Absence of systemic oxidative stress and increased CSF prostaglandin $F_{2\alpha}$ in progressive MS

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ABSTRACT

Objective: We aimed to investigate the role of oxidative stress in the progression of multiple sclerosis (MS).

Methods: We determined by liquid chromatography–tandem mass spectrometry nonenzymatic (F_2-isoprostanes) and enzymatic oxidation products of arachidonic acid (prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$]) in plasma and CSF of 45 controls (other neurologic disease [OND] with no signs of inflammation) and 62 patients with MS. Oxidation products were correlated with disease severity and validated biomarkers of inflammation (chemokine ligand 13; matrix metalloproteinase-9; osteopontin) and axonal damage (neurofilament light protein).

Results: Compared with OND controls, plasma concentrations of F_2-isoprostanes and $PGF_{2\alpha}$ were significantly lower in patients with progressive disease, and decreased with increasing disability score (Expanded Disability Status Scale). In contrast, CSF concentrations of $PGF_{2\alpha}$, but not F_2-isoprostanes, were significantly higher in patients with progressive disease than OND controls ($p < 0.01$). The content of $PGF_{2\alpha}$ in CSF increased with disease severity ($p = 0.044$) and patient age ($p = 0.022$), although this increase could not be explained by age. CSF $PGF_{2\alpha}$ decreased with natalizumab and methylprednisolone treatment and was unaffected by the use of nonsteroidal anti-inflammatory drug in secondary progressive MS. CSF $PGF_{2\alpha}$ did not associate with validated CSF markers of inflammation and axonal damage that themselves did not associate with the Expanded Disability Status Scale.

Conclusions: Our data suggest that MS progression is associated with low systemic oxidative activity. This may contribute to immune dysregulation with CNS inflammation accompanied by increased local cyclooxygenase-dependent lipid oxidation. ***Neurol Neuroimmunol Neuroinflamm* 2016;3:e256; doi: 10.1212/NXI.0000000000000256**

GLOSSARY

F_2-IP = F_2-isoprostane; **MS** = multiple sclerosis; **OND** = other neurologic disease; **$PGF_{2\alpha}$** = prostaglandin F; **ROS** = reactive oxygen species; **RRMS** = relapsing-remitting multiple sclerosis.

Multiple sclerosis (MS) is usually relapsing-remitting at onset, but, with time, a majority of patients convert to a secondary progressive disease course, for which current therapies are ineffective. Recently, increased oxidative stress has been proposed as a pathogenic mechanism leading to progressive MS.[1] However, a decrease in reactive oxygen species (ROS) derived from NADPH oxidase 2 has been associated with more severe experimental autoimmune encephalomyelitis, a model of MS.[2,3] Moreover, disease progression correlates with altered activity of ROS-producing immune cells.[4–6] Thus, changes in local and systemic oxidative stress are of interest for the transition into progressive MS, and we hypothesize that low oxidative stress may promote such progression.

F_2-isoprostanes (F_2-IPs) are considered the gold-standard biomarker of in vivo oxidative stress.[7] They are formed predominantly via nonenzymatic oxidation of arachidonic acid

Supplemental data at Neurology.org/nn

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(20:4). However, the most frequently determined F_2-IP (8-iso-$PGF_{2\alpha}$), can also be generated during enzymatic oxidation of 20:4 to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), involving cyclooxygenase.[8] As cyclooxygenase is significantly induced during inflammation, it can lead to incorrect biomarker assignment and interpretation.[8] Therefore, we quantified the ROS-derived F_2-IP and enzyme-derived $PGF_{2\alpha}$ in plasma and CSF of patients with MS. We correlated these oxidation markers with disease severity, patient age, and other clinical measures. To explore whether CNS 20:4 oxidation changes with treatment, we also analyzed samples from 2 intervention studies of patients with progressive MS treated with natalizumab or methylprednisolone.

METHODS

Materials. Standards of F_2-IP (5[*R*]-$iPF_{2\alpha}$-VI, 5[*S*]-$iPF_{2\alpha}$-VI, 5-$iPF_{2\alpha}$-VI-d_{11}, 8-iso-15[*S*]-$PGF_{2\alpha}$, 8-iso-15[*R*]-$PGF_{2\alpha}$, 8-iso-15[*S*]-$PGF_{2\alpha}$-d_4, and 15[*R*]-$PGF_{2\alpha}$), 15(*S*)-$PGF_{2\alpha}$ (hereafter referred to as $PGF_{2\alpha}$), 20:4, and 20:4-d_8 were from Cayman Chemicals (Ann Arbor, MI). Artificial CSF was from Tocris Bioscience (Bristol, UK). Methanol (Fisher Scientific Inc.), hexane, and ethyl acetate (Lab Scan) were of high-performance liquid chromatography grade, and Bond Elut CertifyII SPE columns were from Agilent Technologies (Santa Clara, CA). Other chemicals were from Sigma, unless indicated otherwise.

