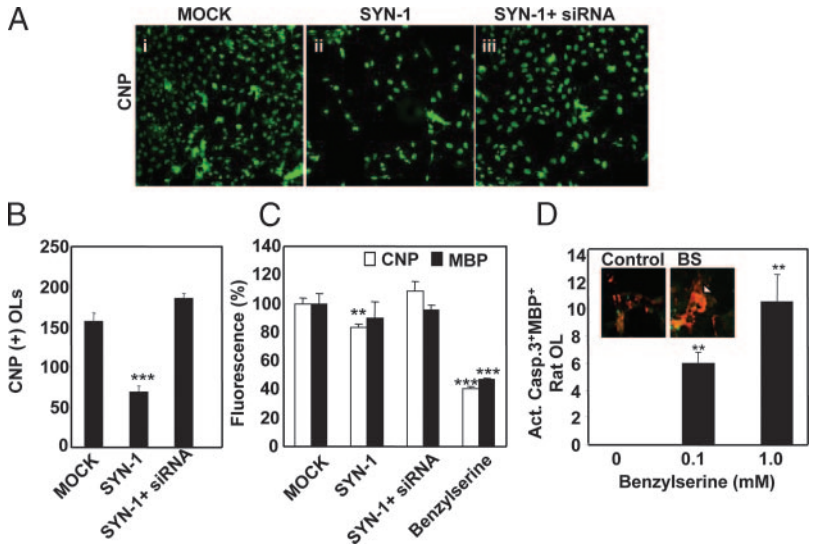

FIGURE 6. Syncytin-1 diminishes oligodendrocyte viability. *A*, Oligodendrocytes treated with supernatants from mock (*i*), Syncytin-1 (SYN-1) overexpressing (*ii*), and Syncytin-1 overexpressing with anti-Syncytin-1 siRNA transduction (*iii*) human fetal astrocytes revealed that the supernatants from Syncytin-1-overexpressing astrocytes were cytotoxic to oligodendrocytes (*ii*). Spontaneous death of oligodendrocytes in cultures ranged from 1.2 to 2.5%. siRNA-mediated knockdown of Syncytin-1 reversed the toxic effects of Syncytin-1-expressing astrocyte supernatants as shown by counts of the number of CNP-positive oligodendrocytes (*B*) as well as quantitative analysis of immunofluorescence depicted as a percent of CNP immunoreactivity (*C*). Immunoreactivity of MBP was not affected by various treatments (*C*). Supernatants from human fetal astrocytes treated with 1 mM benzylserine decreased oligodendrocyte viability as demonstrated by significantly low levels of CNP and MBP immunoreactivity (*C*). Human fetal astrocytes (HFA) were treated with benzylserine dissolved in DMSO or medium containing similar amounts of DMSO. A total of 100 μl of supernatant from HFA was added to rat oligodendrocytes cultured on a chamber slide and incubated for 24 h at 37°C. Oligodendrocytes were immunostained for MBP (red) and activated caspase-3 (green). The number of activated caspase-3 and MBP-positive oligodendrocytes was counted per field and expressed as a ratio of positive cells in benzylserine (BS) containing supernatant to that of DMSO containing supernatant from astrocytes. Results indicate a concentration-dependent increase in oligodendrocyte damage and injury with benzylserine treatment (*D*). (Original magnification, ×400 (*A*)) (***, $p < 0.001$; **, $p < 0.01$).

treatment of oligodendrocytes reduced immunofluorescence levels of both CNP and MBP (Fig. 6*C*), more so than astrocytes expressing Syncytin-1 ($p < 0.01$). Thus, Syncytin-1 expression and inhibition of ASCT reduced oligodendrocyte viability, as evidenced by suppression of myelin protein and cell death.

iNOS and Egr1 suppress ASCT1 in astrocytes

Brains of MS patients display increased expression of iNOS (12, 60) and Egr1 (18), a transcriptional repressor of ASCT1 (61). Given that OASIS induced iNOS in astrocytes, we examined the contributions of iNOS and Egr1 to the expression of ASCT1. Transfection of astrocytes with a vector expressing Syncytin-1 revealed significant induction of Egr1, but not Egr3 and Egr4 ($p < 0.05$; Fig. 7*A*). Treatment of astrocytes with the NO donor, SNP, reduced ASCT1 mRNA ($p < 0.05$; Fig. 7*B*). Expression of Egr1 was also significantly increased by SNP in keeping with earlier studies showing that NO enhanced Egr1 expression (62) ($p < 0.001$; Fig. 7*B*). Our observations were confirmed by quantitative immunofluorescence analysis of Egr1 expression, which was increased in astrocytes after SNP or Syncytin-1 treatment ($p < 0.05$; Fig. 7*C*). SNP treatment decreased ASCT1 expression in a dose-dependent manner in astrocytes ($p < 0.05$; Fig. 7*D*), confirming our PCR results (Fig. 7*B*). The suppression of ASCT1 transcripts observed in Syncytin-1-overexpressing astrocytes was significantly reversed by treatment of cells with a nonspecific NOS inhibitor, L-NAME (Fig. 7*E*). ASCT1 suppression was independent of regulation by cytokines, as both IL-10 and IL-1β significantly increased the transcription of ASCT1 (Fig. 7*F*). Enhanced expression of Egr1 was also observed in MS brain white matter lesions (Fig. 7*H*) compared with non-MS controls (Fig. 7*G*). Egr1 was localized predominantly in the cytoplasm of astrocytes in the white matter (GFAP-positive astrocytes: blue; Egr1: brown) (Fig. 7*H*, *inset*). Thus, these observations indicated that diminished ASCT1 expression on astrocytes was induced by Syncytin-1 and modulated by NO and Egr1.

Syncytin-1-Tg mice exhibit neuroinflammation and induction of ER chaperones

We have previously reported up-regulation of Syncytin-1 in the brains of MS patients relative to age-matched controls (12). To extend the ex vivo data obtained above, we generated Tg mice expressing Syncytin-1, under the control of the *GFAP* promoter (39) in CD-1 mice (Fig. 8*A*). Transgene detection was performed by PCR assay using tail-derived genomic DNA (Fig. 8*B*). As Syncytin-1 Tg mice displayed no disease phenotype, we used a model of MS in which TNF-α was stereotaxically implanted into the corpus callosum (12), an anatomical region frequently exhibiting demyelination in MS patients (63). TNF-α activates GFAP (64) and also enhanced Syncytin-1 expression in astrocytes (Fig. 2*A*). Because Syncytin-1 Tg and Wt littermates stereotaxically implanted with PBS did not demonstrate Syncytin-1 expression (Fig. 8*C*), subsequent comparisons were made between Tg and Wt animals implanted with TNF-α, which significantly induced Syncytin-1 mRNA (Fig. 8*D*) and protein (Fig. 8*E*) expression in brains of Tg mice. To determine whether Syncytin-1 expression in the brain provoked an inflammatory response in mice, we examined TNF-α-implanted Syncytin-1-Tg and Wt littermates, which showed that the inflammatory genes *TNF-α* and *IFN-α* were increased in the implanted Tg mice brains relative to implanted Wt animals (Fig. 8*F*). Induction of proinflammatory molecules was specific to the brain, because treatment of macrophages from Syncytin-1-Tg mice with TNF-α did not induce proinflammatory gene expression differentially compared with Wt-derived macrophages (data not shown). Interestingly, expression of CNP and another

