analysis controlling for age. Statistical analyses were performed using SPSS Statistics for Windows 17.0 software (SPSS Japan, Tokyo, Japan).

Correlations between the BT in the lateral ventricles and rCBF in patient groups and healthy subjects were assessed using the SPM8 (Welcome Department of Imaging Neuroscience, London, UK) software. We evaluated the correlation of each group by 'full factorial' design in SPM8 with age as a covariate, and with the BT in the lateral ventricles as a covariate interacting with the factor 'diagnosis'. Statistical analyses were performed only differences that met the following criteria were deemed significant. In this case, a height threshold of $P < 0.001$ (uncorrected) and the extent threshold of $P < 0.05$ (uncorrected) were adopted.

RESULTS

The demographic and clinical characteristics of the participants are shown in Table 1. There were no significant differences in age and education years among the three groups in each sample.

When the temperature in the lateral ventricles was compared by DWI thermometry, we could not detect a significant difference among the three diagnostic groups (Figure 1). Only the significant negative correlation was detected between the BT in lateral ventricles of schizophrenia and PANSS-general score (correlation coefficient = − 0.54, $P = 0.012$, d.f. = 19). Second, we evaluated the relationships between the BT in the lateral ventricles and rCBF, and there were significant positive correlations in the left anterior cingulate in healthy subjects, and the left orbitofrontal region in patients with bipolar disorder (Figures 2A 2a, 2B, and 2b; Table 2). However, there were significant negative correlations in the right medial frontal region in patients with schizophrenia (Figure 2C and 2c; Table 2). The slope and R^2 value of the linear approximate equation between the rCBF and the temperature in the lateral ventricles were slope = 0.06, R^2 value = 0.53 in healthy subjects (Figure 2a); slope = 0.04, R^2 value = 0.62 in the patients with bipolar disorder (Figure 2b); slope = − 0.08, R^2 value = 0.49 in the patients with schizophrenia (Figure 2c).

DISCUSSION

We found that there were different correlation patterns between BT in the lateral ventricles and CBF in the patients with schizophrenia compared with the patients with bipolar disorder and healthy subjects. To our knowledge, this is the first study focusing on the surrogate of BT and CBF simultaneously determined using magnetic resonance imaging.

Human studies examining acute stroke, brain tumors, and Moyamoya disease have shown a significant difference of BT compared with healthy subjects,[10,28,29] and they indicated that the balance between heat production caused by CMR and heat removal by CBF helps to keep the temperature of the brain constant. A previous study focusing on psychiatric disease reported that all regional correlation coefficients for CMR and CBF were positive in the patients with bipolar disorder just as in the control subjects.[7] Our results revealed that patients with bipolar disorder showed a positive correlation between the BT in the lateral ventricles and the CBF that was the same pattern in healthy subjects. These points may indicate that the thermoregulation system of the healthy subjects is the same as that of patients with bipolar disorder. However, the patients with schizophrenia showed a different correlation between the BT and the CBF. Typically, glucose is the principal energy substrate of the brain. However, it is reported that the brain of depressive patient uses an energy source other than glucose,[30] and other study showed the dysregulation between CMR and CBF in the patients with major depressive disorder.[7] Similarly, the patients with dementia of Alzheimer' type and with the alcohol abuse, who showed the decrement of brain glucose utilization and the increase in acetate uptake in the brain,[31–33] presented with a dysregulation between CMR and CBF.[34,35] The metabolic profiling study[36] and postmortem study[37] indicated the disordered energy metabolism in the brain of schizophrenia. Brain depends exclusively on glycolysis and oxidative phosphorylation to create ATP. Thus, any mitochondrial pathology will have the most pronounced effect on the brain. Recent postmortem studies in schizophrenia have revealed abnormalities in mitochondrial morphology, function, and gene expression.[38] *In vivo* evidence for mitochondrial involvement in schizophrenia derives from magnetic resonance spectroscopy, an imaging technique that allows visualization of energy-related metabolite levels in the brain. *N*-acetyl-aspartate is an amino acid thought to be primarily synthesized in the mitochondria of neurons that can be measured by magnetic resonance spectroscopy. Some of magnetic resonance spectroscopy studies in schizophrenia have reported decreased *N*-acetyl-aspartate levels in several brain regions.[39] Further, it is known that antipsychotics induce the dysfunction of mitochondria.[40] The change in the brain energy metabolism in schizophrenia would alter the coupling of CBF and BT.

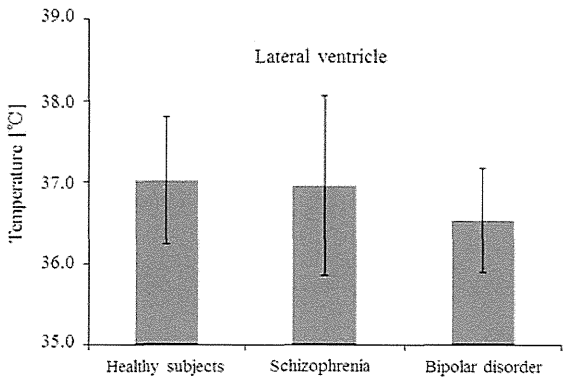

Figure 1. Clinical differences of brain temperature. There was no significant difference of temperature in the lateral ventricles among the three groups.

In this study, the significant positive or negative correlation between the BT of the lateral ventricles and CBF was observed in the medial frontal region and orbitofrontal region. The anterior cerebral artery runs through the neighborhood of the anterior part of the lateral ventricles, and the course of the artery is thought to influence the BT in the lateral ventricles.