Standard protocol approvals, registrations, and patient consents. The ethical review boards of the Karolinska Institute in Sweden (DN: 2009/2107-31-2) approved the study involving 62 patients with MS with a diagnosis according to the revised McDonald criteria[9] and a heterogeneous control group of 45 individuals with other neurologic diseases (ONDs) (see the table for demographic data). These controls with ONDs displayed no clinical and neuroradiologic features of MS and no signs of intrathecal inflammation as shown by the presence of oligoclonal bands, increased immunoglobulin G index, or pleocytosis (higher than upper normal limit, i.e., >5,000 cells/mL). The ethics committee of the Capital Region of Denmark approved the 2 intervention studies with natalizumab[10] (n = 24) and methylprednisolone[11] (n = 30). Written informed consent was obtained from all participants. Disease severity was assessed using the Expanded Disability Status Scale score. CSF was collected during diagnostic workup according to clinical routine,[10,11] and for the Swedish study, venous EDTA blood samples were collected at the same time. Following centrifugation (15 minutes, 2,700*g*, room temperature), the resulting plasma was stored at −80°C until use. CSF mononuclear and polymorphonuclear cells were determined by flow cytometry and albumin with an automated system (Beckman Coulter UniCel DxC 800 Pro). Chemokine CXCL13, matrix metalloproteinase-9, osteopontin, and neurofilament light protein were determined using corresponding ELISA kits.[12] Coded samples were shipped frozen on dry ice to Australia for liquid chromatography–tandem mass spectrometric analysis of F_2-IP and $PGF_{2\alpha}$. Upon receipt, frozen samples were placed immediately at −80°C until analysis was performed blinded within 12 months. The Sydney Local Health District Ethics Review

Table Demographic data of patients and controls in the longitudinal study

Clinical/paraclinical measures	RRMS	SPMS	PPMS	OND
No. of participants	23	24	15	45
Mean age, y (range)	35.9 (21–52)	49.4 (33–62)	55 (35–70)	40.3 (19–82)
Female/male	19/4	15/9	7/8	30/15
Mean disease duration, y (range)	2.7 (1–12)	18.5 (9–37)	6.5 (1–17)	NA
Median EDSS score (range)	1.5 (0–4.5)	5.75 (3.0–8.0)	4.0 (2.0–6.5)	NA
Annualized relapse rate (range)	1.17 (0–4)	0.17 (0–1)	0 (0–0)	NA
Previous DMT, %	13.0	50.0	6.7	2.2[a]
DMT at sample time point, %	13.0	20.8	0	2.2[a]
Mean IgG index (range)	0.93 (0.50–2.35)	0.85 (0.46–1.77)	0.92 (0.48–1.9)	0.50 (0.43–0.62)
Oligoclonal IgG bands (+/−/NA)	20/3/0	21/3/0	12/3/0	2/41/2
Mean CSF albumin quotient (range)	4.4 (2.6–7.6)	6.1 (2.4–12.0)	7.1 (3.1–12.2)	4.5 (2.5–11.2)
No. of brain MRI lesions, %				
0–2	13	0	7	NA
3–5	13	0	0	NA
6–8	17	4	21	NA
≥9	57	96	72	NA
Median contrast-enhancing lesions (range)	1.0 (0–4)	0 (0–0)	0 (0–0)	NA

Abbreviations: DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; IgG = immunoglobulin G; NA = not available; OND = other neurologic disease; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.
Age (in years) refers to the age at sampling time point.
[a] One individual in the OND group used corticosteroid at the sampling time point and before.

Committee (X12-0102 and HREC/12/RPAH/174) approved use and analyses of all human samples.

Sample preparation. Frozen CSF and plasma samples were thawed for 4 minutes at 25°C, then kept on ice at all times. A solution of internal standards (1 ng 8-iso-15[*S*]-$PGF_{2\alpha}$-d_4, 1 ng 5[*R*/*S*]-$iPF_{2\alpha}$-d_{11}, and 2.5 μg 20:4-d_8 in nitrogen-purged ethanol) was added to 1 mL of plasma or CSF and mixed briefly. Samples were subjected to alkaline hydrolysis by adding 1 mL 1 M KOH in methanol containing 0.005% butylated hydroxytoluene, purging each tube with nitrogen, followed by incubation at 37°C for 30 minutes. Samples were acidified with 2 mL of sodium acetate (100 mM, pH 4.6) and the pH-adjusted to 4.6 using HCl. Following centrifugation (10 minutes, 800*g*, 4°C), the supernate was applied to SPE columns preconditioned with 2 mL of methanol, followed by 2 mL of sodium acetate containing 5% (v/v) methanol, pH 7. Columns were washed with 2 mL of methanol/water (1:1, v/v) followed by 2 mL of ethyl acetate/hexane (1:3, v/v). Analytes were eluted with 2 mL of ethyl acetate/methanol (9:1, v/v), evaporated to dryness under nitrogen, and reconstituted in 50 μL of 50% methanol containing 0.01% acetic acid.

Liquid chromatography–tandem mass spectrometry. $PGF_{2\alpha}$, F_2-IP, and 20:4 in CSF and plasma were quantified by liquid chromatography–tandem mass spectrometry (QQQ 6490 mass spectrometer; Agilent Technologies) with an ESI source and multiple-reaction monitoring in negative ionization mode. A Zorbax Eclipse C18 column (2.1 × 50 mm, 1.8 μm; Agilent Technologies) was used for separation at a flow rate of 0.35 mL/min, with a gradient of A: 0.01% acetic acid in water, and B: 100% methanol. Solvent B was increased initially from 50% to 60% B in the first 15 minutes, then to 90% at 15.1 minutes, 100% at 20 minutes, and held for 3 minutes before re-equilibration at 50%. For quantification, specific ion pairs were monitored: 5-series F_2-IP *m/z* 353 → 115; 5-$iPF_{2\alpha}$-VI-d_{11} *m/z* 364 → 115; 15-series F_2-IP *m/z* 353 → 193; 15-F_{2t}-IsoP-d_4 *m/z* 357 → 197; 20:4 *m/z* 303 → 205; 20:4-d_8 *m/z* 311 → 213. Quantification was achieved by peak area comparison with the corresponding internal standard, using Mass Hunter software. Only peaks coeluting with internal standard and with a signal-to-noise ratio of ≥3 (defined as limit of detection) were quantified. Results were expressed as amount of oxidized lipid per volume or 20:4 content. Samples in which F_2-IP and $PGF_{2\alpha}$ were below detection limit were not considered for statistical analyses, resulting in variable n-numbers for different F_2-IP and $PGF_{2\alpha}$. The linearity and reproducibility of the assay was confirmed by spiking plasma or CSF before hydrolysis with authentic standards of 5-$iPF_{2\alpha}$-VI and 15-F_{2t}-IsoP (0.05–2.5 ng/mL) or 20:4 (0.1–100 μg/mL). Intra- and interday coefficients of variation (calculated from the responses of the internal standards) were 1.8%–12.6% and 6.4%–15.2% for the 5-series F_2-IPs, and 3.1%–12.2% and 2.9%–13.5% for the 15 series F_2-IPs, respectively.

