Peroxiredoxins are involved in the pathogenesis of multiple sclerosis and neuromyelitis optica spectrum disorder

Akiyuki Uzawa[1], Masahiro Mori[1], Hiroki Masuda[1], Ryohei Ohtani[1], Tomohiko Uchida[1], Reiji Aoki[1], and Satoshi Kuwabara[1]

[1]Graduate School of Medicine, Chiba University

April 28, 2020

Abstract

Peroxiredoxins (PRXs) are intracellular antioxidative enzymes but work as inflammatory amplifiers under the extracellular condition. To date, the function of PRXs in the pathogenesis of multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) is not fully understood. The aim of this study was to investigate whether PRXs play a role in the pathogenesis of MS and NMOSD. We analyzed levels of PRXs (PRX1, PRX5, and PRX6) in the CSF and serum of 16 patients with MS, 16 patients with NMOSD, and 15 patients with other neurological disorders (ONDs). We identified potential correlations between significantly elevated PRXs levels and the clinical variables in patients with MS and NMOSD. Additionally, pathological analyses of PRXs (PRX1-6) in the central nervous system were performed using the experimental autoimmune encephalomyelitis (EAE), animal model of MS. We found that serum levels of PRX5 and PRX6 in patients with MS and NMOSD were higher compared with those in patients with ONDs ($p < 0.05$). Furthermore, high levels of PRX5 and PRX6 were partly associated with blood–brain barrier dysfunction and disease duration in NMOSD patients. No significant elevation was found in CSF PRXs levels of MS and NMOSD. Spinal cords from EAE mice showed remarkable PRX5 staining, especially in CD45+ infiltrating cells. In conclusion, PRX5 and PRX6 may play a role in the pathogeneses of MS and NMOSD.

Original Article

Peroxiredoxins are involved in the pathogenesis of multiple sclerosis and neuromyelitis optica spectrum disorder

Akiyuki Uzawa, MD, PhD; Masahiro Mori, MD, PhD; Hiroki Masuda, MD, PhD; Ryohei Ohtani, MD, PhD; Tomohiko Uchida, MD, PhD; Reiji Aoki, MD; Satoshi Kuwabara, MD, PhD

Department of Neurology, Graduate School of Medicine, Chiba University

Address correspondence to:

Akiyuki Uzawa, MD, PhDDepartment of Neurology, Graduate School of Medicine, Chiba University1-8-1, Inohana, Chuo-ku, Chiba 260-8670, JapanTelephone number: +81-43-226-2129Fax number: +81-43-226-2160E-mail address: *auzawa@chiba-u.jp*

Running Title: PRXs are involved in the MS and NMOSD pathogeneses

Words : Title, 14; abstract, 222; text, 2427

Figures : 3 (color figures, 2); **Tables** : 2;**References** : 19

Key words: blood brain barrier, CD45, experimental autoimmune encephalomyelitis, multiple sclerosis, neuromyelitis optica spectrum disorder, peroxiredoxin

SUMMARY

Peroxiredoxins (PRXs) are intracellular antioxidative enzymes but work as inflammatory amplifiers under the extracellular condition. To date, the function of PRXs in the pathogenesis of multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) is not fully understood. The aim of this study was to investigate whether PRXs play a role in the pathogenesis of MS and NMOSD. We analyzed levels of PRXs (PRX1, PRX5, and PRX6) in the CSF and serum of 16 patients with MS, 16 patients with NMOSD, and 15 patients with other neurological disorders (ONDs). We identified potential correlations between significantly elevated PRXs levels and the clinical variables in patients with MS and NMOSD. Additionally, pathological analyses of PRXs (PRX1-6) in the central nervous system were performed using the experimental autoimmune encephalomyelitis (EAE), animal model of MS. We found that serum levels of PRX5 and PRX6 in patients with MS and NMOSD were higher compared with those in patients with ONDs ($p < 0.05$). Furthermore, high levels of PRX5 and PRX6 were partly associated with blood–brain barrier dysfunction and disease duration in NMOSD patients. No significant elevation was found in CSF PRXs levels of MS and NMOSD. Spinal cords from EAE mice showed remarkable PRX5 staining, especially in CD45+ infiltrating cells. In conclusion, PRX5 and PRX6 may play a role in the pathogeneses of MS and NMOSD.