Our data could not detect the significant difference of the BT in the lateral ventricle among three diagnostic groups. It is known that schizophrenia patients have dysfunctional thermoregulation (e.g., axillary, corneal, rectal, and oral esophageal),[11–17] though there has been only one study that evaluated the BT of schizophrenic patients *in vivo*. In that report, there was posterior-dominant occipital-frontal temperature gradient in schizophrenics.[17] We calculated the mean BT in the whole lateral ventricle, and did not evaluate in each segmented area. Further study with the measurement of temperature in small segmented lateral ventricles may show the temperature gradient of the schizophrenic brain. We detected the negative correlation between the BT and PANSS-general score, and this point was compatible with the previous one.[17] In addition, it is known that antipsychotics may influence the temperature. Specifically, antipsychotics have been shown to have the capacity to lower core temperature,[41,42] yet the schizophrenic patients in this study were prescribed larger volumes of antipsychotics, and showed higher BTs, compared with the patients with bipolar disorder. The schizophrenic participants in this study also showed relatively low PANSS scores, and PANSS scores have been shown to be negatively correlated with frontal CBF.[43,44] Then, the little decline in CBF may have obscured the change in BT. A further study with controlled antipsychotics and severe schizophrenic patients may show the dysfunctional brain

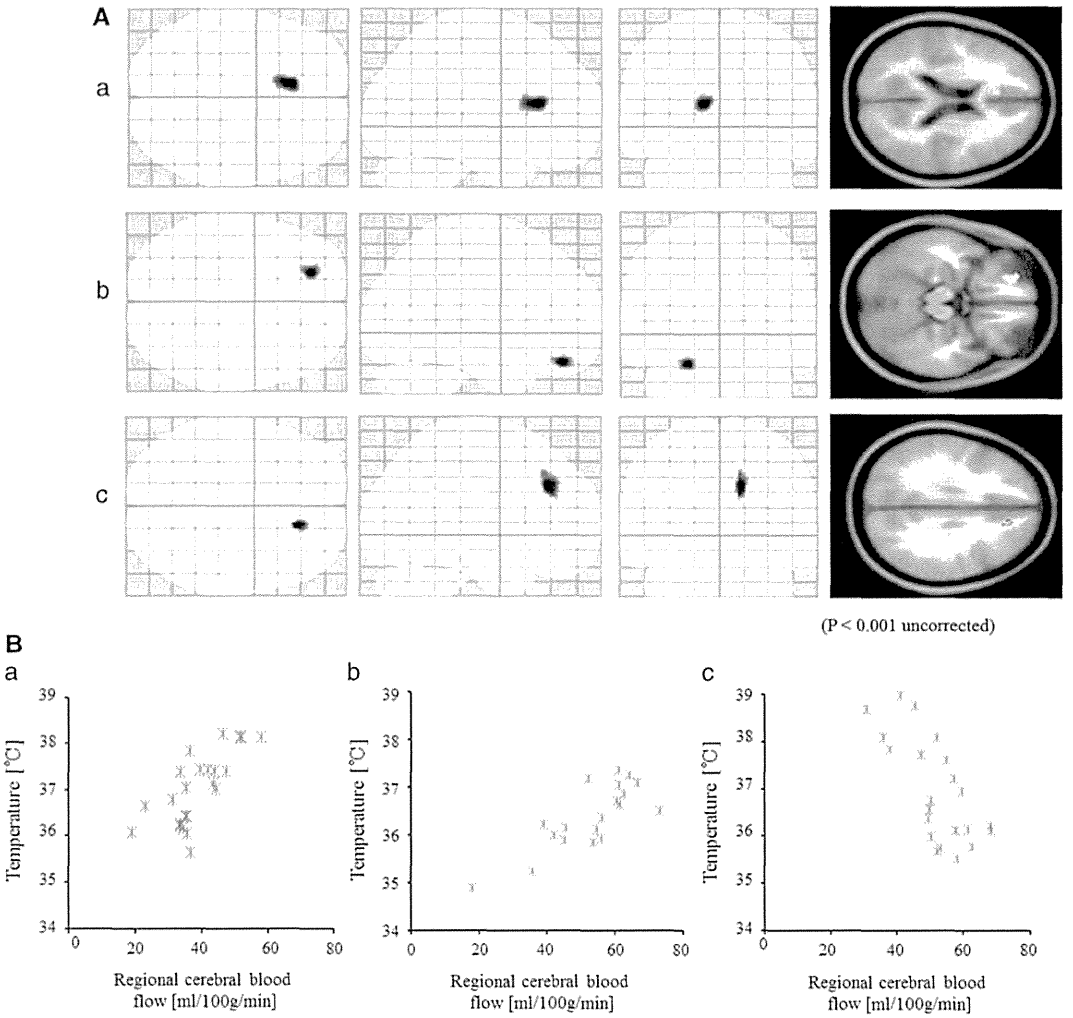

Figure 2. The relationships between the brain temperature and regional cerebral blood flow. Three left images showed the statistically significant regions projected on a glass brain in the three orthogonal planes and one right image showed the regions on the standard T1-weighted image. (**A**) There were significant positive correlations in the left anterior cingulate in healthy subjects (shown in yellow). (a) The scatter plot of them. (**B**) There were significant positive correlations in the left orbitofrontal region in patients with bipolar disorder (shown in red). (b) The scatter plot of them. (**C**) There were significant negative correlations in the right medial frontal region in patients with schizophrenia (shown in blue). (c) The scatter plot of them.

Table 2. Regions of statistically significant correlations between regional cerebral blood flow and temperature of lateral ventricles using age as nuisance variable

Group	*Cluster size*	*Z score*	*x*	*y*	*z*	*Brain region*
Positive correlation						
Healthy subjects	184	4.52	−10	26	16	Left anterior cingulate gyrus
Patients with bipolar disorder	82	4.33	−22	44	−20	Left orbitofrontal gyrus
Negative correlation						
Patients with schizophrenia	99	3.94	14	34	34	Right medial frontal gyrus

thermoregulation. Besides, this study contained relatively small sample size, then the degrees of freedoms for each of their correlations in the individual groups were low; 19 for schizophrenia patients, 16 for bipolar disorder, and 20 for healthy controls. The validity of the SPM is strongly dependent on the degrees of freedom, and experiments should be designed such that d.f. $\geqslant 30$.[45] Future study with a larger number of subjects would be necessary to verify this study.