Statistical analyses. Statistical analyses were performed using GraphPad Prism version 6.0 for Macintosh (San Diego, CA). For comparison of median values between >2 groups, Kruskal-Wallis test with Dunn posttest was used. Because this was an exploratory study, no adjustment for multiple comparisons was made. Correlation analyses were performed using Spearman ranked correlation at 95% confidence interval. For the intervention studies, statistical significance was determined by the Wilcoxon matched-pairs signed rank test.

RESULTS

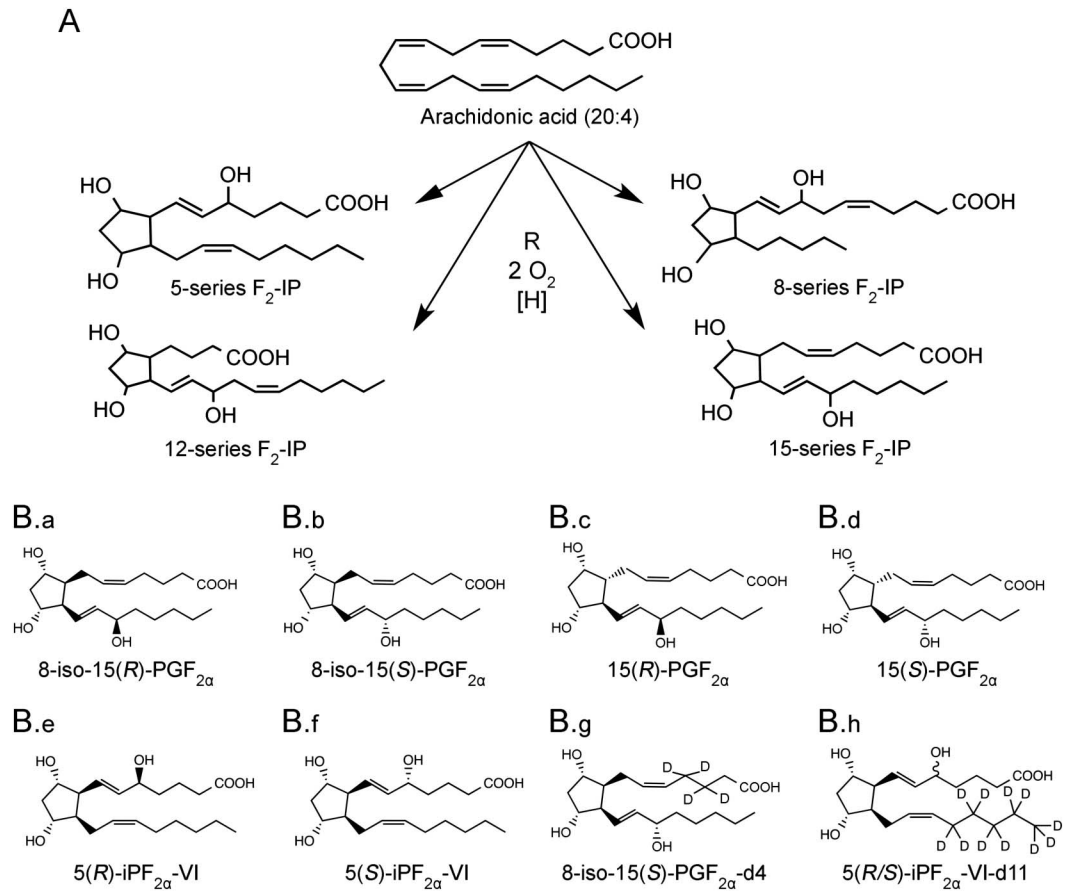
Plasma F_2-IPs decrease with MS progression. We first examined systemic oxidative stress in progressive MS by measuring the concentrations of 20:4, F_2-IP, and $PGF_{2\alpha}$ in plasma of controls with OND and patients with MS at various disease stages. The method used allowed for quantification of 4 series of F_2-IP (figure 1A). Because of the limited availability of authentic standards required for unambiguous assignment of chromatographic peaks, we restricted our analysis to F_2-IP species for which standards were available commercially (figure 1B).

Plasma concentrations of F_2-IP and $PGF_{2\alpha}$ were significantly lower in patients with progressive MS compared to patients with relapsing-remitting MS (RRMS) and OND controls (figure 2). This was true for individual 5- and 15-species and for the sum of products detected for each IP series. The amount of the oxidation products formed can be affected by the concentration of substrate[13]; however, plasma 20:4 concentrations did not vary between controls with OND and patients with MS (figure 2G). These results indicate that MS progression is not associated with increased systemic oxidative stress.