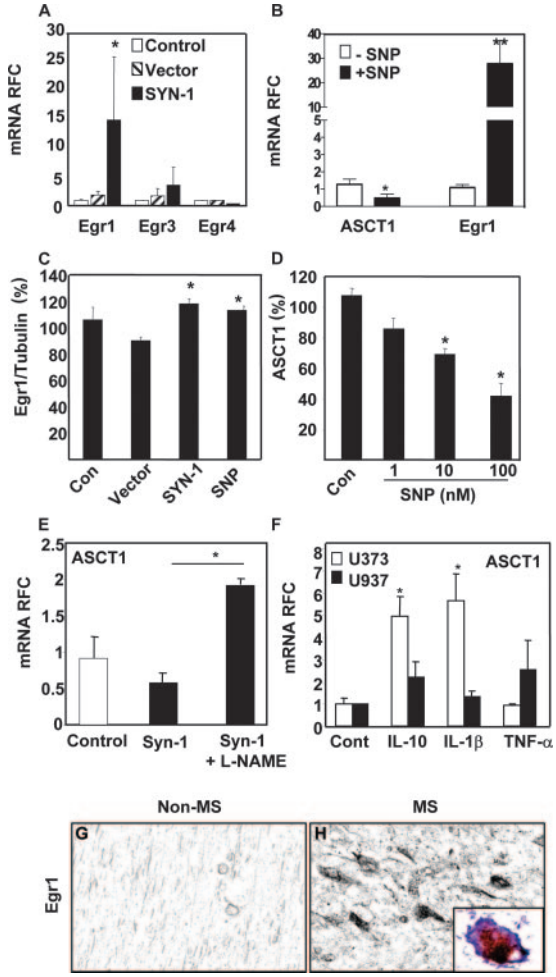

FIGURE 7. iNOS and Egr1 suppress ASCT1 abundance. Expression of Syncytin-1 (SYN-1) in astrocytes induced the expression of the repressive transcription factor Egr1, but not Egr3 and Egr4, (*A*) compared with mock (Control) or empty vector (Vector) transfected cells. Treatment of astrocytes with the NO donor, SNP (+SNP) significantly decreased ASCT1 expression, concurrently increasing the expression of Egr1 compared with untreated cells (−SNP) (*B*). Quantitative immunofluorescence of Egr1 showed significant induction in astrocytes treated with soluble Syncytin-1 (SYN-1) or SNP relative to supernatants from empty vector (Vector) or mock (Control) transfected HEK293T cells (*C*). SNP dose-dependently suppressed ASCT1 in astrocytes, measured by quantitative immunofluorescence analysis (*D*). The suppression of ASCT1 in astrocytes expressing Syncytin-1 was reversed when cells were treated with the nonselective NOS inhibitor, L-NAME (5 μM) (*E*). Astrocytes (U373) and monocytoid cells (U937) were treated with IL-10, IL-1β, and TNF-α (10 ng/ml) for 24 h and expression of ASCT1 was examined by real-time RT-PCR, which revealed a significant increase with IL-10 and IL-1β, but not with TNF-α treatment, in astrocytes but not monocytoid cells (*F*). MS brains (*H*) revealed increased expression of Egr1 in astrocytes in the white matter compared with non-MS controls (*G*). (Original magnification, ×400 (*G* and *H*); *inset H*, ×1000) (**, $p < 0.01$; *, $p < 0.05$).

marker of oligodendrocyte/myelination, ceramide galactosyltransferase (CGT), were significantly reduced in Tg mice (Fig. 8*F*). Brain tissue from TNF-α-implanted Tg mice also revealed significant induction of ER chaperone gene transcripts, ERp57, OASIS, and GADD153/CHOP, compared with Wt littermates (Fig. 8*G*), while both BiP and PERK showed a trend toward increased transcript levels. OASIS, which is induced during astrocytic ER stress (31) (Fig. 3*A*) and in acute lesions of MS patients (Fig. 4*A*), was also increased in the brain of Syncytin-1-Tg mice (Fig. 8*G*). Expression of BiP and GADD153/CHOP proteins was increased in