In conclusion, we showed that patients with schizophrenia, but not those with bipolar disorder, exhibited dysfunctional

thermoregulation in the brain. Brain temperature is highly dependent on CMR and CBF, and thus uncoupling of CMR and CBF may occur in schizophrenics.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Journal of Affective Disorders 165 (2014) 59–63

Contents lists available at ScienceDirect

Journal of Affective Disorders

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

Research report

Characteristic distributions of regional cerebral blood flow changes in major depressive disorder patients: A pseudo-continuous arterial spin labeling (pCASL) study

Miho Ota [a,*], Takamasa Noda [b], Noriko Sato [c], Kotaro Hattori [a], Toshiya Teraishi [a], Hiroaki Hori [a], Anna Nagashima [a], Keigo Shimoji [c], Teruhiko Higuchi [d], Hiroshi Kunugi [a]

[a] Department of Mental Disorder Research, National Institute of Neuroscience, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo, Japan
[b] Department of Psychiatry, National Institute of Neuroscience, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo, Japan
[c] Department of Radiology, National Institute of Neuroscience, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo, Japan
[d] National Center of Neurology and Psychiatry, National Institute of Neuroscience, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan

ARTICLE INFO

Article history:
Received 25 March 2014
Accepted 11 April 2014
Available online 21 April 2014

Keywords:
Biological parametric mapping
Cerebral blood flow
Major depressive disorder
Pseudo-continuous arterial spin labeling
Schizophrenia

ABSTRACT

Background: Most previous studies that examined regional cerebral blood flow (rCBF) abnormalities in major depressive disorder (MDD) required the injection of radioisotopes into subjects. Here by using magnetic resonance imaging (MRI) with the pseudo-continuous arterial spin labeling (pCASL) method which does not require radioisotopes, we examined rCBF in patients with MDD in comparison with that in patients with schizophrenia and healthy subjects, taking the regional cerebral gray matter volume into account.
Methods: Subjects were 27 patients with MDD, 42 with schizophrenia and 43 healthy volunteers who underwent 3-T MRI with pCASL. Obtained pCASL imaging data were subject to the voxel-by-voxel statistical analysis.
Results: There were significant reductions of rCBF in the right inferior prefrontal cortex and anterior cingulate cortices (ACCs) in the MDD patients compared with the healthy controls. When compared with the schizophrenic patients, the MDD patients showed lower rCBF in the subgenual ACC and higher rCBF in left occipital region.
Limitation: The abnormalities of rCBF in MDD were known to reverse during symptom remission. Further study with follow-up period would bring the perception about the treatment response.
Conclusion: The rCBF reduction in the subgenual region may be a specific functional abnormality to MDD patients, which may provide a biological marker for MDD. The MRI with pCASL method is a promising tool to detect rCBF abnormalities controlling for gray matter volume in psychiatric disorders.

1. Introduction

Major depressive disorder (MDD) is a common disorder, and the lifetime prevalence was reported to be 8–12% (Andrade et al., 2003). Structural brain abnormalities in areas involved in emotional processing including the dorsolateral prefrontal region, orbitofrontal region, cingulate cortex, temporal region, hippocampus and striatum have been reported in MDD (reviewed in Arnone et al., Bora et al., 2012; Murphy and Frodl, 2011; Sexton et al., 2009). Previous studies using nuclear medicine techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) showed significant reductions of regional cerebral blood flow (rCBF) and metabolism in the frontal, parietal, and temporal regions of patients with MDD (Drevets et al., 2002; Mayberg et al., 2000; Smith and Cavanagh, 2005).

Arterial spin labeling (ASL) magnetic resonance imaging (MRI) is a novel noninvasive (i.e., non-radioactive) technique that can measure rCBF by taking advantage of arterial water as a freely diffusible tracer. This technique has recently been applied to detect functional abnormalities of the brain in MDD patients (Duhameau et al., 2010; Ho et al., 2013; Järnum et al., 2011; Lui et al., 2009; Walther et al., 2012). Some of these studies showed rCBF reduction in the frontal regions (Ho et al., 2013; Lui et al., 2009) and anterior cingulate cortex (ACC) (Walther et al., 2012). However, the others found no significant reduction in MDD patients (Duhameau et al., 2010; Järnum et al., 2011). Importantly, the limited spatial resolution of ASL images precludes accurate rCBF measurements because

* Correspondence to: Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan. Tel.: +81 42 341 2712; fax: +81 42 346 2094.
E-mail address: ota@ncnp.go.jp (M. Ota).

http://dx.doi.org/10.1016/j.jad.2014.04.032
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the partial volume effects on ASL cause an underestimation of activity in small brain structures. Since many MDD patients have altered brain structures as described above, their rCBF images are likely to be influenced by the partial volume effect. To our knowledge, however, no ASL study in MDD has thus far taken account of thise partial volume effect.

In this study, we examined differences in rCBF between patients with MDD and healthy controls by a recently developed method, pseudo-continuous ASL (pCASL), taking the regional cerebral gray matter volume into account (Dai et al., 2008; Wu et al., 2007). In addition, depressive features or syndromes are often manifested in patients with schizophrenia. It has been estimated that depression is manifested in 21 to 74% of acute patients with recent onset schizophrenia and in 13 to 50% of patients with chronic schizophrenia, while depressive features have been found in even higher rates, up to 80%, in patients with schizophrenia (Kollias et al., 2008). So it would be useful if there is a method to differentiate between MDD and schizophrenia by using a neuroimaging method. We previously reported MRI-pCASL study on schizophrenia that showed a significant reduction of rCBF in the left inferior frontal cortex (Ota et al., 2014). So we evaluated rCBF of MDD patients in comparison with that of schizophrenia patients as well.

2. Methods

2.1. Participants

Subjects were 27 patients with MDD, 42 patients with schizophrenia and 43 age- and gender-matched healthy subjects. The subjects partially overlapped with those in the previous report (Ota et al., 2014). A consensus diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) criteria (American Psychiatric Association, 1994), by a research psychiatrist (MO, HH, or TT). The MDD patients were rated with Hamilton Depression Rating scale (HAM-D) for their depressive symptoms (Hamilton, 1960), and the schizophrenic patients were done with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). MDD patients who were in remission, as defined by the total score on the HAM-D of less than 8, were not enrolled in the study (Mayberg et al., 2005). Daily doses of antidepressants were converted to imipramine equivalents, and daily doses of antipsychotics including depot antipsychotics were converted to chlorpromazine equivalents using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999).