$PGF_{2\alpha}$ but not F_2-IP is increased in CSF from patients with progressive MS. We next determined 20:4 and 20:4 oxidation products in CSF from patients with MS and OND controls. Concentrations of different F_2-IP species (figure 3, A–E) and 20:4 (figure 3G) were comparable in controls and patients. However, CSF of patients with progressive MS contained significantly higher concentrations of $PGF_{2\alpha}$ than CSF of patients with RRMS and OND controls (figure 3F). Normalizing the F_2-IP and $PGF_{2\alpha}$ data to CSF 20:4 did not change the overall results, consistent with the similar concentrations of 20:4 in controls and patients with MS. These results indicate that MS is associated with an increase in enzymatic oxidation of 20:4 rather than general oxidative stress in the CNS.

Associations among $PGF_{2\alpha}$, disease severity, CSF biomarkers, and age. In CSF, the concentration of $PGF_{2\alpha}$ increased with disease severity assessed by the Expanded Disability Status Scale score, irrespective of whether $PGF_{2\alpha}$ was standardized to CSF volume (r_s = 0.280, p = 0.044) (not shown) or normalized to 20:4 (figure e-1A at Neurology.org/nn). Such association was not observed for 8-iso-15(*S*) $PGF_{2\alpha}$ (figure e-1B) and other F_2-IP (not shown). In contrast to $PGF_{2\alpha}$, none of the validated CSF biomarkers determined correlated with disease severity (figure e-1, C–I). The CSF concentration of $PGF_{2\alpha}$ (ng/μg 20:4) increased with patient age (figure e-1J), although it remained higher in progressive patients compared with age-matched controls with OND (figure e-1K). Also, CSF $PGF_{2\alpha}$ remained higher in patients with secondary progressive MS taking nonsteroidal anti-inflammatory drugs compared with OND controls (figure e-1L). $PGF_{2\alpha}$

Figure 1 Arachidonic acid (20:4) oxidation pathways



(A) General structures of the 4 series of F_2-isoprostanes formed from 20:4 via radical (R•) mediated oxidation. (B) Chemical structures of the 20:4 oxidation products studied (B.a–B.f) and corresponding heavy isotope-labeled internal standards (B.g and B.h). Compounds B.a–B.e are formed via nonenzymatic oxidation of 20:4, whereas B.f is formed enzymatically and nonenzymatically.

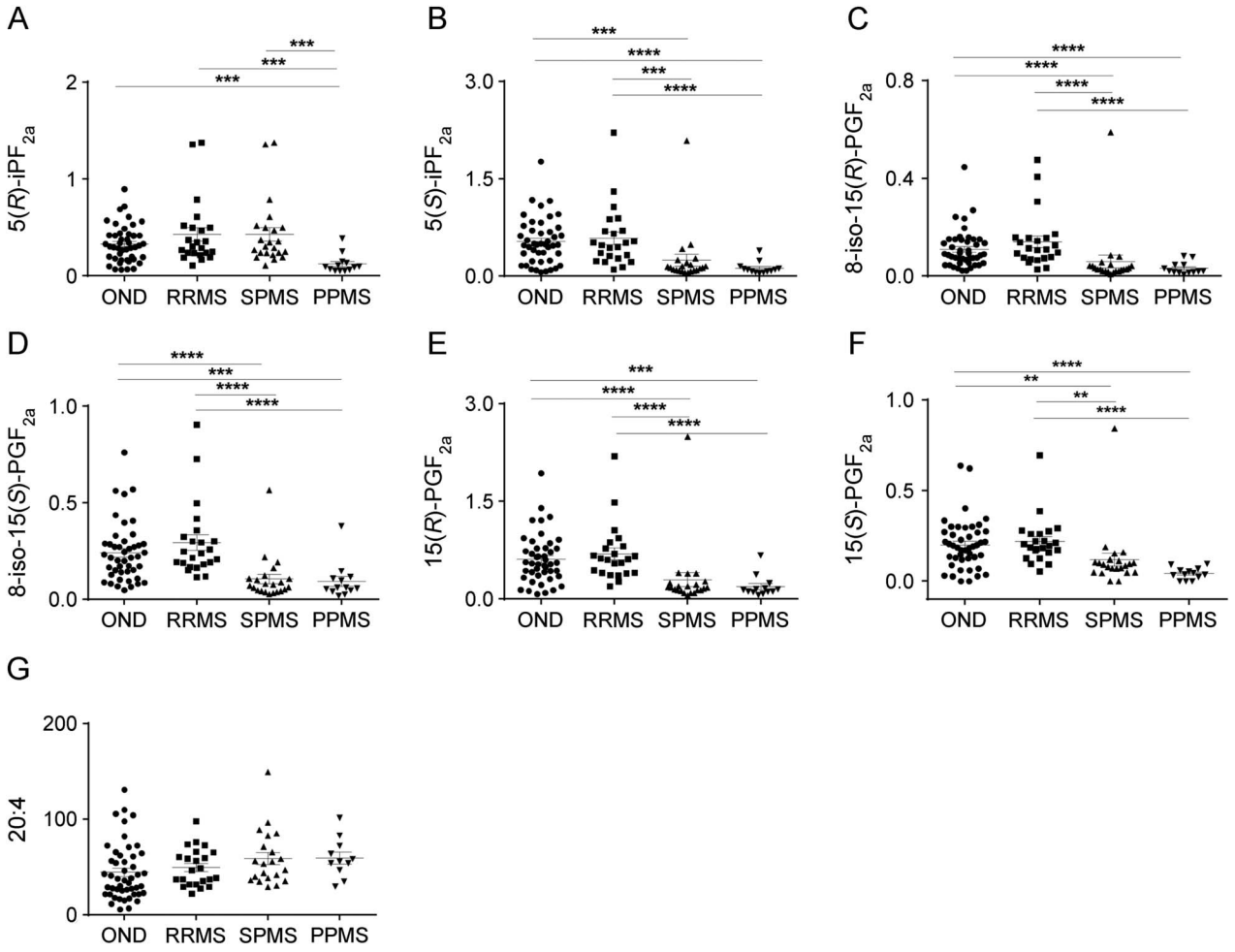
did not correlate significantly with any of the biomarkers determined (figure e-2).