INTRODUCTION

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are demyelinating inflammatory diseases of the central nervous system (CNS) [1,2]. Many previous studies have revealed the important roles of inflammatory mediators, including cytokines, in the pathogenesis of MS and NMOSD [3-8]. Substances derived from damaged autologous tissues, called damage-associated molecular patterns (DAMPs), are attracting attention in the field of autoimmune diseases because they function as inflammatory mediators when released from cells [9,10]. We previously reported increased levels of high mobility group box 1 (HMGB1), a DAMP, in the serum of MS patients and the cerebrospinal fluid (CSF) from MS and NMOSD [4]. We also reported a significant positive correlation between CSF HMGB1 levels and CSF cells in patients with MS and NMOSD [4]. These findings indicate that HMGB1 may be involved with inflammation in MS and NMOSD. Also, the administration of anti-HMGB1 monoclonal antibodies ameliorated clinical symptoms, CNS inflammation, demyelination, and serum IL-17 upregulation in experimental autoimmune encephalomyelitis (EAE) [11]. These findings suggest that HMGB1 may control autoimmune responses by stimulating the release of inflammatory cytokines in MS. Recently, peroxiredoxins (PRXs), which are intracellular antioxidant enzymes, attract attention as novel DAMPs. Extracellular PRXs also induce the production of inflammatory cytokines and triggers inflammation; the pro-inflammatory effects of PRXs are reported to be stronger than that of HMGB1 [12]. Besides increasing inflammation, PRXs was reported to affect the blood–brain barrier function [13], which is another important factor in the pathogenesis of MS and NMOSD [14]. We speculate that PRXs could be key players in the inflammatory response of demyelinating CNS disorders, including MS and NMOSD, and could be a future therapeutic target. However, the exact function of PRXs in the pathogenesis of MS and NMOSD is not yet fully understood. The main aim of this study is to determine whether PRXs are involved in promoting inflammatory processes and affecting the blood–brain barrier function in patients with MS and NMOSD.

MATERIALS AND METHODS

Patients

Patients with relapsing–remitting MS (n = 16) [1] and anti-aquaporin 4 (AQP4) antibody-positive NMOSD (n = 16) [2] were included in this study. Fifteen patients (nine men; six women; mean age, 61.4 years) with other neurological disorders (ONDs), including nine patients with amyotrophic lateral sclerosis and six with spinocerebellar degeneration, were recruited as controls. The following patient variables were reviewed: gender, age, disease duration, Kurtzke's expanded disability status scale (EDSS) scores, the presence of serum anti-AQP4 antibody [15], positivity for oligoclonal bands, CSF cell counts, CSF protein, CSF/serum albumin ratio (albumin quotient, Qalb), and immunomodulatory treatment at the time of sampling. The ethics committee of the Chiba University School of Medicine in Chiba, Japan, approved the study (Approved No. 842). Written informed consent was obtained from all study subjects.

PRX1, PRX5, and PRX6 measurements in patients with MS and NMOSD

Commercial enzyme-linked immunosorbent assay (ELISA) kits were only available for the detection of PRX1, PRX5, and PRX6 (MyBioSource, Inc, San Diego, CA, USA). CSF samples were obtained during the active disease phase (within 1 month of clinical attack and before treatment for the attack) from patients with MS (n = 16) and NMOSD (n = 16) and patients with ONDs (n = 15). Serum samples were simultaneously obtained in 10 patients with MS, 10 with NMOSD, and 10 with ONDs at the time of CSF sampling. All samples were stored at -80°C until use. The CSF and serum levels of PRX1, PRX5, and PRX6 were measured using the ELISA kits according to the manufacturer's instructions.

Correlations between the PRXs levels and clinical parameters in patients

The possible associations between the significantly elevated levels of PRXs and clinical variables such as the duration of disease, EDSS, CSF cell counts, CSF protein levels, and Qalb, were examined in patients with MS and NMOSD.