Controls were recruited from the community through local magazine advertisements and our website announcement. These participants were interviewed for enrollment by a research psychiatrist using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998). Participants were excluded if they had a prior medical history of central nervous system disease or severe head injury, or if they met the criteria for substance abuse or dependence. Those individuals who demonstrated a history of psychiatric illness or contact with psychiatric services were excluded from the control group.

After the study was explained to each participant, his or her written informed consent was obtained for participation in the study. This study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. MRI data acquisition and processing

Imaging was performed on a 3-T MR system (Philips Medical Systems, Best, the Netherlands). 3D T1-weighted images and pCASL images were acquired as the same parameter as described previously (Ota et al., 2014). For measurement of the magnetization of arterial blood and also for segmentation purposes, an EPI M0 image was obtained separately with the same geometry and the same imaging parameters as the pCASL without labeling.

2.3. Postprocessing of the ASL data

Because the pCASL and M0 images were acquired separately, the image signal intensities of both were corrected for data scaling. Corrected data were transferred to a workstation and analyzed using ASLtbx software (Wang et al., 2008) running on statistical parametric mapping 5 (SPM5). For the rCBF calculations, we added the attenuation correction for the transversal relaxation rate of gray matter to the original equation. Details of this process are described elsewhere (Ota et al., 2013a).

The mean rCBF image derived using the ASLtbx software contained some patchy noise, and thus we used a median filter (a nonlinear digital filtering technique). In median filtering, the neighboring pixels are ranked according to their intensity, and the median value becomes the new value for the central pixel. Since the slice gap that we used was somewhat large, simple 2D median filtering (3 voxels × 3 voxels) was used. To evaluate rCBF voxel-basically, we normalized the mean rCBF images to the standard space. First, each individual 3D-T1 image was coregistered and resliced to its own M0 image. Next, the coregistered 3D-T1 image was normalized to the "avg152T1" image regarded as the anatomically standard image using with the DARTEL (diffeomorphic anatomical registration using exponentiated lie) registration method (Ashburner, 2007). Finally, the transformation matrix was applied to the mean rCBF images treated with the median filter. The spatially normalized images were resliced with a final voxel size of approx. 4 × 4 × 8 mm. Each map was then spatially smoothed with a 4-mm full-width at half-maximum Gaussian kernel in order to decrease spatial noise and compensate for the inexactitude of normalization.

2.4. Statistical analysis

Statistical analyses were performed using SPM5 software. Differences in rCBF among 3 diagnostic groups were assessed using the age and gender as non-imaging nuisance variables and the individual normalized gray matter volume image as an imaging nuisance covariate using Biological Parametric Mapping (BPM) (Casanova et al., 2007). Only differences that met the following criteria were deemed significant: a seed level of $p < 0.05$ (false discovery rate [FDR] correction for multiple comparisons) and a cluster level of $p < 0.05$ (uncorrected).

3. Results

Demographic and clinical characteristics of the participants are shown in Table 1. There was no significant difference in age or gender among the 3 diagnostic groups.

There were significant rCBF reductions in the ACCs and right inferior prefrontal cortex in the patients with MDD compared with the controls (Fig. 1). We found significant rCBF reductions in the ACC, bilateral prefrontal cortex, left superior temporal cortex, and bilateral occipital cortex in the patients with schizophrenia compared with the controls, which is consistent with the results of our previous study (Fig. 2) (Ota et al., 2014). When the 2 patient groups were compared, the MDD patients showed significantly lower rCBF in subgenual ACC (Fig. 3A) and higher rCBF in left occipital cortex (Fig. 3B) compared with the schizophrenic patients.

Table 1
Demographic and clinical characteristics of subjects.

Variable	MDD $n=27$			Schizophrenia $n=42$			Healthy subjects $n=43$			p Value
	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Gender (M:F)	13	:	14	22	:	20	21	:	22	0.93
Age (years)	38.9	±	9.9	39.2	±	11.5	37.4	±	12.7	0.75
Antidepressant medication (mg/day)[a]	####	±	75.7							
Antipsychotic medication (mg/day)[b]				627.1	±	503.0				
HAM-D	16.6	±	7.3							
PANSS total				63.4	±	18.9				

MDD; major depressive disorder.
HAM-D; Hamilton's rating scale for depression.
PANSS; positive and negative synptom scale.
[a] Impramine equivalent.
[b] Chlorpromazine equivalent.

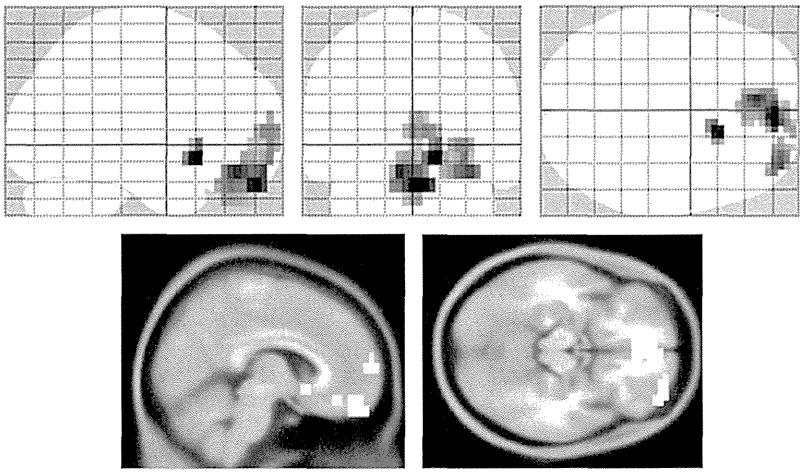

Fig. 1. Regional cerebral blood flow (rCBF) changes in major depressive disorder (MDD). There were significant reductions of rCBF in the right inferior prefrontal and anterior cingulate cortices (ACCs) compared with healthy subjects ($p < 0.05$, (false discovery rate [FDR])).