In plasma, the content of $PGF_{2\alpha}$ and each individual F_2-IP species decreased with increasing disease severity (figure e-3, A–F). Plasma $PGF_{2\alpha}$ decreased with patient age (figure e-3G) but it remained significantly lower in progressive patients compared with age-matched OND controls (figure e-3H). As a result, the CSF/plasma ratio of $PGF_{2\alpha}$ and F_2-IP (except for 8-iso-15[*R*] $PGF_{2\alpha}$) were higher in patients with MS who had progressive disease compared with controls with OND (figure e-3, I–N), despite a decrease in the CSF/plasma ratio of 20:4 (figure e-3O). These data suggest that the disease-associated increase in CSF $PGF_{2\alpha}$ originates from increased local enzymatic oxidation of 20:4 rather than circulating $PGF_{2\alpha}$. This interpretation is consistent with the absence of MS-associated increase in the CSF/plasma albumin ratio (figure e-3P).

Treatment effect on CSF lipid oxidation in progressive MS. To assess whether lipid oxidation in CNS is affected by current MS therapies, we determined CSF F_2-IP from patients with progressive MS treated with natalizumab or methylprednisolone.[10,11] F_2-IPs were affected variably by natalizumab: compared with baseline, the contents of 5(*R*)-$iPF_{2\alpha}$, 5(*S*)-$iPF_{2\alpha}$, 8-iso-15(*R*)-$PGF_{2\alpha}$, and 8-iso-15(*S*)-$PGF_{2\alpha}$ (figure 4, A–D) decreased significantly following 60 weeks of treatment, while 15(*R*)-$PGF_{2\alpha}$ remained unchanged ($p = 0.563$; figure 4E). In contrast to nonenzymatic 15(*R*)-$PGF_{2\alpha}$, its corresponding stereoisomer and enzymatic product, 15(*S*)-$PGF_{2\alpha}$, was significantly decreased after treatment compared with baseline ($p = 0.002$; figure 4F). CSF concentrations of 20:4 did not vary significantly between baseline and treatment (data not shown).

We repeated the above analyses in a separate set of CSF samples from patients with progressive MS at baseline and 60 weeks after treatment with methylprednisolone.[11] In this cohort of 23 patients, $PGF_{2\alpha}$ was detected in CSF samples of only 7 patients. In all of these patients, methylprednisolone treatment also significantly decreased $PGF_{2\alpha}$ ($p = 0.031$) without changing the content of 20:4 ($p = 0.819$). Similar to

Figure 2 Decrease in F_2-isoprostanes in blood plasma of patients with progressive MS