EAE induction and pathological analyses

To identify associations between PRXs and CNS inflammation, we isolated the spinal cords from EAE mice for pathological analysis. EAE was induced in mice using the same method described in our previous study [16]. Simply, a total of 200 μg myelin oligodendrocyte glycoprotein peptide 35–55 in complete Freund's adjuvant containing killed Mycobacterium tuberculosis H37Ra was subcutaneously administered to wild-type C57BL/6 mice (10 weeks old, female) (day 1). Then, the mice received intraperitoneal injections of 400 ng pertussis toxin (days 1 and 2). EAE mice were scored using the following scale: 0.0 = no clinical signs, 1.0 = partial paralysis of the tail, 2.0 = limp tail and mild bilateral hind leg paralysis, 3.0 = limp tail and complete paralysis of the hind legs, 4.0 = limp tail and complete hind leg and partial front leg paralysis, and 5.0 = complete hind and front leg paralysis. In this study, we used spinal cords from EAE mice (n = 2) whose score was 4.0 on day 18 to investigate the role of PRXs in the established inflammatory CNS lesions. Untreated normal (naive) mice (n = 2) were used as controls. All experimental animal procedures were approved by the Institutional Animal Care and Use Committee of Chiba University (Approved No. 1-9).

Histopathological examinations were performed using paraffin-embedded sections of spinal cords, and the sections were stained with hematoxylin and eosin (HE), mouse anti-glial fibrillary acidic protein (GFAP) (Novocastra, NCL-GFAP-GA5), rabbit anti-PRX1 (ProteinTech Group, 15816-1-AP), rabbit anti-PRX2 (ProteinTech Group, 10545-2-AP), rabbit anti-PRX3 (ProteinTech Group, 10664-1-AP), rabbit anti-PRX4 (ProteinTech Group, 10703-1-AP), rabbit anti-PRX5 (ProteinTech Group, 17724-1-AP), rabbit anti-PRX6 (ProteinTech Group, 13585-1-AP), rat anti-CD3 (Abcam, ab56313), and rat anti-CD45 (Santa Cruz Biotechnology, SC-53665). Alexa Fluor594 chicken anti-rabbit IgG (Invitrogen, A21442) and goat anti-rat secondary antibody Alexa Fluor488 (ThermoFisher, A-11006) were used as secondary antibodies.

Statistical analyses

Statistical analyses were performed using the JMP Pro 12.0.1 software (SAS Institute Inc., Cary, North Carolina, USA). Groups were compared using the Mann–Whitney U -test for unpaired continuous variables. Spearman's rank correlation coefficient was used to test correlations between variables. P -values of <0.05 were considered statistically significant.

RESULTS

Clinical profiles of patients

The clinical characteristics of the patients with MS, NMOSD, and ONDs are summarized in Table 1. The age, duration of disease, positivity for serum anti-AQP4 antibodies, negativity for oligoclonal bands, CSF cells, CSF protein, and Qalb were higher in patients with NMOSD than those in patients with MS. The ages of patients with ONDs were similar to those of the NMOSD patients but higher than those of MS patients. CSF cells, CSF protein, and Qalb were higher in MS and NMOSD patients compared with patients with ONDs.

CSF and serum PRXs levels in patients

CSF PRX1 levels were 2.10 ± 2.72 (mean ± SD), 1.27 ± 1.54, and 1.84 ± 1.68 ng/ml; CSF PRX5 levels were 1.17 ± 0.28, 2.20 ± 4.74, and 1.26 ± 0.41 ng/ml; and CSF PRX6 levels were 3.94 ± 5.49, 2.15 ± 1.91, and 2.42 ± 3.70 ng/ml in patients with NMOSD, MS, and ONDs, respectively. There were no significant differences in the levels of PRXs in the CSF among the groups of patients (Figure 1).