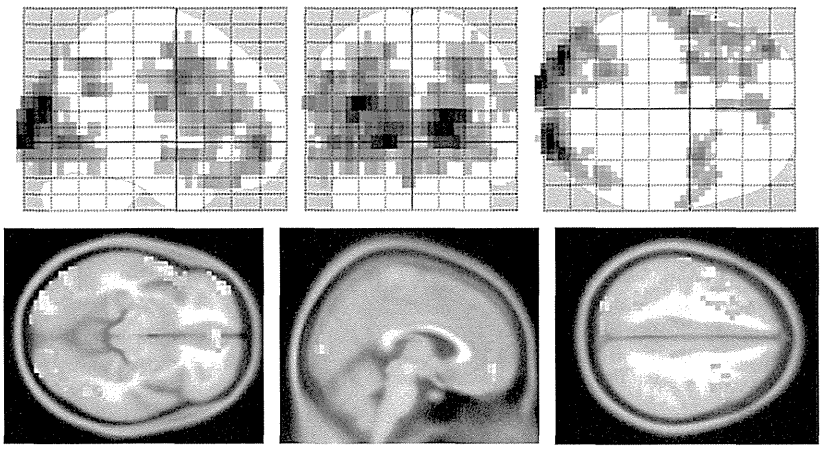

Fig. 2. Regional CBF changes in schizophrenia. There were significant reductions of rCBF in the bilateral prefrontal and occipital cortices, left temporal cortex, and ACC compared with healthy subjects ($p < 0.05$, FDR).

4. Discussion

We examined rCBF changes inMDD patients compared with healthy subjects and schizophrenia patients. By using the pCASL method and the regional gray matter volume correction, we found significant changes of rCBF in cingulate and frontal regions in MDD patients. To our knowledge, this is the first study of ASL-based rCBF changes in MDD patients that took the regional gray matter volume into account. In addition, we found differences in rCBF between MDD and schizophrenia patients by using this method.

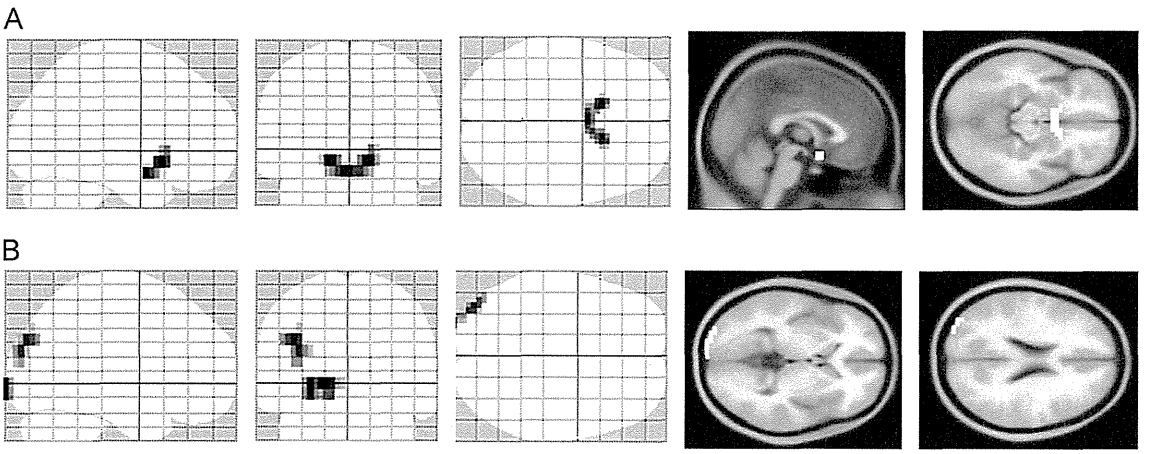

Fig. 3. Comparison of rCBF between the patients with MDD and schizophrenia. The MDD patients showed the lower rCBF in the subgenual ACC (A) and the higher rCBF in left occipital region (B) than schizophrenic patients. ($p < 0.05$, FDR).

Many MRI studies have focused on structural brain volume changes in MDD (reviewed in Arnone et al., 2012; Bora et al., 2012), and some studies have reported altered functions by using ASL (Ho et al., 2013; Lui et al., 2009; Walther et al., 2012). In addition, studies using PET and SPECT found that patients with MDD have decreased rCBF and metabolism (see review; Fitzgerald et al., 2008). We observed reduction of rCBF in the inferior prefrontal cortex in the MDD relative to healthy subjects. These findings mirror previous ASL studies showing lower baseline frontal rCBF (Ho et al., 2013; Lui et al., 2009; Walther et al., 2012). Hypoactivity in frontal areas has been strongly linked to psychomotor retardation (Galynker et al., 1998; Bench et al., 1992), and impaired executive functioning (e.g., attention, working memory, and decision making) (Paelecke-Habermann et al., 2005; Fossati et al., 2002). Together with findings from these studies, our results suggest that reduced perfusion in these regions may be associated with some of the cognitive and motor symptoms found in MDD. Our finding of hypoperfusion in the ACC of MDD patients is consistent with several perfusion and metabolism studies of MDD (Fitzgerald et al., 2008; Ho et al., 2013; Walther et al., 2012). The ACC has been shown to be involved in the processing of emotion and motivation (Carter et al., 1999). Thus, dysfunction in the ACC may underlie some of the core affective symptoms seen in MDD. Specifically, previous studies have shown that there were structural (Coryell et al., 2005; Costafreda et al., 2009; Lee et al., 2011; Wagner et al., 2011) and functional (Drevets et al., 1997; Liotti et al., 2002) dysfunctions in the subgenual ACC of MDD. And some studies showed that such change in the subgenual ACC was noticeable not in schizophrenia but in MDD, compared with healthy subjects (Coryell et al., 2005; Ota et al., 2013b). In line, we detected the lower rCBF in the subgenual ACC of MDD patients than schizophrenic patients, which is consistent with the preceding results.

ASL studies of schizophrenia revealed several rCBF changes (Horn et al., 2009; Pinkham et al., 2011; Scheef et al., 2010; Walther et al., 2011). However, the results of these studies differ substantially. For the frontal and temporal cortices, three and two out of these four studies consistently reported reduced rCBF, which is compatible with our results. We found rCBF reduction in bilateral occipital cortices of the individuals with schizophrenia, which is consistent with the study by Pinkham et al. (2011) in medicated schizophrenic patients and the study by Scheef et al. (2010) in drug-free subjects. Several studies obtained evidence of deficits of schizophrenia in visual processing, using electroencephalography (EEG) (Butler et al., 2001, 2005; Doniger et al., 2002), and other studies documented the abnormal EEG activities in the occipital lobe of patients with schizophrenia (Spencer et al., 2003, 2004). Thus, it seems likely that the occipital lobe is involved in some aspects of the pathophysiology of schizophrenia.