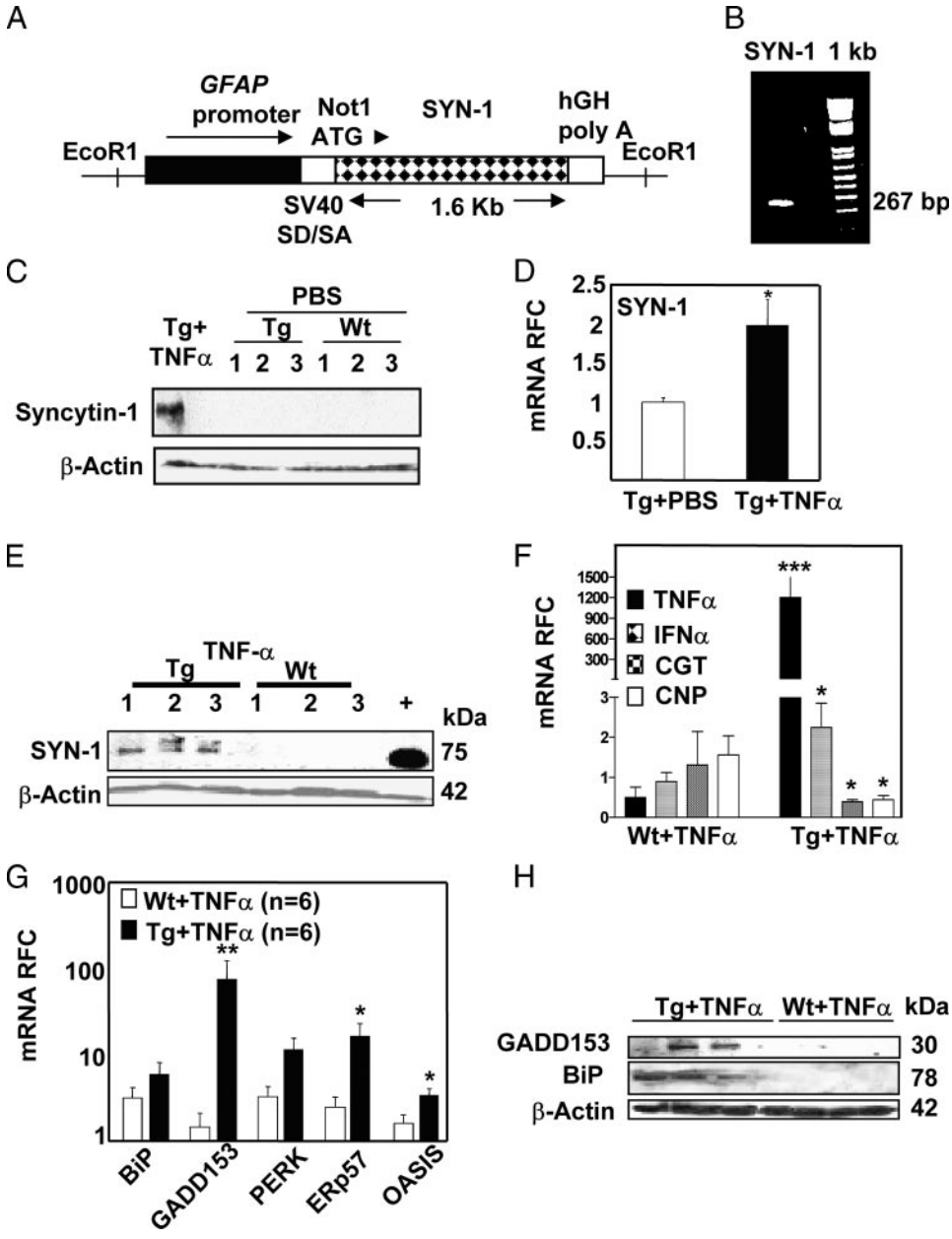

FIGURE 8. Syncytin-1-Tg mice show neuroinflammation and ER stress. The *Syncytin-1* gene was cloned into pFGH vector containing the GFAP promoter (*A*). Transgene integration was confirmed by genotyping revealing a 267-bp product (SYN-1) (*B*). Implantation of PBS into the corpus callosum of Syncytin-1-Tg mice did not induce Syncytin-1 expression compared with implantation with TNF-α, where the Syncytin-1 immunoreactive band is seen in *lane 1* (*C*). TNF-α implantation induced Syncytin-1 transcript levels in Tg mice compared with PBS-implanted Tg mice (*D*). TNF-α implantation induced Syncytin-1 immunoreactivity on Western blot in Tg mice brains but not in Wt littermates (*E*). TNF-α implantation also enhanced levels of the proinflammatory genes, *TNF-α* and *IFN-α*, and decreased levels of the oligodendrocyte markers, CGT and CNP, in Syncytin-1-Tg mice relative to Wt littermates (*F*). TNF-α implantation significantly enhanced levels of ER chaperone genes, particularly *GADD153/CHOP*, *ERp57*, and *OASIS*, in the brains of Syncytin-1-Tg mice relative to Wt littermates (*G*). These latter results were also confirmed by Western blot analysis, which revealed induction of GADD153/CHOP and BiP in Syncytin-1-Tg mice brains (*H*). (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

brains of Syncytin-1-Tg mice (Fig. 8*H*). Thus, the Syncytin-1-Tg mice exhibited neuroinflammation with induction of several ER chaperones, similar to observations in MS brains.

Syncytin-1-Tg animals show diminished myelin proteins and neurobehavioral deficits

The brains of TNF-α-implanted Syncytin-1-Tg mice revealed astrocytosis, demonstrated by increased GFAP immunoreactivity and astrocyte hypertrophy (Fig. 9*A*, *i* and *ii*). Iba-1 immunoreactivity on monocytoid cells was also augmented in the brains of Tg mice compared with Wt littermates (Fig. 9*A*, *iii* and *iv*). Brains of Tg mice displayed increased numbers of infiltrating CD3-positive T cells relative to controls (Fig. 9*A*, *v* and *vi*). Importantly, Syncytin-1-positive astrocytes (Fig. 9*Aviii*, *inset*) in brains of Tg mice demonstrated increased iNOS immunoreactivity (Fig. 9*Aviii*), relative to Wt littermates (Fig. 9*Avii*), which supported the notion that iNOS and ER stress induction are closely coupled (65). Analysis of CNP immunoreactivity in the corpus callosum demonstrated a marked reduction in Syncytin-1-Tg mice (Fig. 9*Ax*) compared with Wt littermates implanted with TNF-α (Fig. 9*Aix*), confirming earlier observations (12). The Syncytin-1-Tg mice, thus, did not possess a hypomyelination phenotype, but rather a specific demyelination because CNP immunostaining in the corpus callosum was unaffected in sites remote from TNF-α implantation. Expression of the ER chaperone, BiP, was observed in both groups (Fig. 9*A*, *xi* and *xii*), while GADD153/CHOP expression was prominently expressed in oligodendrocytes in brains of Syncytin-1-Tg mice (Fig. 9*A*, *xiv* and *xiii*, *inset*) compared with Wt littermates implanted with TNF-α (Fig. 9*Axiii*). Expression of the ER stress protein, GADD153/CHOP, in oligodendrocytes corroborates recent observations in murine retrovirus-induced oligodendropathy (26). Complementing these findings, Syncytin-1-Tg mice implanted with TNF-α displayed significantly lower ASCT1 transcript levels but ASCT2 did not differ between groups. This finding corresponded to higher iNOS mRNA levels in the Tg mice (Fig. 9*B*), emphasizing our immunohistochemical analysis. This effect appeared to be specific to iNOS because transcript levels of neuronal NOS did not differ between Wt and Tg mice implanted with TNF-α (data

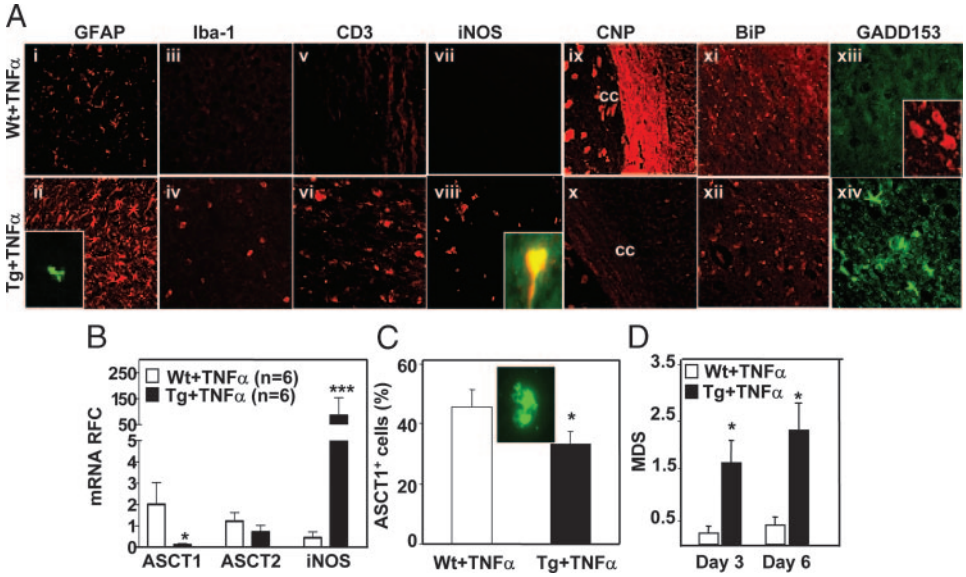

FIGURE 9. Syncytin-1-Tg mice display neuroinflammation, oligodendrocyte loss and neurobehavioral abnormalities. *A*, Syncytin-1-Tg mice implanted with TNF-α revealed astrocytosis (GFAP immunoreactivity) (*ii*, *inset*), microgliosis (Iba-1 immunoreactivity) (*iv*), infiltrating CD3-positive T cells (*Avi*), and iNOS immunoreactivity in Syncytin-1-positive cells (*viii*, *inset*) compared with Wt littermates (*i*, *iii*, *v*, and *vii*). CNP expression was reduced in the corpus callosum (CC) of Syncytin-1-Tg mice implanted with TNF-α (*x*) compared with Wt littermates (*ix*). BiP expression was observed in Wt and Tg mice (*xi* and *xii*), while GADD153/CHOP expression was prominently expressed in oligodendrocytes in brains of Syncytin-1-Tg mice (*xiv* and *xiii*, *inset*) compared with Wt littermates implanted with TNF-α (*xiii*). TNF-α implantation induced iNOS but reduced ASCT1 expression, but not ASCT2, in Syncytin-1-Tg mice relative to Wt littermates (*B*). Analysis of ASCT1-immunopositive cells in the corpus callosum (*C*, *inset*) revealed significantly lower numbers in TNF-α-implanted brains of Syncytin-1-Tg mice compared with Wt littermates (*C*). Mean deficit scores (MDS) revealed significantly high MDS scores for Syncytin-1-Tg mice implanted with TNF-α compared with Wt littermates (*D*). (***, $p < 0.001$; *, $p < 0.05$) (Original magnification, $\times$400 (*A*); *inset* (*A* and *C*), $\times$1000).

