Journal of Neuroimmunology 259 (2013) 92–95

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

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

Short communication

Serum amyloid A level is increased in neuromyelitis optica and atypical multiple sclerosis with smaller T2 lesion volume in brain MRI

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ARTICLE INFO

Article history:
Received 4 January 2013
Received in revised form 7 March 2013
Accepted 15 March 2013

Keywords:
Multiple sclerosis
Neuromyelitis optica
Serum amyloid A
T helper 17 cells
Barkhof criteria
T2 lesion volume

ABSTRACT

Serum amyloid A (SAA) is known to promote the development of T helper 17 cells (Th17) and can be a critical mediator of disease pathogenesis. We analyzed SAA levels in 40 patients with multiple sclerosis (MS) and related disorders, and 10 with non-inflammatory neurological disease (NIND) as controls. We found that SAA levels were significantly increased in neuromyelitis optica (NMO) patients and relapsing and remitting MS (RRMS) patients showing atypical phenotype with spinal cord lesions and smaller T2 lesion volume in brain MRI, resembling NMO. Therefore, SAA levels can be associated with clinical phenotypes in MS and NMO.

1. Introduction

Serum amyloid A (SAA), an acute phase reactant synthesized by hepatocytes in response to cytokines and other regulatory factors, is a known inflammatory and disease activity marker. It is also a critical mediator of disease pathogenesis and promotes the development of T helper 17 cells (Th17) and induces Th17-associated cytokines (He et al., 2006; Ivanov et al., 2009; Migita et al., 2010). Conversely, many recent studies have shown Th17 in a pathogenic role in several autoimmune diseases, including MS and neuromyelitis optica (NMO) (Ishizu et al., 2005; Brucklacher-Waldert et al., 2009; Durelli et al., 2009; Herges et al., 2012). In this study, we measured SAA levels in patients with MS, NMO, and clinically isolated syndrome (CIS) and compared the SAA levels across the groups. Furthermore, we investigated the association between SAA levels and clinical phenotype, especially the T2 lesion volume (T2LV) in brain MRI.

2. Materials & methods

2.1. Subjects and clinical assessment

From August 2010 to October 2012, we consecutively enrolled 40 patients: 12 with CIS, 20 with relapsing and remitting MS (RRMS), and 8 with NMO, including NMO spectrum disorder. Age and sex-matched patients (n = 10) with non-inflammatory neurological disease (NIND) were enrolled as controls. The NIND group included 2 patients with stroke, 2 with epilepsy, 1 with panic disorder, 3 with psychosomatic disorder, 1 with tension-type headache, and 1 with alcoholic myelopathy. A patient was diagnosed with CIS if the patients had the first monosymptomatic clinical demyelinating attack typical of MS without any evidence of dissemination in time. All MS patients fulfilled the McDonald criteria 2010 (Polman et al., 2011). None of the patients with RRMS had anti-aquaporin 4 (AQP4) antibodies. Some patients with CIS who were clinically suspected for NMO were evaluated, and we confirmed that they did not have anti-AQP4 antibodies. Although only 3 patients were definite cases of NMO according to the Wingerchuk criteria (Wingerchuk et al., 2006), all NMO patients had anti-AQP4 antibodies. Anti-AQP 4 antibodies were measured by a previously described cell-based assay (Takahashi et al., 2007). Neurological examination of each patient was conducted every 3 months.

2.2. Blood sample collection

Blood samples from 6 RRMS patients were obtained during the relapse phase before corticosteroid administration, whereas blood samples from 8 RRMS patients, who were defined as having a stable status for at least 3 months, were obtained during the remission phase. In the remaining 6 RRMS patients, blood samples were obtained during both phases. The duration between relapse onset and the blood draw was 2.7 ± 2.6 days. Blood samples from CIS

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http://dx.doi.org/10.1016/j.jneuroim.2013.03.004

Table 1
Clinical and demographic characteristics of study patients.

	NIND n = 10	NMO n = 8	RRMS-rel n = 12	RRMS-rem n = 14	CIS n = 12	p
Age (years)	37.5 ± 16.0	55.9 ± 18.4	34.6 ± 8.5	34.9 ± 7.9	43.2 ± 14.4	0.046
Female (%)	7 (70)	7 (87.5)	9 (75.0)	11 (78.6)	10 (83.3)	0.92
DMT/IST (%)	–	4 (50.0)	5 (41.2)	7 (50.0)	–	0.91
Disease duration (years)	–	2.6 ± 4.1	4.2 ± 5.1	7.6 ± 5.7	–	0.082
CRP (mg/dl)	0.09 ± 0.20	0.049 ± 0.050	0.23 ± 0.29	0.043 ± 0.039*	0.073 ± 0.10	0.25
Optic nerve lesion (%)	–	4 (50.0)	4 (33.3)	5 (50.0)	3 (25.0)	0.54
Spinal cord lesion (%)	–	6 (75.0)	9 (75.0)	10 (71.4)	7 (58.3)	0.83
Optic nerve & spinal cord lesion (%)	–	3 (37.5)	2 (16.7)	3 (21.4)	1 (8.3)	0.50
LESCL (%)	–	6 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.000017

NIND, other non-inflammatory neurological diseases; NMO, neuromyelitis optica; RRMS, relapsing and remitting multiple sclerosis; rel, relapsing; rem, remitting; CIS, clinically isolated syndrome; DMT, disease modifying therapy; IST, immunosuppresive therapy; CRP, C reactive protein; LESCL, longitudinally extensive spinal cord lesion.
* n = 13.

patients were obtained before corticosteroid administration, during the first demyelination episode. The SAA level in the blood samples was measured by the latex immunity measuring method (SRL, Japan), and the C-reactive protein (CRP) level was determined by routine blood examination. SAA and serum CRP levels were also measured in 10 age- and sex-matched controls with NIND.