There was limitation in this study. The abnormalities of rCBF in MDD were known to reverse during symptom remission (see review; Drevets, 2000). Our MDD patients showed the HAM-D score of > 8, depressed non-remitters, and they showed the lower rCBF than healthy subjects. Further study with follow-up period that provides the information about the response to therapy would bring the prediction about the treatment response.

In conclusion, our pCASL study with partial volume effect correction demonstrated hypoactivity in the right inferior prefrontal area and cingulate cortex in MDD patients. The rCBF reduction in the subgenual region was specific in MDD patients compared with not only the healthy subjects, but also with schizophrenic patients. This point may provide objective biological information pertaining to the clinical diagnosis of schizophrenia and MDD. Finally, the present study demonstrate that the MRI with pCASL method is a promising tool to detect rCBF abnormalities controlling for gray matter volume in psychiatric disorders.

Role of funding source

The founding source had no involvement.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgement

This study was supported by Health and Labor Sciences Research Grant (Comprehensive Research on Disability, Health, and Welfare) (M.O. and H.K.), Intramural Research Grant (24-11) for Neurological and Psychiatric Disorders of NCNP (M.O. and H.K.), and "Understanding of molecular and environmental bases for brain health" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.K.).

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Clinical & Experimental Neuroimmunology

Clinical and Experimental Neuroimmunology **5** (2014) 137–148

REVIEW ARTICLE

Towards understanding the role of orphan nuclear receptor NR4A2 in Th17 cell-mediated central nervous system autoimmunity: An experimental approach using an animal model of multiple sclerosis

Shinji Oki

Department of Immunology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

Correspondence
Shinji Oki, PhD, Department of Immunology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan.
Tel: +81-42-341-2711
Fax: +81-42-346-1753
Email: soki@ncnp.go.jp

Received: 31 March 2014; revised: 20 April 2014; accepted: 21 April 2014.

Abstract

Although details of its pathogenesis remain elusive, multiple sclerosis (MS) is now widely accepted as an autoimmune disease of the central nervous system (CNS) in which autoreactive helper T cells play a pivotal role in triggering pathogenic cascades. Recently developed drugs and ongoing clinical trials clearly reflect the significance of targeting pathogenic immune cells, such as T helper 17 (Th17) cells, for MS treatment. Through comprehensive gene expression profiling analysis, we previously showed that the orphan nuclear receptor, NR4A2, is selectively upregulated in peripheral blood T cells from relapsing–remitting MS patients. Furthermore, using experimental autoimmune encephalomyelitis, an animal model of MS, we have shown that NR4A2 is selectively upregulated in peripheral blood T cells and T cells from inflamed CNS tissues. T cells expressing NR4A2 *in vivo* were induced only when immunized with self-peptide, not with irrelevant exogenous peptides. Accordingly, interleukin-17 (IL-17)-producing helper T cells exclusively express NR4A2, whether or not they secrete interferon (IFN)-γ, suggesting that NR4A2-expressing T cells represent a pathogenic Th17 subset during autoimmunity. Therefore, NR4A2 could be a useful biomarker to estimate pathogenic Th17 cell behavior in MS patients. In addition, a blockade of NR4A2 expression in differentiating Th17 cells with small interfering RNA not only abolished IL-17 secretion, but also Th17-related genes, such as IL-21, c-Maf and IL-23 receptor. Finally, *in vivo* administration of NR4A2-specific small interfering RNA significantly ameliorated experimental autoimmune encephalomyelitis, implying that NR4A2 is essential for triggering MS/experimental autoimmune encephalomyelitis, and could serve as a novel therapeutic target of the diseases. (Clin. Exp. Neuroimmunol. doi: 10.1111/cen3.12128, May 2014)

Introduction

Multiple sclerosis (MS) is a complex disease of the central nervous system (CNS) in which inflammatory and neurodegenerative processes cause intermittent neurological disorder and subsequent progression of debilitating symptoms. In general, MS is categorized into several major disease forms, including the most common form, relapsing–remitting MS (RR-MS), which sometimes exacerbates into secondary progressive MS (SP-MS), and the less frequent form primary progressive MS (PP-MS).[1] Although the etiology and specific causes of MS are not well understood, the susceptibility for individuals to develop MS could be attributed to both genetic (e.g. disease susceptibility genes) and environmental (e.g. external and internal environmental microorganisms) factors. Recent genome-wide association studies (GWAS) have identified many potential risk loci and multiple variants that might have a key role

in disease susceptibility.[2,3] Of note, these studies highlight a number of immunologically relevant genes, particularly those linked to helper T cell differentiation and function, suggesting the intrinsic participation of T cell components for MS pathogenesis.[4] As such, interest in interventions against autoreactive T cells to treat MS has grown, resulting in recently-developed therapies and ongoing clinical trials, such as those using natalizumab (humanized anti-α 4 integrin monoclonal antibody), fingolimod (FTY720) and glatiramer acetate, all of which are aimed to mitigate excessive immune responses of autoreactive T cells.

Organ-specific autoimmune diseases, including MS, emerge when autoreactive T cells primed in the periphery infiltrate into the CNS where reactivation of those T cells *in situ* initiates local damage and drives the recruitment of other inflammatory components (macrophages, B cells, granulocytes etc.). In early studies of MS, the major cause of organ-specific autoimmunity was believed to be the induction of immune responses by Th1 cells secreting interferon (IFN)-γ. However, recent studies have shown that autoimmune responses mediated by T helper 17 (Th17) cells secreting interleukin (IL)-17 might play key roles in the induction of autoimmune diseases. Accordingly, the role of Th17 cells in MS has been highlighted in recent years, and a large body of research has shown that such T cell responses could potentiate the pathogenesis of CNS-specific autoimmune inflammation; for example, elevated IL-17 responses and increased IL-17-secreting T cell numbers have been detected in MS patients, and correlate with active MS relapses.[5,6] Furthermore, Th17 responses have also been observed in the case of experimental autoimmune encephalomyelitis (EAE). Th17 responses appear to be critical for the induction of EAE as the severity of EAE was greatly reduced in mice lacking IL-23, IL-23R, IL-17 or IL-17R.[7–10] Interestingly, the CNS milieu in established EAE provides signals that preferentially drive Th17 responses.[11] As diverse sites, such as secondary lymphoid organs and peripheral circulation, as well as tertiary lymphoid-like structures, particularly those in the target organ (s), are involved in the development of organ-specific autoimmunity, the dynamics of the process by which pathogenic Th17 cells develop in EAE and MS have not yet been fully elucidated.