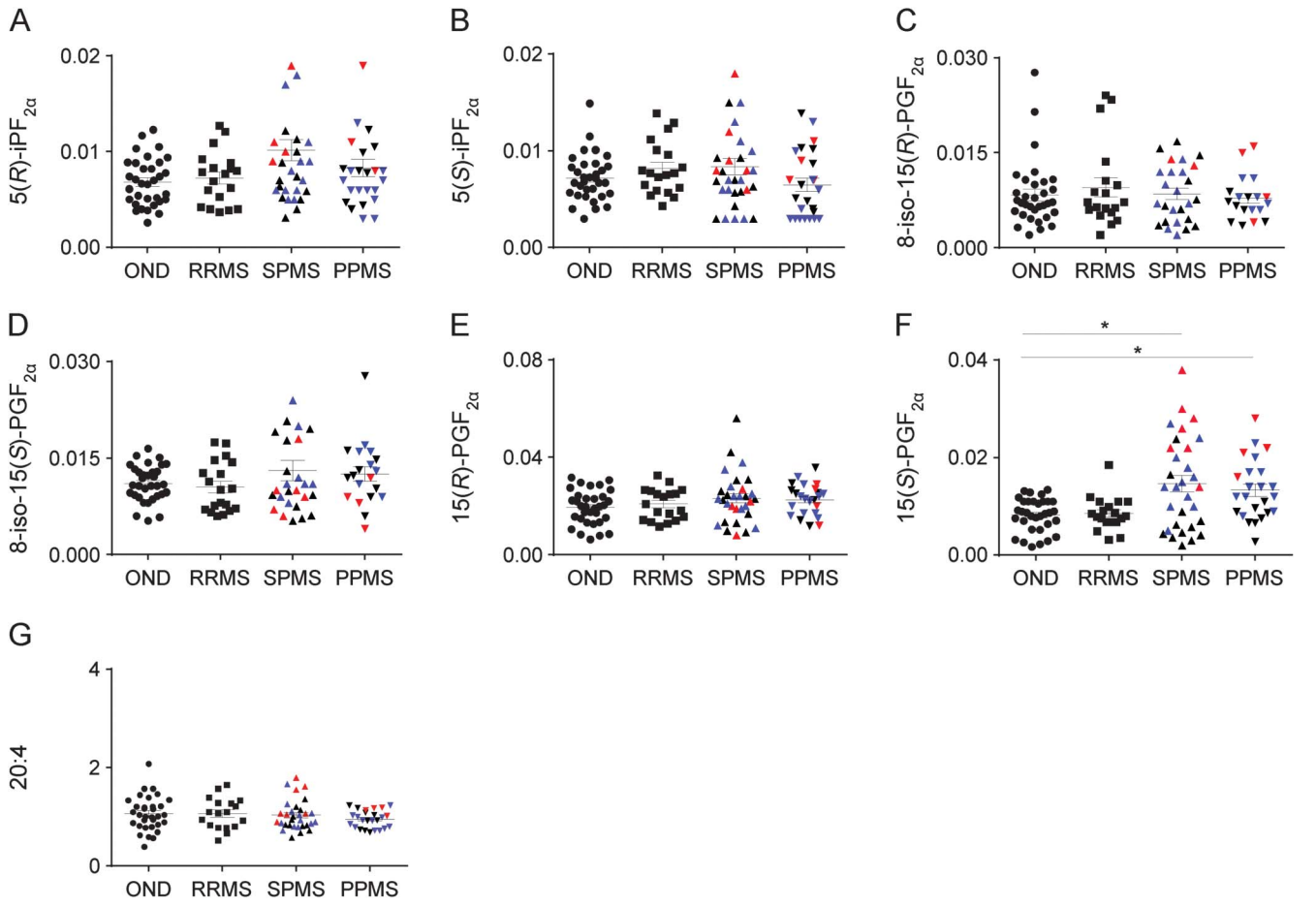
Results show values (ng/mL plasma) for the specific 5-series products 5(*R*)-iPF$_{2\alpha}$ (A) and 5(*S*)-iPF$_{2\alpha}$ (B), the specific 15-series products 8-iso-15(*R*)-PGF$_{2\alpha}$ (C), 8-iso-15(*S*)-PGF$_{2\alpha}$ (D), and 15(*R*)-PGF$_{2\alpha}$ (E), as well as the enzymatic product 15(*S*)-PGF$_{2\alpha}$ (F) and the precursor arachidonic acid, 20:4 (G). Patients were classified as follows: ONDs (n = 45), RRMS (n = 23), SPMS (n = 24), and PPMS (n = 15). A heterogeneous group of individuals with ONDs (n = 45) were used as control with the following diagnoses: unspecified sensory disturbances (n = 18), different psychological symptoms (n = 6), dizziness (n = 1), unspecified headache (n = 4), chronic idiopathic fatigue (n = 1), balance disturbance (n = 1), visual disturbance (n = 2), muscle diseases (n = 2), trigeminal neuralgia (n = 1), ulcerative colitis (n = 1), spinal stenosis (n = 1), stroke (n = 1), polymyositis (n = 1), postcommotion syndrome (n = 1), unspecific sarcoidosis (n = 1), hypokalemia (n = 1), hypesthesia (n = 1), and Bechterew disease (n = 1). Data show results for individual participants and mean ± SEM. Statistical significance was determined by the Kruskal-Wallis multiple comparison test. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. MS = multiple sclerosis; OND = other neurologic disease; PPMS = primary progressive MS; RRMS = relapsing-remitting MS; SPMS = secondary progressive MS.

the situation with natalizumab, CSF F_2-IPs were generally but not consistently lower at 60 weeks compared with baseline, and this did reach statistical significance only in the case of 8-iso-15(*S*)-PGF$_{2\alpha}$ ($p = 0.031$). Together, these results suggest that enzymatic lipid oxidation in CNS of progressive MS is decreased by modulating the enzymatic activity of inflammatory cells (with methylprednisolone) or blocking their migration into the CNS (with natalizumab).

DISCUSSION There are 2 main new findings in the present study. First, compared with OND controls, the concentration of PGF$_{2\alpha}$ in CSF is elevated in participants with progressive MS, and it increases with disease progression while it is decreased by treatment with natalizumab and methylprednisolone. The disease-associated increase in PGF$_{2\alpha}$ does not simply reflect the heightened state of inflammation and breakdown of the blood–brain barrier typical of RRMS.[14] Rather, it may represent pathologic responses to CNS resident cells, as disease severity did not correlate with CSF inflammatory cells and biomarkers, or the albumin ratio. Together, the data suggest that cyclooxygenase-mediated formation of PGF$_{2\alpha}$ may have a role in the pathogenesis of progressive MS.

Second, plasma concentrations of F_2-IP were significantly lower in patients with progressive MS than OND controls and patients with RRMS. This is consistent with progressive MS being a consequence of impaired NADPH oxidase-dependent oxidative

Figure 3 Concentrations of F_2-isoprostane in CSF of controls with OND (n = 33) and patients with multiple sclerosis who had different disease courses



Disease courses included RRMS (n = 19), SPMS (n = 31), and PPMS (n = 25). Results show values (ng/mL CSF) for the specific 5-series products: 5(*R*)-iPF$_{2\alpha}$ (A) and 5(*S*)-iPF$_{2\alpha}$ (B); the specific 15-series products: 8-iso-15(*R*)-PGF$_{2\alpha}$ (C), 8-iso-15(*S*)-PGF$_{2\alpha}$ (D), 15(*R*)-PGF$_{2\alpha}$ (E), and 15(*S*)-PGF$_{2\alpha}$ (F), as well as arachidonic acid (G). Red symbols: second KI batch; black symbols: third KI batch; blue symbols: baseline samples from the Danish NAPMS Study. Data are shown for individuals and as means ± SEM. Statistical significance was determined by the Kruskal-Wallis multiple comparison test. *$p < 0.05$. OND = other neurologic disease; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