2.3. MRI assessment and quantifying the T2 lesion volume

RRMS and NMO patients underwent brain MRI scans using a 1.5 T MR system (GE Healthcare, Japan) every 3 to 6 months. A conventional MRI protocol comprised the following: a T1-weighted 3 dimensional spin-echo (SE) sequence with and without gadolinium injection (repetition time (TR), 580 ms; echo time (TE), 14.00 ms; flip angle, 70°; matrix size, 256 × 256; field of view (FOV), 220 × 220 mm; slice thickness, 6 mm); a fast SE sequence with T2-weighted contrasts (TR, 4000 ms; TE, 89.54 ms; flip angle, 90°; matrix size, 256 × 256; FOV, 220 × 220 mm; slice thickness, 6 mm); and a fluid-attenuated inversion recovery sequence (TR, 9202 ms; TE, 125 ms; flip angle, 90°; matrix size, 256 × 256; FOV, 220 × 220 mm; slice thickness, 6 mm).

Brain images from the scan taken around the time that blood samples were obtained were visualized and analyzed using SepINRIA v 1.8.X (http://www-sop.inria.fr/asclepios/software/SepINRIA/), which is free software for analyzing brain MRI in MS patients. The number of T2 lesions and the T2LV were segmented manually. The Barkhof criteria (number of T2 and periventricular lesions and presence of gadolinium enhancement, infratentorial, juxtacortical, and spinal lesions) (Barkhof et al., 1997) were also analyzed.

2.4. Statistics

Continuous data across different patient groups were compared using analysis of variance, whereas nominal data were compared using Fisher's exact test. SAA levels across different patient groups were compared using the Kruskal–Wallis test, followed by post-hoc analysis using pairwise Mann–Whitney *U* tests with Holm's correction. T2LV between 2 patient groups was compared using the *t* test.

3. Results

NMO patients were significantly older than those with other diseases (Table 1). The frequency of optic nerve and spinal cord involvement was similar among patients with NMO, relapsing RRMS, remitting RRMS and CIS (Table 1). Because disease-modifying therapy (DMT) or immunosuppressive therapy (IST) may attenuate SAA levels, we determined whether each patient received any DMTs/ISTs and found no significant differences among the groups (Table 1). We excluded the possibility that a concomitant inflammatory condition also affected SAA levels, because CRP levels were similar among the groups (Table 1).

SAA levels were significantly increased in NMO patients compared to NIND patients (Fig. 1), whereas SAA levels in CIS or RRMS patients were not. Furthermore, in RRMS patients, SAA levels during the relapse phase tended to be higher than those in the remission phase, but the difference was not significant (p = 1.00). Since the SAA levels in RRMS patients were highly variable, we defined SAA level thresholds as the mean SAA level for NIND patients plus 2 standard deviations (SD) and grouped the RRMS patients as $RRMS^{highSAA}$ and $RRMS^{lowSAA}$. Intriguingly, all the patients in the $RRMS^{highSAA}$ group had spinal cord lesions. Furthermore, these patients had relatively few brain lesions, and 66.7% (4 out of 6) did not fulfill the Barkhof criteria (Fig. 2A). To confirm this idea, we quantified T2LV in the brain MRI of patients with MS and NMO. As expected, we found that $RRMS^{highSAA}$ patients had significantly smaller T2LV than the $RRMS^{lowSAA}$ patients (Fig. 2B, 14,360 ± 12,580 mm^3 and 4722 ± 3570 mm^3, respectively). If we extended the analysis to all the enrolled MS/NMO patients, we also observed significantly smaller

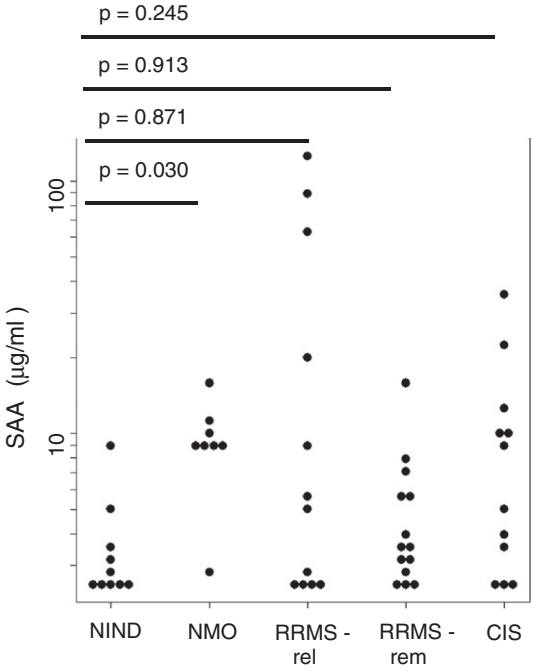

Fig 1. SAA levels in NMO, RRMS, and CIS. Compared to NIND patients, NMO patients had significantly increased SAA levels whereas RRMS and CIS patients showed only mild but insignificant increases. Note the variation in the SAA levels in RRMS patients.