Through comprehensive gene expression profiling analysis, we previously showed that NR4A2, an orphan nuclear receptor that plays a versatile role in many aspects of biological and pathological responses, is selectively upregulated in the peripheral blood T cells of RR-MS patients in remission compared with healthy subjects.[12] Using EAE, an animal model of MS, we have further shown that NR4A2 is selectively upregulated in both T cells isolated from peripheral blood and those infiltrating into the CNS, but not from T cells in secondary lymphoid organs, such as the spleen and draining lymph nodes.[13,14] Here, the possible link between Th17 cells expressing NR4A2 and their pathogenic properties for CNS autoimmunity is summarized, the molecular mechanism of NR4A2-mediated Th17 cell differentiation is discussed, and the potential clinical application of NR4A2 as a novel therapeutic target for CNS autoimmunity with experimental data using small interfering RNA (siRNA) targeting the NR4A2 gene is suggested.

Th17 cells and EAE, an animal model of MS

Naive CD4+ T cells differentiate into Th1 cells on antigenic exposure in the presence of IL-12, and, once differentiated, Th1 cells maintain their phenotype even in different cytokine milieus, suggesting the relative robustness of the Th1 phenotype. In contrast, inflammatory processes crucial for Th17 differentiation are currently less understood. Originally, Th17 differentiation *in vitro* was observed after stimulation of naive T cells in the presence of transforming growth factor (TGF)-β in combination with IL-6.[15,16] However, IL-21 has also been reported to act as a differentiation factor by supporting the expansion of developing Th17 cells in an autocrine manner, and as an inducer of IL-23R expression on Th17 cells.[17] In that case, IL-23 could act as a critical factor for the stabilization and maturation of the phenotype of Th17 cells that express IL-23R on their cell surface.[18] In addition, a combination of different cytokines, such as IL-1β, IL-6 and IL-23, in the absence of TGF-β induces differentiation of IL-17-producing cells that apparently have an increased pathogenicity compared with conventional Th17 cells obtained by cultures with TGF-β and IL-6.[19] Therefore, it is not necessarily clear which differentiation pathway is critical for the physiological emergence of pathogenic T cells secreting IL-17 *in vivo*, and all of the factors described here could contribute to the differentiation of those cells to a greater or lesser extent.

It is well known that Th17 cells express the master transcriptional regulator retinoic acid-related orphan receptor γt (RORγt), and the deletion of the RORγt gene leads to impaired Th17 cell differentiation and reduces the severity of EAE development.[16] Pheno-

typically, Th17 cells show a greater degree of context-dependent plasticity[20,21] associated with a higher *in vivo* survival and self-renewal capacity.[22] Using fate-reporter animals, IFN-γ producing T cells that appeared in the CNS of EAE mice were shown to be almost exclusively derived from cells that formerly produced IL-17.[23] The conversion of IL-17-producing T cells into IFN-γ producers during EAE appears to confer an increased pathogenic phenotype to those T cells, accompanied by downregulation of RORγt and upregulation of T-bet. This suggests that the tracking of RORγt expression in T cells is not necessarily enough to identify ongoing pathogenic responses resulting from Th17 cells in CNS autoimmunity. In addition, the development of conventional Th17 cells and distinct T cell subsets producing IL-17 cells requires RORγt. Deletion of RORγt results in impaired differentiation of all types of IL-17-producing T cell subsets, as well as the impaired development of other newly-identified immune cell subsets, lymphoid tissue inducer (LTi) cells and a part of type 3 innate lymphoid cells (ILC3) that also express RORγt.[24,25] It is conceivable that not all of those RORγt-positive T cells producing IL-17 are necessarily involved in pathogenesis of CNS autoimmunity. Therefore, identification of novel molecular marker(s) that exclusively represent pathogenic IL-17-producing T cells is highly desirable.

EAE is a prototype autoimmune disease model that has greatly contributed to elucidating the pathogenesis of MS.[26] EAE can be induced in laboratory animals by active immunization with myelin antigens or by passive transfer of myelin antigen-reactive T cells. Th1 cells reactive to myelin basic protein (MBP), proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG) are capable of inducing clinical and pathological manifestations of EAE after transfer into naive mice; thus, Th1 cells producing IFN-γ have long been believed to play a central role in the pathogenesis of MS. However, the "Th1 disease" dogma has been challenged by contradicting results showing that gene-targeted mice deficient in IFN-γ[27,28] or IFN-γ receptor, and mice deficient for IL-12 signaling, are still susceptible to EAE. Subsequently, it was shown that IL-23, and not IL-12, is essential for the development of EAE,[7] resulting in the identification of pathogenic IL-23-dependent Th17 cells that produce the unique inflammatory cytokine, IL-17.[29] Currently, it is widely accepted that Th17 cells play an important role in the development of inflammatory autoimmune diseases either independently or collaboratively with Th1 cells.

What is NR4A2?