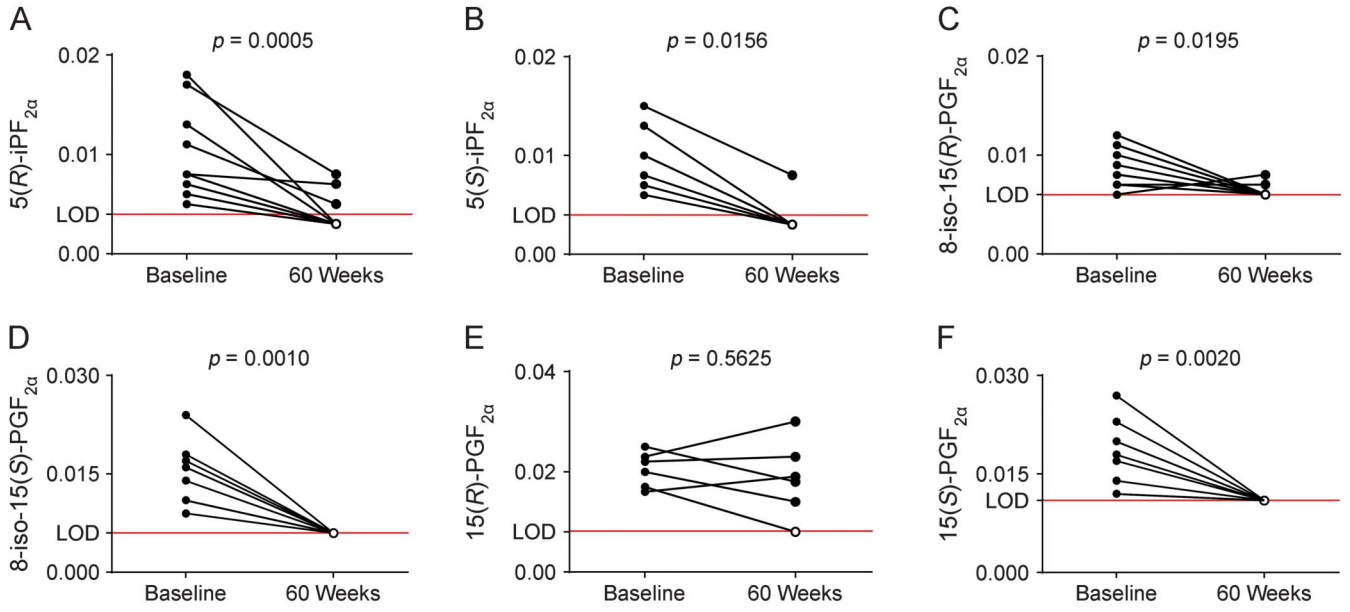
dampening of systemic immune activation.[2,3] A potential implication of these findings is that the use of antioxidants may be counterproductive, and that instead agents increasing NADPH oxidase activity may be worth considering.

Although not commonly recognized, our observation of increased CSF PGF$_{2\alpha}$ in patients with progressive MS is in agreement with previous reports. Thus, cyclooxygenase-2 is expressed in CD64$^+$ macrophages/microglia in actively demyelinating human lesions[15] and in damaged or apoptotic oligodendrocytes.[16] Cyclooxygenase-derived products in addition to PGF$_{2\alpha}$ (PGE$_2$, PGI$_2$, and PGD$_2$) are elevated in the CSF of patients with MS[17] and animal models of the disease.[18] Moreover, cyclooxygenase inhibition improves MS symptoms in models of the disease.[19]

The synthesis of prostaglandins is complex,[20] with their biological effects mediated via specific cellular receptors. Our findings, combined with previous reports, suggest inhibition of prostaglandin synthesis or their action as a potential drug target(s) for the treatment of MS. Because selective cyclooxygenase inhibitors can have severe side effects,[21] a better understanding of the processes that link elevated CSF PGF$_{2\alpha}$ to MS is required to identify appropriate therapeutic targets to treat MS.[18]

In contrast to previous reports of increased oxidative stress in patients with MS,[22,23] we found no evidence for MS being associated with an increase in CSF F_2-IP, considered the "gold standard" for the assessment of in vivo oxidative stress.[7] Consistent with our findings, others[24,25] also reported no significant difference between patients with MS and controls in their CSF content of 8,12-iPF$_{2\alpha}$-VI (5-series F_2-IP), determined by mass spectrometry. However, an increase in the CSF concentration of 8-iso-15(*S*)-PGF$_{2\alpha}$ was reported in the CSF of patients with MS with F_2-IP determined by ELISA.[26–28] We attribute

Figure 4 Natalizumab treatment decreases $PGF_{2\alpha}$ in CSF of patients with secondary progressive MS and primary progressive MS