Serum PRX1 levels were 13.88 ± 20.74, 7.24 ± 10.50, and 2.28 ± 3.98 ng/ml; serum PRX5 levels were 5.47 ± 6.54, 1.77 ± 1.28, and 0.98 ± 0.00 ng/ml; and serum PRX6 levels were 43.81 ± 41.21, 22.72 ± 11.47, and 6.25 ± 4.75 ng/ml in patients with NMOSD, MS, and ONDs, respectively. Serum PRX5 and PRX6 levels in patients with NMOSD and MS were significantly higher than those in patients with ONDs (PRX5: NMOSD vs. ONDs, P = 0.013; MS vs. ONDs, P = 0.031, PRX6: NMOSD vs. ONDs, P = 0.0003; MS vs. ONDs, P = 0.0009) (Figure 1).

Correlations between elevated serum levels of PRX5 and PRX6 and clinical variables in patients

Table 2 shows possible associations between significantly elevated serum levels of PRX5 and PRX6 and clinical parameters in patients with MS and NMOSD. In MS patients, no significant correlation was observed between serum PRX5 and PRX6 levels and clinical variables (CSF cell counts, CSF protein levels, Qalb, EDSS scores, duration of disease, or oligoclonal band positivity). Among NMOSD patients, serum PRX5 levels correlated positively with disease duration (rs = 0.7198, p = 0.0189); and serum PRX6 levels correlated positively with CSF protein levels (rs = 0.6727, p = 0.0330), Qalb (rs = 0.6727, p = 0.0330), and the duration of disease (rs = 0.6748, p = 0.0323). Significant correlations between serum PRX5 and PRX6 levels were confirmed in patients with NMOSD (rs = 0.7047, p = 0.0229) but not MS patients (rs = 0.5924, p = 0.0711).

Immunohistochemical staining of PRXs in EAE spinal cords

HE staining revealed cellular infiltration in the meninges and parenchyma of EAE spinal cords (Fig. 2). Among the PRXs families (PRX 1-6), only PRX5 staining was confirmed in EAE spinal cords (Fig. 2). PRX5 staining was observed in CD45+ infiltrating cells in the inflammatory lesions (Fig. 3).

DISCUSSION

In this study, we identified the elevations of serum PRX5 and PRX6 levels in patients with MS and NMOSD and their associations with blood–brain barrier dysfunction and disease duration in NMOSD patients. Pathological analyses of the spinal cords from EAE mice showed remarkable PRX5 staining in CD45+ infiltrating cells. These results indicate that PRXs may play some role in CNS inflammation during MS and NMOSD.

PRXs have recently received attention as novel DAMPs. Among the PRX subtypes (PRX1-6), PRX1, PRX2, PRX5, and PRX6 are expressed in the brain and can trigger the release of several cytokines. Furthermore, PRX5 has the strongest effect on the activation of Th17 activation via the secretion of IL-23 [12]. Th17 cells play a dominant role in the development of EAE [17] and are also thought to be involved in the pathogenesis of MS and NMOSD [5]. Therefore, we must consider that PRXs may play a role in triggering autoimmunity during MS and NMOSD.

Thus far, some papers about the role of PRXs in patients with MS have been published but not in patients with NMOSD. Holley et al. reported that PRX5+ hypertrophic reactive astrocytes were observed in the acute and chronic brain lesions of MS patients. They also speculated that ongoing oxidative stress occurred during the acute and chronic phases of MS, and PRX5 was upregulated in astrocytes to neutralize oxidative stress [18]. Voigt et al. reported that PRX2 was upregulated mainly in astrocytes of white matter lesions. Furthermore, its expression level was positively correlated with the degree of inflammation and oxidative stress in patients with MS, which suggests that PRX2 contributes to the resistance of astrocytes against oxidative damage [19]. Yun et al. reported that PRX6 was strongly expressed by cells with astrocyte-like morphology in the MS lesions of human patients [13]. The increased PRX6 expression in astrocytes of MS patients reduced MMP9 expression, fibrinogen leakage, chemokines, and free radical stress, leading to