not shown). Furthermore, the number of ASCT1-positive cells was reduced in Syncytin-1-Tg mice implanted with TNF-α compared with Wt littermates (Fig. 9*C*). Stereotaxic implantation of TNF-α into the corpus callosum of Syncytin-1-Tg animals resulted in significant neurobehavioral deficits compared with TNF-α-implanted Wt littermates at days 3 and 6 postimplantation (Fig. 9*D*). The mean deficit scores were obtained from a combination of three behavioral tests described previously (41). TNF-α-implanted Tg mice grasped a horizontal rod for a significantly shorter length of time compared with the Wt littermates. In addition, Tg mice were sufficiently impaired that they could not hold on to an inverted screen and reach the screen edge, while Wt littermates reached the edges of the inverted screen more quickly. Lastly, Tg mice implanted with TNF-α exhibited mean delays in the time taken to cross a cantilevered beam, compared with Wt littermates, suggesting that the Tg mice showed diminished motor activity and exploratory behavior. Thus, in vivo Syncytin-1 overexpression resulted in neuroinflammation characterized by induction of iNOS and activation of ER chaperones, particularly OASIS and subsequent down-regulation of the Syncytin-1 receptor, ASCT1, resulting in suppression of myelin proteins and neurobehavioral abnormalities.

Discussion

Sustained activation of the innate immune cells within the CNS in concert with persistent adaptive immune responses promotes neural injury during MS, independent of the roles of infiltrating T and B cells (66, 67). Herein, overexpression of a human endogenous retrovirus envelope protein, Syncytin-1, in astrocytes exerted neuropathogenic effects by inducing the ER chaperone, OASIS, with ensuing down-regulation of the Syncytin-1 receptor, ASCT1, accompanied by the release of oligodendrocyte cytotoxins (Fig. 10). Activation of OASIS-induced iNOS and the transcriptional repressor Egr1 in astrocytes contributed to diminished ASCT1 expression (Fig. 10) observed in clinical samples, cell culture models, and a new Tg mouse model of MS. Given that HERVs represent 8% of the human genome and are able to express biologically active proteins (40), the present studies underscore the potential impact of *HERV* gene expression on disease mechanisms.

Previous studies have demonstrated that Syncytin-1 expression in astrocytes increases proinflammatory molecules (12). However, Syncytin-1 expression in astrocytes is also induced by an inflammatory milieu, highlighting a vicious cycle with deleterious implications for oligodendrocytes. Moreover, excessive or chronic ER stresses result in apoptotic cell death (31), which is a feature of MS, including mounting evidence for the role of ER stress in affecting oligodendrocyte viability (26). The current studies implicate astrocytes in the pathogenesis of MS, which is plausible assuming the pivotal role of astrocytes in maintaining homeostasis in the CNS through regulation of neuroinflammatory, neurotrophic, and neurotoxic factors (68). Because ER stress responses can regulate both inflammation and cell survival (69), the induction of ER chaperones in the brains of Syncytin-1-Tg mice may contribute to enhanced inflammation observed in these animals. Although the expression and function of the protective ER chaperone OASIS have not been elucidated in detail (31), this study indicates that OASIS is induced in MS brain tissue and in astrocytes overexpressing Syncytin-1 with pathogenic effects on oligodendrocytes, possibly mediated by reduced expression of ASCT1.

Diminished expression of ASCT1 in brains of MS patients in conjunction with increased expression of Syncytin-1 prompted the examination of relative levels of ASCT1 and ASCT2 in astrocytes expressing Syncytin-1. Interestingly, cellular differentiation increased ASCT1 receptor expression in macrophages,

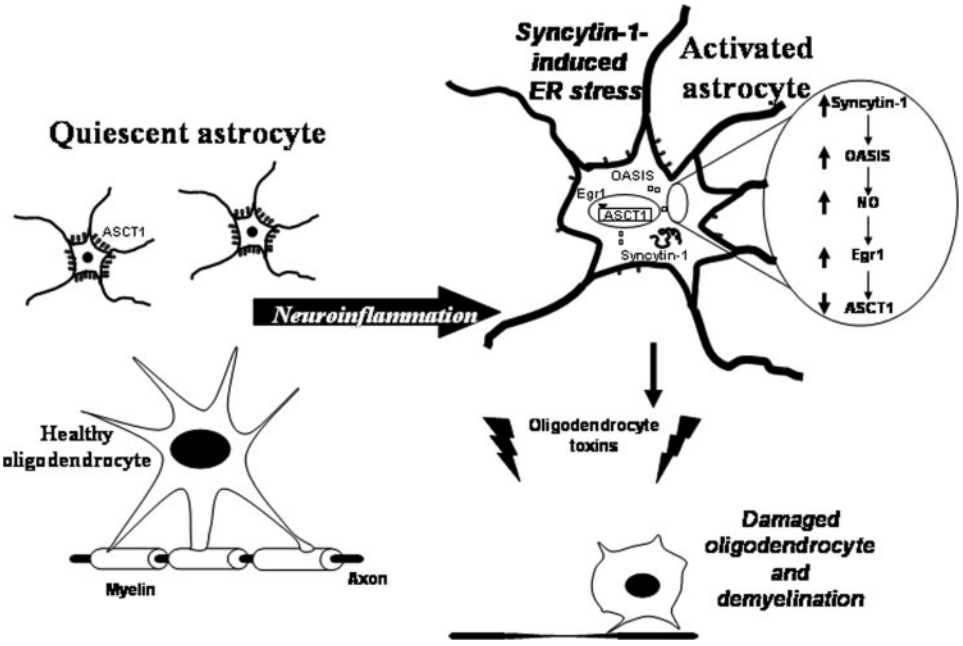

FIGURE 10. Model of Syncytin-1-mediated neuropathogenesis. Astrocytes overexpressing Syncytin-1 undergo ER stress, including induction of OASIS. Activation of iNOS by OASIS leads to increased production of NO, which augments Egr1 expression with ensuing suppression of ASCT1, a receptor for Syncytin-1 and an amino acid transporter. Reduced ASCT1 function causes oligodendrocyte injury and death through the regulation of cytotoxic molecule release.

which may have important implications for viral entry. Syncytin-1 has been reported to form infectious pseudotyped viruses with HIV-1, but not MuLV (47). We used a similar pseudotyping strategy to investigate the permissiveness of different neural cells to Syncytin-1-mediated cell entry. Interestingly, Syncytin-1-mediated entry of the pseudotyped virus was restricted to astrocytes and macrophages, although neurons also expressed the putative receptors for Syncytin-1. Given the ubiquity of their expression, it was expected that all cell types were permissive to the Syncytin-1-pseudotyped virus. However, the present negligible infection of human neurons raises the possibility of other (co)receptors mediating viral envelope engagement or perhaps other inhibitory mechanisms including viral interference (70). However, these studies confirmed the functional properties of the present Syncytin-1 clone.