Results are shown for the specific F_2-IP 5-series: 5(*R*)-$iPF_{2\alpha}$ (n = 12) (A) and 5(*S*)-$iPF_{2\alpha}$ (n = 7) (B); and the 15-series: 8-iso-15(*R*)-$PGF_{2\alpha}$ (n = 10) (C), 8-iso-15(*S*)-$PGF_{2\alpha}$ (n = 11) (D), and 15(*R*)-$PGF_{2\alpha}$ (n = 6) (E), as well as the enzymatic oxidation product 15(*S*)-$PGF_{2\alpha}$ (n = 10) (F), respectively, before (baseline) and after treatment with natalizumab (60 weeks) with data expressed as ng/mL CSF. Data show results for individual participants and mean ± SEM. Open circle symbols represent values at or below detection limit. Dotted line represents the average LOD for each product. Statistical significance was determined by Wilcoxon matched-pairs signed rank test. LOD = limit of detection; MS = multiple sclerosis.

these apparent discrepancies to differences in the methods used to determine F_2-IP. It is well known that results from ELISA are not directly comparable to those determined by mass spectrometry–based assays,[29] with the former being less specific and commonly yielding higher values for 8-iso-15(*S*)-$PGF_{2\alpha}$ than mass spectrometry–based assays. Thus, our findings are overall consistent with the literature and strongly suggest that oxidative stress is not increased in the CSF of patients with MS, and it does not appear to have a role in disease progression. While our study did not detect an increase in CSF F_2-IP in patients with MS, it does not exclude the possibility that localized oxidative stress and damage to brain tissue contribute to the lesions observed in patients with MS.[30]

MS lesions are thought to arise as a result of the migration of immune cells through the blood–brain barrier, resulting in an inflammatory cascade that leads to myelin loss, axonal damage, and neuronal death.[31] Binding of leukocytes α4-integrin to VCAM-1 on the vascular endothelium facilitates the migration of leukocytes across the blood–brain barrier.[32] The humanized α4-integrin monoclonal antibody natalizumab blocks the recruitment of circulating immune cells to the CNS[33] and it has recently been shown to reduce intrathecal inflammation and tissue damage in patients with progressive MS.[11] The observed decrease in enzymatic and some nonenzymatic markers of 20:4 oxidation in the CSF after natalizumab treatment suggests that intrathecal pathogenic events are triggered by systemic immune cells entering the CNS and this may be amenable to treatment. Consequently, the reduced lipid oxidation following natalizumab treatment is driven by decreased intrathecal inflammation. The finding of decreased CSF $PGF_{2\alpha}$ following treatment with the glucocorticoid methylprednisolone[34] is also important since we found that treatment was associated with clinical improvement as well as improvement in magnetization transfer ratio measurements in brain MRI studies.[11] This may reflect a direct effect of methylprednisolone on the biosynthesis of $PGF_{2\alpha}$ rather than a general inhibition of intrathecal inflammation since we found little effect of monthly methylprednisolone pulse treatment on a panel of other biomarkers in progressive MS.[11] The lack of correlation between CSF $PGF_{2\alpha}$ and validated inflammatory biomarkers, and the differences in their response to treatments, further suggests that multiple pathologic mechanisms coexist in patients with MS contributing to the complexity of this disease.

Our results indicate the potential utility of CSF $PGF_{2\alpha}$ as a biomarker of progressive MS. However, the causal role of any abnormal finding in MS, such as an increase in CSF $PGF_{2\alpha}$, will need experimental support because it can also represent events secondary to the disease process, for example a reduced level of physical activity resulting from ambulation handicap. Currently used MRI biomarkers are adequate for

patients with RRMS but do not reflect the complex pathogenesis of progressive MS. Although mass spectrometry–based determination of $PGF_{2\alpha}$ is labor-intense, the specificity and reproducibility of the quantitative data for the 6 prostanoids investigated (figure 1B), over several separate batches of analyses and involving 3 independent cohorts of patients from 2 different sites, cannot be achieved with other laboratory methods.

The decrease in plasma concentration of several types of F_2-IP in patients with MS is important. If replicated, plasma F_2-IP could serve as a more easily obtainable biomarker for progressive MS. The data are consistent with rodent studies that imply a genetic deficiency in NADPH oxidase–derived ROS to contribute to progressive MS via inadequate oxidative dampening of immune competent cells.[2,3] Thus, there is a possible role for regulation of the oxidative burst in humans, and genetic characteristics may be decisive in whether, how soon, and how serious the development of progressive MS ensues. To date, genetic studies have largely been restricted to incidence of disease, while correlation with disease severity and progression remains to be determined.

This study shows that $PGF_{2\alpha}$ is significantly increased in the CSF of patients with progressive MS and that this is associated with disease severity and amenable to treatment, but independent of age. Since $PGF_{2\alpha}$ has biological activities, such as modulation of immunologic effector cell functions[35,36] and vasoconstriction,[37] our results warrant future studies addressing whether cyclooxygenase-derived oxidation products of 20:4, including $PGF_{2\alpha}$, contribute to MS pathogenesis. In addition, the surprisingly low systemic concentrations of F_2-IP deserve further attention regarding potential mechanisms of disease progression, its treatment, and in relation to their use as systemic biomarker.

AUTHOR CONTRIBUTIONS

M. Lam: study design, analyses or interpretation of data, drafting or revising the manuscript. G. Maghzal: analyses or interpretation of data, drafting or revising the manuscript. M. Khademi: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. F. Piehl: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. R. Ratzer: study design or conceptualization, drafting or revising the manuscript. J. Romme Christensen: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. F. Sellebjerg: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. T. Olsson: study design and conceptualization, analyses or interpretation of data, drafting or revising the manuscript. R. Stocker: study design and conceptualization, analyses or interpretation of data, drafting or revising the manuscript. All authors accept responsibility for conduct of research and give final approval.

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