decreased blood–brain barrier disruption [13]. These findings suggest that PRX6 expression may represent a therapeutic way to restrict inflammation in the CNS and potentiate oligodendrocyte survival. Therefore, PRX6 may have potential as a new neuroprotective therapy for MS [13]. Taken together, upregulated PRXs may help protect astrocytes and maintain blood–brain barrier function. However, no paper has described the protein levels of PRXs in patients with MS and NMOSD thus far. In our study, serum PRX5 and PRX6 levels were significantly elevated in MS and NMOSD patients and partly associated with Qalb (as a marker of blood–brain barrier function), CSF protein, and disease duration in NMOSD patients. In general, longer disease duration correlates with more severe blood–brain barrier dysfunction in MS and NMOSD patients. Additionally, serum PRX6 levels were significantly associated with PRX5 levels in NMOSD patients. Our findings indicate that serum PRX6 and PRX5 may be associated with blood–brain barrier dysfunction in NMOSD, like pathological analyses in patients with MS [13]. However, there was no significant elevation in PRX levels within the CSF of MS and NMOSD patients. Further studies are needed to confirm the definite mechanism of PRXs in patients with MS and NMOSD.

Conversely, there is a limited number of papers regarding the role of PRXs in EAE. It has been reported that mRNA levels of PRX1, PRX3, and PRX6 were increased in the spinal cords of EAE mice compared with that of control mice. Also, PRX6 was strongly expressed on cells with astrocyte-like morphology in EAE lesions [13]. PRX6-transgenic EAE mice exhibited less severe pathology, which indicates that the upregulation of astrocytic PRX6 has an important role in inhibiting the destruction of myelin via microglial activation, blood–brain barrier breakdown, and immune cell infiltration [13]. Similar to pathological analyses in patients with MS [13], upregulated PRXs inside astrocytes may protect blood–brain barrier function and inhibit CNS inflammation. Contrariwise, PRXs have an opposite face: having the ability to induce inflammatory cytokines production as inflammatory mediators [12]. In our study, only PRX5 was upregulated in CD45+ cells (likely monocytes), which had infiltrated into the spinal cord lesions of EAE mice. We speculate that CD45+ cells may accumulate in the lesion and amplify the inflammatory response via PRX5. Another possible explanation is that CD45+ cells were dealing with ongoing oxidative stress. Although previous papers described the upregulation of astrocytic PRX6 in EAE [13], we did not observe the expression of PRXs in astrocytes.

Some limitations of our study need to be addressed. First, increased PRXs levels were not confirmed in the CSF but only the serum from patients with MS and NMOSD. The small sample size in our study may explain this discrepancy between serum and CSF levels of PRXs. Also, it is still unclear as to which cells secreted PRXs into the serum. Additional basic research studies are required to determine the source of these PRXs. Finally, our pathological findings of EAE were different from previous reports; EAE spinal cords showed PRX5 expression in CD45+ infiltrating cells but no PRX expression in astrocytes. One possible explanation for this discrepancy is species-specific differences between humans and mice. It is also possible that pathological characteristics may differ according to disease status. Further studies are needed to confirm the definite mechanism between PRXs and EAE pathogenesis.

From our results, we suggest that the elevations of serum PRX5 and PRX6 levels were associated with the pathogenesis of MS and NMOSD and partially responsible for blood–brain barrier dysfunction in patients with NMOSD. We also showed that upregulated PRX5 in CD45+ infiltrating cells amplify inflammation in EAE. In summary, the expression of PRXs was significantly altered in CNS inflammatory demyelinating diseases, which suggests that they may trigger of blood–brain barrier disruption or CNS inflammation. Consequently, the development of new treatment targeting PRXs may reduce symptoms of MS and NMOSD.

ACKNOWLEDGMENTS

Funding : This work was supported by JSPSKAKENHI Grant Number JP16K09691.

Conflict of interest statement: The authors declare no conflicts of interest.

Authorship:

All authors were involved in collecting clinical data, drafting the article, or revising it critically for important intellectual content and have read and approved the final version of the manuscript.

REFERENCES

1. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292-302.

2. Wingerchuk DM, Banwell B, Bennett JL, *et al.* International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015; 85: 177-189.

3. Uzawa A, Mori M, Arai K, et al. Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. *Mult Scler*2010; 16: 1443-1452.

4. Uzawa A, Mori M, Masuda S, Muto M and Kuwabara S. CSF high-mobility group box 1 is associated with intrathecal inflammation and astrocytic damage in neuromyelitis optica. *J Neurol Neurosurg Psychiatry* 2013; 84: 517-522.