The current report demonstrates that brain cells express ASCT1 and ASCT2, providing the first human in vivo description of their expression in the CNS. Furthermore, expression of ASCT2 was low in the adult brain (71) but our observations of ASCT2 immunostaining in human brains reveals its expression in microglia. ASCT1 was chiefly expressed in GFAP-positive astrocytes, but not in neurons, oligodendrocytes, or microglia of mice (72), similar to our observations in human brains. Importantly, there was selective down-regulation of ASCT1 in MS brain white matter. Of interest, ASCT1 was also found to be significantly down-regulated in glial cells treated with 7-ketocholesterol, which is increased in MS brains and is formed by oxidative stress-damaged myelin (73), supporting the present findings.

To elucidate the mechanism of ASCT1 down-regulation, we hypothesized that Syncytin-1-mediated inflammation and ER stress might contribute to diminished receptor expression. Expression of OASIS in astrocytes resulted in induction of iNOS and suppression of ASCT1. Hence, it was assumed that NO might play a role because NO donors are known to modulate ASCT2 expression (74). The rationale for using NO donors was that Syncytin-1 mediates NO production and the formation of peroxynitrites (12), both of which induce ER stress (75). iNOS expression and overproduction of NO in astrocytes of MS demyelinating lesions also contributes to inflammation and tissue injury (12, 60). Indeed, SNP, an NO donor, diminished ASCT1 expression in astrocytes, but also induced Egr1, a potential repressor protein of ASCT1 in neural cells (61, 76). Of note, Egr1 also suppresses TNF-α (77), which may have pathogenic consequences in MS due to the protective nature of this proinflammatory cytokine at low concentrations (78). Indeed, Egr1 DNA-binding activity has been associated with oligodendrocyte death (79). Because iNOS and Egr1 are significantly enhanced in brain lesions of MS patients (18) with concurrent down-regulation of ASCT1, we might have also identified a potential role for Egr1 in MS neuropathogenesis. Thus, suppression of ASCT1 was likely brought about by OASIS-mediated iNOS expression through the production of Egr1.

The induction of ER chaperones in Syncytin-1-expressing astrocytes and brain of MS patients might be a mechanism directed to achieving cellular homeostasis; failure to initiate ER stress or protracted ER stress leads to cell death. For example, GADD153/CHOP promotes recovery from ER and oxidative stress by inducing the expression of GADD34 and ER oxidoreductin 1, respectively, but prolonged expression results in release of cytotoxic reactive oxygen species (15). This suggests that the mechanism of oligodendrocyte killing seen in our previous study (12) might be due to OASIS-mediated production of free radicals in astrocytes as OASIS is upstream of GADD153/CHOP. Thus, it appears that OASIS might bind to the promoter of BiP during the early stages of ER stress, as described by others (31) and enhance BiP expression leading to the release of reactive oxygen species and induction of iNOS as described previously (30). Additional ER stress pathways, including phosphorylation of the sensor protein PERK as well as XBP-1 splicing, are potential topics for future experiments. Nevertheless, we have shown that a key ER stress sensor, OASIS, was induced in brains of MS patients and astrocytes expressing Syncytin-1, might be the principal mechanism by which astrocytes expressing Syncytin-1 affect myelin protein expression.

The present results suggest a specific down-regulation of ASCT1 in MS because another astrocytic amino acid transporter, EAAT1 (80), was unaffected by Syncytin-1. A further implication of these findings is that compromised expression of

ASCT1 in the brain, particularly in astrocytes, might affect oligodendrocyte viability and myelin protein expression. ASCT1 is the principal transport system involved in the secretion of L-serine (81), a potent astrocyte-derived neurotrophic factor essential for myelination (82) and neuronal survival (83). In addition, ASCT1 also regulates D-serine and cysteine levels, which exert neurotoxic effects (7). Thus, diminished myelin protein expression observed in our study may be due to withdrawal of trophic amino acids or excess extracellular levels of toxic amino acids. These pathogenic processes require further investigation.

Because the expression of ASCT1 in astrocytes was suppressed by Syncytin-1, a potential mechanism might be its regulation by NO or peroxynitrites, as described for the HIV-1 coreceptors, CXCR4 and CCR5 (84). Alternatively, diminished ASCT1 expression in astrocytes might be a protective host cell response to minimize interaction with viruses and thereby limit immune-mediated pathology (85) or a receptor interference mechanism as in the case of CD4 down-regulation by HIV-1 to prevent superinfection (20). Retrovirus receptor interference and down-regulation proceeds from direct interactions between the virus and the receptor or through indirect (intracellular) mechanisms, possibly involving redox regulation of the receptor (84). Nevertheless, loss of ASCT1 might be compensated by ASCT2 due to the functional redundancy between ASCT1 and ASCT2 (4). This possibility was further emphasized by our observation of increased ASCT2 expression in astrocytes transfected with OASIS (Fig. 4*C*). Moreover, our observation that inhibition of both ASCT1 and ASCT2 in astrocytes by benzylserine affected myelin protein expression (Fig. 6*C*) indicated that soluble free radical-induced factors released by astrocytes might be responsible.

Enhanced neuroinflammatory responses in the brains of Syncytin-1-Tg mice as well as recruitment of CD3-positive T cells were evident in our model of MS, suggesting the potential role of Syncytin-1 to chemoattract T cells into the brain and mediate immunopathology in conjunction with neurobehavioral abnormalities. In addition to adenomatous polyposis coli and GSTPi, oligodendrocyte markers that were earlier shown to be affected by Syncytin-1 (12), we now show that CNP, an early marker of myelination and oligodendrocyte viability, was also suppressed in our Tg model compared with MBP, a late marker. Supporting our observation is the finding that CNP, but not MBP, is regulated by redox reactants (86).

Hence, these studies have identified a distinct pathway involved in the pathogenesis of MS, wherein Syncytin-1 induces OASIS in astrocytes, which results in NO production and concurrent suppression of ASCT1 in astrocytes, leading to diminished myelin protein expression (Fig. 10). Moreover, the present mouse model of MS expressing a HERV glycoprotein in astrocytes could be used to dissect the pathogenic mechanisms of MS without the constraints reported for other MS models (87).

Acknowledgments

We thank Robyn Flynn and Neda Shariat for technical assistance, Dr. Farshid Noorbakhsh for useful discussions, and Dr. Iain Campbell (University of Sydney, Sydney, Australia) for the pFGH vector.

Disclosures

The authors have no financial conflict of interest.