5. Uzawa A, Mori M and Kuwabara S. Cytokines and chemokines in neuromyelitis optica: pathogenetic and therapeutic implications.*Brain Pathol* 2014; 24: 67-73.

6. Khaibullin T, Ivanova V, Martynova E, *et al.* Elevated levels of proinflammatory cytokines in cerebrospinal fluid of multiple sclerosis patients. *Front Immunol* 2017; 8: 531.

7. Michael BD, Elsone L, Griffiths MJ, *et al.* Post-acute serum eosinophil and neutrophil-associated cytokine/chemokine profile can distinguish between patients with neuromyelitis optica and multiple sclerosis; and identifies potential pathophysiological mechanisms - a pilot study. *Cytokine* 2013; 64: 90-96.

8. Wang KC, Lee CL, Chen SY, *et al.* Distinct serum cytokine profiles in neuromyelitis optica and multiple sclerosis. *J Interferon Cytokine Res* 2013; 33: 58-64.

9. Harris HE and Raucci A. Alarmin(g) news about danger: workshop on innate danger signals and HMGB1. *EMBO Rep* 2006; 7: 774-778.

10. Fiuza C, Bustin M, Talwar S, et al. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. *Blood* 2003; 101: 2652-2660.

11. Uzawa A, Mori M, Taniguchi J, Masuda S, Muto M and Kuwabara S. Anti-high mobility group box 1 monoclonal antibody ameliorates experimental autoimmune encephalomyelitis. *Clin Exp Immunol* 2013; 172: 37-43.

12. Shichita T, Hasegawa E, Kimura A, *et al.* Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain.*Nat Med* 2012; 18: 911-917.

13. Yun HM, Park KR, Kim EC and Hong JT. PRDX6 controls multiple sclerosis by suppressing inflammation and blood brain barrier disruption. *Oncotarget* 2015; 6: 20875-20884.

14. Masuda H, Mori M, Uchida T, Uzawa A, Ohtani R and Kuwabara S. Soluble CD40 ligand contributes to blood-brain barrier breakdown and central nervous system inflammation in multiple sclerosis and neuromyelitis optica spectrum disorder. *J Neuroimmunol* 2017; 305: 102-107.

15. Sugimoto K, Mori M, Liu J, *et al.* The accuracy of flow cytometric cell-based assay to detect anti-myelin oligodendrocyte glycoprotein (MOG) antibodies determining the optimal method for positivity judgement. *J Neuroimmunol* 2019; 336: 577021.

16. Uzawa A, Mori M, Masuda H, Ohtani R, Uchida T and Kuwabara S. Recombinant thrombomodulin ameliorates experimental autoimmune encephalomyelitis by suppressing high mobility group box 1 and inflammatory cytokines. *Clin Exp Immunol* 2018; 193: 47-54.

17. Komiyama Y, Nakae S, Matsuki T, et al. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol* 2006; 177: 566-573.

18. Holley JE, Newcombe J, Winyard PG and Gutowski NJ. Peroxiredoxin V in multiple sclerosis lesions: predominant expression by astrocytes. *Mult Scler* 2007; 13: 955-961.

19. Voigt D, Scheidt U, Derfuss T, Brück W and Junker A. Expression of the antioxidative enzyme peroxiredoxin 2 in multiple sclerosis lesions in relation to inflammation. *Int J Mol Sci* 2017; 18: 760.

Table 1. Clinical manifestations of patients with MS, NMOSD, and ONDs

	MS (n = 16)	NMOSD (n = 16)	ONDs (n = 15)
Men:women	4:12	2:14	9:6
Age, y	36.5 (18–51)	61.0 (27–86)	61.4 (42–75)
Disease duration, y	4.9 (0–20)	5.8 (0–40)	-
EDSS	6.0 (1.0–9.0)	6.3 (1.5–9.0)	-
Positive AQP4 antibody	0/16 (0%)	16/16 (100%)	-
Positive OCB	10/14 (71%)	2/14 (14%)	-
CSF cells, /mm3	3.5 (0–43)	4.7 (1–64)	0.7 (0–3)
CSF TP, mg/dL	32 (19–50)	51 (17–160)	37 (18–71)
Qalb, $\times 10^{-2}$	4.9 (2.7–11.7)	6.0 (2.8–37.0)	4.0 (2.7–6.2)
Immunomodulating therapy	4/16 (25%)	5/16 (31%)	0/15 (0%)