References

1. Frohman, E. M., M. K. Racke, and C. S. Raine. 2006. Multiple sclerosis–the plaque and its pathogenesis. *N. Engl. J. Med.* 354: 942–955.
2. Hartung, H. P., and P. Rieckmann. 1997. Pathogenesis of immune-mediated demyelination in the CNS. *J. Neural. Transm.* 50(Suppl.): 173–181.
3. Custer, S. K., G. A. Garden, N. Gill, U. Rueb, R. T. Libby, C. Schultz, S. J. Guyenet, T. Deller, L. E. Westrum, B. L. Sopher, and A. R. La Spada. 2006. Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport. *Nat. Neurosci.* 9: 1302–1311.
4. Lavillette, D., M. Marin, A. Ruggieri, F. Mallet, F. L. Cosset, and D. Kabat. 2002. The envelope glycoprotein of human endogenous retrovirus type W uses a divergent family of amino acid transporters/cell surface receptors. *J. Virol.* 76: 6442–6452.
5. Dolinska, M., B. Zablocka, U. Sonnewald, and J. Albrecht. 2004. Glutamine uptake and expression of mRNA's of glutamine transporting proteins in mouse cerebellar and cerebral cortical astrocytes and neurons. *Neurochem. Int.* 44: 75–81.
6. Yamamoto, T., I. Nishizaki, T. Nukada, E. Kamegaya, S. Furuya, Y. Hirabayashi, K. Ikeda, H. Hata, H. Kobayashi, I. Sora, and H. Yamamoto. 2004. Functional identification of ASCT1 neutral amino acid transporter as the predominant system for the uptake of L-serine in rat neurons in primary culture. *Neurosci. Res.* 49: 101–111.
7. Weiss, M. D., C. Rossignol, C. Sumners, and K. J. Anderson. 2005. A pH-dependent increase in neuronal glutamate efflux in vitro: possible involvement of ASCT1. *Brain Res.* 1056: 105–112.
8. Blond, J. L., D. Lavillette, V. Cheynet, O. Bouton, G. Oriol, S. Chapel-Fernandes, B. Mandrand, F. Mallet, and F. L. Cosset. 2000. An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J. Virol.* 74: 3321–3329.
9. Ruprecht, K., K. Obojes, V. Wengel, F. Gronen, K. S. Kim, H. Perron, J. Schneider-Schaulies, and P. Rieckmann. 2006. Regulation of human endogenous retrovirus W protein expression by herpes simplex virus type 1: implications for multiple sclerosis. *J. Neurovirol.* 12: 65–71.
10. Antony, J. M., M. Izad, A. Bar-Or, M. Vodjgani, K. G. Warren, and C. Power. 2006. Quantitative analysis of human endogenous retrovirus-W env in neuroinflammatory diseases. *AIDS Res. Hum. Retroviruses* 22: 1253–1259.
11. Mameli, G., V. Astone, K. Khalili, C. Serra, B. E. Sawaya, and A. Dolei. 2007. Regulation of the syncytin-1 promoter in human astrocytes by multiple sclerosis-related cytokines. *Virology* 362: 120–130.
12. Antony, J. M., G. van Marle, W. Opii, D. A. Butterfield, F. Mallet, V. W. Yong, J. L. Wallace, R. M. Deacon, K. Warren, and C. Power. 2004. Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. *Nat. Neurosci.* 7: 1088–1095.
13. Sasaki, H., H. Sato, K. Kuriyama-Matsumura, K. Sato, K. Maebara, H. Wang, M. Tamba, K. Itoh, M. Yamamoto, and S. Bannai. 2002. Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. *J. Biol. Chem.* 277: 44765–44771.
14. Qiang, W., J. M. Cahill, J. Liu, X. Kuang, N. Liu, V. L. Scofield, J. R. Voorhees, A. J. Reid, M. Yan, W. S. Lynn, and P. K. Wong. 2004. Activation of transcription factor Nrf-2 and its downstream targets in response to moloney murine leukemia virus ts1-induced thiol depletion and oxidative stress in astrocytes. *J. Virol.* 78: 11926–11938.
15. Lin, W., A. Kemper, J. L. Dupree, H. P. Harding, D. Ron, and B. Popko. 2006. Interferon-γ inhibits central nervous system remyelination through a process modulated by endoplasmic reticulum stress. *Brain* 129: 1306–1318.
16. Kaufman, R. J. 1999. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev.* 13: 1211–1213.
17. Rao, R. V., and D. E. Bredesen. 2004. Misfolded proteins, endoplasmic reticulum stress and neurodegeneration. *Curr. Opin. Cell. Biol.* 16: 653–662.
18. Mycko, M. P., R. Papoian, U. Boschert, C. S. Raine, and K. W. Selmaj. 2004. Microarray gene expression profiling of chronic active and inactive lesions in multiple sclerosis. *Clin. Neurol. Neurosurg.* 106: 223–229.
19. Bauer, J., M. Bradl, M. Klein, M. Leisser, T. L. Deckwerth, H. Wekerle, and H. Lassmann. 2002. Endoplasmic reticulum stress in PLP-overexpressing transgenic rats: gray matter oligodendrocytes are more vulnerable than white matter oligodendrocytes. *J. Neuropathol. Exp. Neurol.* 61: 12–22.
20. Michel, N., I. Allespach, S. Venzke, O. T. Fackler, and O. T. Keppler. 2005. The Nef protein of human immunodeficiency virus establishes superinfection immunity by a dual strategy to downregulate cell-surface CCR5 and CD4. *Curr. Biol.* 15: 714–723.
21. Wang, H., E. Klamo, S. E. Kuhmann, S. L. Kozak, M. P. Kavanaugh, and D. Kabat. 1996. Modulation of ecotropic murine retroviruses by *N*-linked glycosylation of the cell surface receptor/amino acid transporter. *J. Virol.* 70: 6884–6891.
22. Qiang, W., X. Kuang, J. Liu, N. Liu, V. L. Scofield, A. J. Reid, Y. Jiang, G. Stoica, W. S. Lynn, and P. K. Wong. 2006. Astrocytes survive chronic infection and cytopathic effects of the ts1 mutant of the retrovirus Moloney murine leukemia virus by upregulation of antioxidant defenses. *J. Virol.* 80: 3273–3284.
23. Kim, H. T., K. Waters, G. Stoica, W. Qiang, N. Liu, V. L. Scofield, and P. K. Wong. 2004. Activation of endoplasmic reticulum stress signaling pathway is associated with neuronal degeneration in MoMuLV-ts1-induced spongiform encephalomyelopathy. *Lab. Invest.* 84: 816–827.
24. Dimcheff, D. E., S. Askovic, A. H. Baker, C. Johnson-Fowler, and J. L. Portis. 2003. Endoplasmic reticulum stress is a determinant of retrovirus-induced spongiform neurodegeneration. *J. Virol.* 77: 12617–12629.

25. Dimcheff, D. E., M. A. Faasse, F. J. McAtee, and J. L. Portis. 2004. Endoplasmic reticulum (ER) stress induced by a neurovirulent mouse retrovirus is associated with prolonged BiP binding and retention of a viral protein in the ER. *J. Biol. Chem.* 279: 33782–33790.
26. Clase, A. C., D. E. Dimcheff, C. Favara, D. Dorward, F. J. McAtee, L. E. Parrie, D. Ron, and J. L. Portis. 2006. Oligodendrocytes are a major target of the toxicity of spongiogenic murine retroviruses. *Am. J. Pathol.* 169: 1026–1038.
27. Johnston, J. B., C. Silva, J. Holden, K. G. Warren, A. W. Clark, and C. Power. 2001. Monocyte activation and differentiation augment human endogenous retrovirus expression: implications for inflammatory brain diseases. *Ann. Neurol.* 50: 434–442.
28. Katsumata, K., H. Ikeda, M. Sato, A. Ishizu, Y. Kawarada, H. Kato, A. Wakisaka, T. Koike, and T. Yoshiki. 1999. Cytokine regulation of env gene expression of human endogenous retrovirus-R in human vascular endothelial cells. *Clin. Immunol.* 93: 75–80.
29. Perron, H., F. Lazarini, K. Ruprecht, C. Pechoux-Longin, D. Seilhean, V. Sazdovitch, A. Creange, N. Battail-Poirot, G. Sibai, L. Santoro, et al. 2005. Human endogenous retrovirus (HERV)-W ENV and GAG proteins: physiological expression in human brain and pathophysiological modulation in multiple sclerosis lesions. *J. Neurovirol.* 11: 23–33.
30. Xu, W., L. Liu, I. G. Charles, and S. Moncada. 2004. Nitric oxide induces coupling of mitochondrial signalling with the endoplasmic reticulum stress response. *Nat. Cell Biol.* 6: 1129–1134.
31. Kondo, S., T. Murakami, K. Tatsumi, M. Ogata, S. Kanemoto, K. Otori, K. Iseki, A. Wanaka, and K. Imaizumi. 2005. OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. *Nat. Cell. Biol.* 7: 186–194.
32. Tsutsui, S., J. Schnermann, F. Noorbakhsh, S. Henry, V. W. Yong, B. W. Winston, K. Warren, and C. Power. 2004. A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. *J. Neurosci.* 24: 1521–1529.
33. Power, C., S. Henry, M. R. Del Bigio, P. H. Larsen, D. Corbett, Y. Imai, V. W. Yong, and J. Peeling. 2003. Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. *Ann. Neurol.* 53: 731–742.
34. Power, C., P. A. Kong, T. O. Crawford, S. Wesselingh, J. D. Glass, J. C. McArthur, and B. D. Trapp. 1993. Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood-brain barrier. *Ann. Neurol.* 34: 339–350.
35. Power, C., P. A. Kong, and B. D. Trapp. 1996. Major histocompatibility complex class I expression in oligodendrocytes induces hypomyelination in transgenic mice. *J. Neurosci. Res.* 44: 165–173.
36. Yong, V. W. A. J. 1997. Culture of glial cells from human brain biopsies. In *Protocols for Neural Cell Culture.* R. A. Fedoroff S, ed. Humana, Totowa, pp. 81–96.
37. Riches, D. W., and G. A. Underwood. 1991. Expression of interferon-β during the triggering phase of macrophage cytocidal activation: evidence for an autocrine/paracrine role in the regulation of this state. *J. Biol. Chem.* 266: 24785–24792.
38. Zhang, K., F. Rana, C. Silva, J. Ethier, K. Wehrly, B. Chesebro, and C. Power. 2003. Human immunodeficiency virus type 1 envelope-mediated neuronal death: uncoupling of viral replication and neurotoxicity. *J. Virol.* 77: 6899–6912.
39. Stalder, A. K., M. J. Carson, A. Pagenstecher, V. C. Asensio, C. Kincaid, M. Benedict, H. C. Powell, E. Masliah, and I. L. Campbell. 1998. Late-onset chronic inflammatory encephalopathy in immune-competent and severe combined immune-deficient (SCID) mice with astrocyte-targeted expression of tumor necrosis factor. *Am. J. Pathol.* 153: 767–783.
40. Cheynet, V., A. Ruggieri, G. Oriol, J. L. Blond, B. Boson, L. Vachot, B. Verrier, F. L. Cosset, and F. Mallet. 2005. Synthesis, assembly, and processing of the Env ERVWE1/syncytin human endogenous retroviral envelope. *J. Virol.* 79: 5585–5593.
41. Guenther, K., R. M. Deacon, V. H. Perry, and J. N. Rawlins. 2001. Early behavioural changes in scrapie-affected mice and the influence of dapsone. *Eur. J. Neurosci.* 14: 401–409.
42. Boston-Howes, W., S. L. Gibb, E. O. Williams, P. Pasinelli, R. H. Brown, Jr., and D. Trotti. 2006. Caspase-3 cleaves and inactivates the glutamate transporter EAAT2. *J. Biol. Chem.* 281: 14076–14084.
43. Rolland, A., E. Jouvin-Marche, C. Viret, M. Faure, H. Perron, and P. N. Marche. 2006. The envelope protein of a human endogenous retrovirus-W family activates innate immunity through CD14/TLR4 and promotes Th1-like responses. *J. Immunol.* 176: 7636–7644.
44. Gegelashvili, M., A. Rodriguez-Kern, I. Pirozhkova, J. Zhang, L. Sung, and G. Gegelashvili. 2006. High-affinity glutamate transporter GLAST/EAAT1 regulates cell surface expression of glutamine/neutral amino acid transporter ASCT2 in human fetal astrocytes. *Neurochem. Int.* 48: 611–615.
45. Chen, C. P., K. G. Wang, C. Y. Chen, C. Yu, H. C. Chuang, and H. Chen. 2006. Altered placental syncytin and its receptor ASCT2 expression in placental development and pre-eclampsia. *BJOG* 113: 152–158.
46. Marin, M., D. Lavillette, S. M. Kelly, and D. Kabat. 2003. *N*-linked glycosylation and sequence changes in a critical negative control region of the ASCT1 and ASCT2 neutral amino acid transporters determine their retroviral receptor functions. *J. Virol.* 77: 2936–2945.
47. An, D. S., Y. Xie, and I. S. Chen. 2001. Envelope gene of the human endogenous retrovirus HERV-W encodes a functional retrovirus envelope. *J. Virol.* 75: 3488–3489.
48. Lock, C., G. Hermans, R. Pedotti, A. Brendolan, E. Schadt, H. Garren, A. Langer-Gould, S. Strober, B. Cannella, J. Allard, et al. 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat. Med.* 8: 500–508.
49. Noorbakhsh, F., Q. Tang, S. Liu, C. Silva, G. van Marle, and C. Power. 2006. Lentivirus envelope protein exerts differential neuropathogenic effects depending on the site of expression and target cell. *Virology* 348: 260–276.
50. Mattson, M. P., and D. D. Taub. 2004. Ancient viral protein enrages astrocytes in multiple sclerosis. *Nat. Neurosci.* 7: 1021–1023.
51. Sato, H., M. Tamba, K. Kuriyama-Matsumura, S. Okuno, and S. Bannai. 2000. Molecular cloning and expression of human xCT, the light chain of amino acid transport system xc. *Antioxid. Redox. Signal* 2: 665–671.
52. Kanai, Y., and H. Endou. 2001. Heterodimeric amino acid transporters: molecular biology and pathological and pharmacological relevance. *Curr. Drug Metab.* 2: 339–354.
53. Qin, S., C. Colin, I. Hinners, A. Gervais, C. Cheret, and M. Mallat. 2006. System Xc- and apolipoprotein E expressed by microglia have opposite effects on the neurotoxicity of amyloid-β peptide 1–40. *J. Neurosci.* 26: 3345–3356.
54. Murakami, T., S. Kondo, M. Ogata, S. Kanemoto, A. Saito, A. Wanaka, and K. Imaizumi. 2006. Cleavage of the membrane-bound transcription factor OASIS in response to endoplasmic reticulum stress. *J. Neurochem.* 96: 1090–1100.
55. Bhat, N. R., D. L. Feinstein, Q. Shen, and A. N. Bhat. 2002. p38 MAPK-mediated transcriptional activation of inducible nitric-oxide synthase in glial cells: roles of nuclear factors, nuclear factor κB, cAMP response element-binding protein, CCAAT/enhancer-binding protein-β, and activating transcription factor-2. *J. Biol. Chem.* 277: 29584–29592.
56. Barnett, M. H., and J. W. Prineas. 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann. Neurol.* 55: 458–468.
57. Kuhlmann, T., C. Lucchinetti, U. K. Zettl, A. Bitsch, H. Lassmann, and W. Bruck. 1999. Bcl-2-expressing oligodendrocytes in multiple sclerosis lesions. *Glia* 28: 34–39.
58. Noorbakhsh, F., S. Tsutsui, N. Vergnolle, L. A. Boven, N. Shariat, M. Vodjgani, K. G. Warren, P. Andrade-Gordon, M. D. Hollenberg, and C. Power. 2006. Proteinase-activated receptor 2 modulates neuroinflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. *J. Exp. Med.* 203: 425–435.
59. Grewer, C., and E. Grabsch. 2004. New inhibitors for the neutral amino acid transporter ASCT2 reveal its Na^+-dependent anion leak. *J. Physiol.* 557: 747–759.
60. Bo, L., T. M. Dawson, S. Wesselingh, S. Mork, S. Choi, P. A. Kong, D. Hanley, and B. D. Trapp. 1994. Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. *Ann. Neurol.* 36: 778–786.
61. Kanai, Y., and M. A. Hediger. 2004. The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. *Pflugers Arch.* 447: 469–479.
62. Rupprecht, H. D., Y. Akagi, A. Keil, and G. Hofer. 2000. Nitric oxide inhibits growth of glomerular mesangial cells: role of the transcription factor EGR-1. *Kidney Int.* 57: 70–82.
63. Coombs, B. D., A. Best, M. S. Brown, D. E. Miller, J. Corboy, M. Baier, and J. H. Simon. 2004. Multiple sclerosis pathology in the normal and abnormal appearing white matter of the corpus callosum by diffusion tensor imaging. *Mult. Scler.* 10: 392–397.
64. Zhang, L., W. Zhao, B. Li, D. L. Alkon, J. L. Barker, Y. H. Chang, M. Wu, and D. R. Rubinow. 2000. TNF-α induced over-expression of GFAP is associated with MAPKs. *Neuroreport* 11: 409–412.
65. Kawahara, K., S. Oyadomari, T. Gotoh, S. Kohsaka, H. Nakayama, and M. Mori. 2001. Induction of CHOP and apoptosis by nitric oxide in p53-deficient microglial cells. *FEBS Lett.* 506: 135–139.
66. Prat, A., and J. Antel. 2005. Pathogenesis of multiple sclerosis. *Curr. Opin. Neurol.* 18: 225–230.
67. Trapp, B. D. 2004. Pathogenesis of multiple sclerosis: the eyes only see what the mind is prepared to comprehend. *Ann. Neurol.* 55: 455–457.
68. Volterra, A., and J. Meldolesi. 2005. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* 6: 626–640.
69. Zhang, K., X. Shen, J. Wu, K. Sakaki, T. Saunders, D. T. Rutkowski, S. H. Back, and R. J. Kaufman. 2006. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* 124: 587–599.
70. Ponferrada, V. G., B. S. Mauck, and D. P. Wooley. 2003. The envelope glycoprotein of human endogenous retrovirus HERV-W induces cellular resistance to spleen necrosis virus. *Arch. Virol.* 148: 659–675.
71. Kekuda, R., P. D. Prasad, Y. J. Fei, V. Torres-Zamorano, S. Sinha, T. L. Yang-Feng, F. H. Leibach, and V. Ganapathy. 1996. Cloning of the sodium-dependent, broad-scope, neutral amino acid transporter Bo from a human placental choriocarcinoma cell line. *J. Biol. Chem.* 271: 18657–18661.
72. Sakai, K., H. Shimizu, T. Koike, S. Furuya, and M. Watanabe. 2003. Neutral amino acid transporter ASCT1 is preferentially expressed in L-Ser-synthetic/storing glial cells in the mouse brain with transient expression in developing capillaries. *J. Neurosci.* 23: 550–560.
73. Hackel, D. 2005. Activation of microglia by injured neurons-signal pathways of oxidatively modified lipids. PhD thesis, Otto von Guericke University of Magdeburg, Magdeburg, Magdeburg, p. 169.
74. Uchiyama, T., Y. Matsuda, M. Wada, S. Takahashi, and T. Fujita. 2005. Functional regulation of Na^+-dependent neutral amino acid transporter ASCT2 by S-nitrosothiols and nitric oxide in Caco-2 cells. *FEBS Lett.* 579: 2499–2506.
75. Dickhout, J. G., G. S. Hossain, L. M. Pozza, J. Zhou, S. Lhotak, and R. C. Austin. 2005. Peroxynitrite causes endoplasmic reticulum stress and apoptosis in human vascular endothelium: implications in atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* 25: 2623–2629.
76. Tatarowicz, W. A., C. E. Martin, A. S. Pekosz, S. L. Madden, F. J. Rauscher, 3rd, S. Y. Chiang, T. A. Beerman, and N. W. Fraser. 1997. Repression of the HSV-1 latency-associated transcript (LAT) promoter by the early growth response