AQP4 = aquaporin 4, CSF = cerebrospinal fluid, EDSS = expanded disability status scale, MS = multiple sclerosis, NMOSD = neuromyelitis optica spectrum disorder; OCB = oligoclonal bands, ONDs = other neurological disorders, Qalb = albumin quotient

Values show median (range) unless indicated.

Table 2. The relationships between serum PRX5 and PRX6 levels and clinical variables in patients with MS and NMOSD.

	MS (n = 10)		NMOSD (n = 10)	
	Serum PRX5	Serum PRX6	Serum PRX5	Serum PRX6
CSF cells	rs = 0.127 (p = 0.727)	rs = -0.006 (p = 0.987)	rs = 0.614 (p = 0.059)	rs = 0.212 (p = 0.556)
CSF protein	rs = -0.278 (p = 0.436)	rs = -0.064 (p = 0.860)	rs = 0.356 (p = 0.313)	rs = 0.673 (p = 0.033*)
Qalb	rs = -0.416 (p = 0.266)	rs = -0.142 (p = 0.715)	rs = 0.407 (p = 0.243)	rs = 0.673 (p = 0.033*)
EDSS	rs = 0.062 (p = 0.865)	rs = 0.543 (p = 0.105)	rs = -0.111 (p = 0.760)	rs = 0.275 (p = 0.442)
Disease duration	rs = 0.198 (p = 0.583)	rs = -0.055 (p = 0.881)	rs = 0.720 (p = 0.019*)	rs = 0.675 (p = 0.032*)
Serum PRX5	-	rs = 0.592 (p = 0.071)	-	rs = 0.705 (p = 0.023*)
Serum PRX6	rs = 0.592 (p = 0.071)	-	rs = 0.705 (p = 0.023*)	-

CSF = cerebrospinal fluid, EDSS = expanded disability status scale, MS = multiple sclerosis, NMOSD = neuromyelitis optica spectrum disorder; Qalb = albumin quotient, PRX = peroxiredoxin

*statistically significant

FIGURE LEGENDS

Figure 1. CSF and serum levels of PRXs in patients No significant differences in the CSF levels of peroxiredoxins (PRXs) were identified among patients with multiple sclerosis (MS) (n = 16), neuromyelitis optica spectrum disorder (NMOSD) (n = 16), and other neurological disorders (ONDs) (n = 15). Serum PRX5 and PRX6 levels were significantly elevated in patients with MS (n = 10) and NMOSD (n = 10) compared with patients with ONDs (n = 10). Dashed lines indicate mean values. *P < 0.05; **P < 0.01.

Figure 2. Pathological findings of the EAE spinal cords Hematoxylin and eosin (HE), glial fibrillary acidic protein (GFAP), and peroxiredoxins (PRXs) immunoreactivity in the spinal cords of normal (A) and experimental autoimmune encephalomyelitis (EAE) mice (at day 18) (B: lower magnification, C: higher magnification) were performed (n = 2 in each group, representative images are shown). Inflammation and PRX5 staining were remarkable in EAE mice but not in normal mice. Black blank squares indicate the area of high magnification. Bars indicate 100 μm.

Figure 3. Immunofluorescent staining of the EAE spinal cords (A, B): Peroxiredoxin 5 (PRX5) (red), CD3/CD45 (green), and DAPI (blue) staining of the spinal cords of experimental autoimmune encephalomyelitis (EAE) (representative images are shown). PRX5 and CD45+ cells were confirmed in EAE spinal cords but CD3 positive cells were not identified. (C): Merged images of PRX5 and CD45 (yellow) and PRX5 and DAPI (purple). PRX5 was expressed in CD45+ cells. White blank squares indicate the area of high magnification. Bars indicate 100 μm.