(EGR) proteins: involvement of a binding site immediately downstream of the TATA box. *J. Neurovirol.* 3: 212–224.
77. Di Battista, J. A., J. Martel-Pelletier, and J. Pelletier. 1999. Suppression of tumor necrosis factor (TNF-α) gene expression by prostaglandin E_2: role of early growth response protein-1 (Egr-1). *Osteoarthritis Cartilage* 7: 395–398.
78. Korner, H., D. S. Riminton, D. H. Strickland, F. A. Lemckert, J. D. Pollard, and J. D. Sedgwick. 1997. Critical points of tumor necrosis factor action in central nervous system autoimmune inflammation defined by gene targeting. *J. Exp. Med.* 186: 1585–1590.
79. FitzGerald, U. F., T. Gilbey, S. Brodie, and S. C. Barnett. 2003. Transcription factor expression and cellular redox in immature oligodendrocyte cell death: effect of Bcl-2. *Mol. Cell. Neurosci.* 22: 516–529.
80. Storck, T., S. Schulte, K. Hofmann, and W. Stoffel. 1992. Structure, expression, and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain. *Proc. Natl. Acad. Sci. USA* 89: 10955–10959.
81. Yamamoto, T., I. Nishizaki, S. Furuya, Y. Hirabayashi, K. Takahashi, S. Okuyama, and H. Yamamoto. 2003. Characterization of rapid and high-affinity uptake of L-serine in neurons and astrocytes in primary culture. *FEBS Lett.* 548: 69–73.
82. Sundaram, K. S., and M. Lev. 1984. Inhibition of sphingolipid synthesis by cycloserine in vitro and in vivo. *J. Neurochem.* 42: 577–581.
83. Furuya, S., and M. Watanabe. 2003. Novel neuroglial and glioglial relationships mediated by L-serine metabolism. *Arch. Histol. Cytol.* 66: 109–121.
84. Saccani, A., S. Saccani, S. Orlando, M. Sironi, S. Bernasconi, P. Ghezzi, A. Mantovani, and A. Sica. 2000. Redox regulation of chemokine receptor expression. *Proc. Natl. Acad. Sci. USA* 97: 2761–2766.
85. Ramakrishna, C., C. C. Bergmann, K. V. Holmes, and S. A. Stohlman. 2004. Expression of the mouse hepatitis virus receptor by central nervous system microglia. *J. Virol.* 78: 7828–7832.
86. Jana, M., and K. Pahan. 2005. Redox regulation of cytokine-mediated inhibition of myelin gene expression in human primary oligodendrocytes. *Free Radic. Biol. Med.* 39: 823–831.
87. Sriram, S., and I. Steiner. 2005. Experimental allergic encephalomyelitis: a misleading model of multiple sclerosis. *Ann. Neurol.* 58: 939–945.