NR4A2, also known as Nurr1, is a nuclear receptor family member that, to date has no known endogenous ligand (Fig. 1). The members of the NR4A subfamily (NR4A1/Nur77, NR4A2/Nurr1 and NR4A3/NOR-1) are mostly expressed at very low levels in a wide variety of metabolically-demanding and energy-dependent tissues, such as skeletal muscle, adipose tissue, heart, kidney, liver and brain.[30] On particular stimulation, high levels of NR4A expression are induced in these tissues, reminiscent of immediate early genes. The diversity of signals that lead to this expression suggests that NR4A2 functions in a manner highly dependent on cell type and context. NR4A2 is primarily expressed in the CNS, particularly in the cortex, ventral midbrain, brain stem and the spinal cord, and it appears to have important functions in both the development and specific responses of dopaminergic neurons.[31,32] Therefore, many studies regarding NR4A2 have focused on the functional analysis of NR4A2 and its relevance to the pathology of Parkinson's disease. Indeed, mutations in the NR4A2 gene are well known to be associated with familial Parkinson's disease, reflecting the essential role for NR4A2 in the development and survival of neuronal organization of substantia nigra.[33,34] In contrast, much less attention has been paid to the functional role of NR4A2 in T cells. More than a decade ago, NR4A1 and NR4A3 were shown to mediate apoptotic processes of mature and immature T cells.[35–37] However, these studies do not provide insight into the functional implications of upregulated expression of NR4A2 in T cells. Recently, NR4A2 has come into the spotlight as a pivotal pathogenic component for modification of inflammatory milieus of rheumatoid arthritis, atherosclerosis and cancer, which will be discussed later in more detail.[38,39] Conversely, NR4A2 expression has also been implicated in reducing immune responses, including a potential role in neuroprotection from inflammation and repression of matrix metalloproteinases in joint inflammation, suggesting diverse roles for this transcription factor that are altered in a cell-type and context-dependent manner.[40,41]

Nuclear receptors are composed of several conserved functional domains including DNA-binding domain (DBD) with two zinc fingers in the N-terminal region of the molecule and the ligand-binding domain (LBD) in the C-terminal region with a less conserved structure. In the absence of specific ligands, most of the nuclear receptors are inactive by

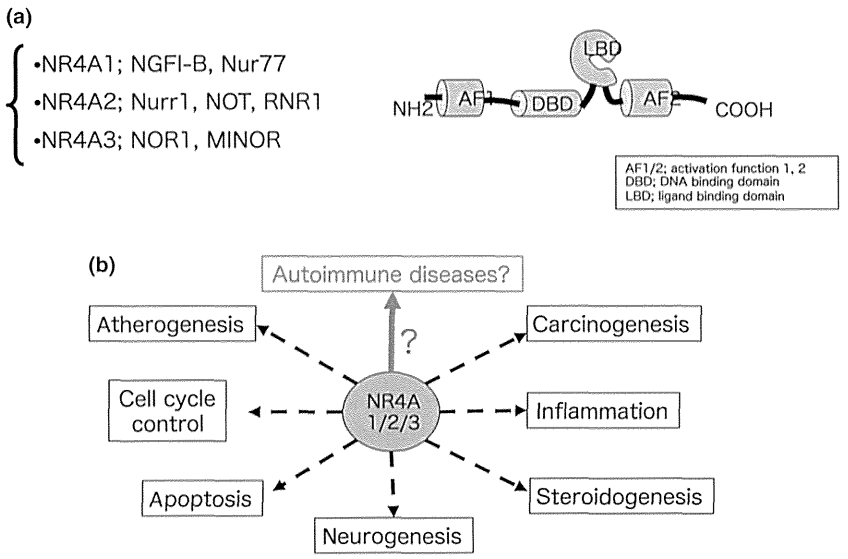

Figure 1 Versatile function of NR4A2 in a variety of biological and pathological responses. (a) Members of the NR4A family of nuclear receptors and their typical molecular structure. (b) Schematic summary of the organ and tissue-specific biological and pathological roles of the NR4A2.

interactions with co-repressor proteins. On ligand binding to a hydrophobic cleft in the LBD, a conformational repositioning occurs at the C-terminal amphipathic α-helix (H12) of the LBD that provides a well-defined surface (activation-function 2 [AF-2]) recognized by co-activator proteins, leading to the formation of multiprotein complex mediating gene activation, such as histone acetylation and chromatin modifications. However, NR4A2 encodes unusual and atypical LBD that lack canonical ligand binding properties.[42] Therefore, NR4A2 is believed to be a ligand-independent and constitutively active receptor, and its activity is tightly controlled at the level of transcription, post-transcriptional modification and multivalent complex formation with other molecules. The DNA-binding motif for the NR4A family members is the octanucleotide 5′-A/TAAAGGTCA (NGFI-B response element [NBRE]), where NR4A2 binds as monomers and homodimers. The pro-opiomelanocortin gene promoter contains another class of transcriptional targets for homodimers: Nur-responsive element (NurRE), with an inverted repeat, the NBRE-related octanucleatide, AAAT(G/A)(C/T)CA. NR4A1 and NR4A2 also bind as heterodimers with the retinoid X receptor (RXR) and bind a motif called DR-5. In addition, multivalent complex formation of NR4A2 with other transcription factors enables it to show non-canonical DNA binding.[43,44]

NR4A2-deficent neonates typically die at birth as a result of a severe defect in respiratory function despite having intact NR4A1/3 genes, suggesting a unique functional property for NR4A2.[45,46] Because of the selective expression of NR4A2 in the CNS, most of the target genes of NR4A2 known to date are limited to its role in this region. For example, NR4A2 is shown to play a role in the transcriptional activation of tyrosine hydroxylase involved in the synthesis of dopamine.[47,48] Another group of NR4A2 target genes reside in those relevant to bone formation, such as osteopontin and osteocalcin.[49,50] It is suggested that NR4A1 and NR4A3 are expressed in the thymus and mediate T cell receptor-mediated T cell apoptosis, but the distribution and function of NR4A2 in immune cells has not been extensively studied. Accordingly, a recent report showed that NR4A family proteins have essential roles in regulatory T (Treg) cell development by inducing promoter activity of the forkhead box P3 (Foxp3) gene;[51] however, NR4A2 itself was shown to be much less effective for transcription of the Foxp3 gene and subsequent Treg differentiation than other NR4A family members.[52] Meanwhile, a series of reports have suggested pivotal roles for NR4A family members, especially the NR4A2 subtype, in inflammatory responses, and they are aberrantly expressed in inflamed synovial tissue of patients with rheumatoid arthritis, psoriatic skin and atherosclerotic lesions. Therefore, NR4A receptors might contribute to the cellular processes that control inflammatory disorders including autoimmunity.

NR4A2 in MS

MS has an autoimmune pathology that is initiated by the development of autoimmune T cells reactive to myelin antigens, such as MBP, MOG and PLP. Immunologically, naïve T cells differentiate into

encephalitogenic T cells on encountering those with myelin autoantigens. Such encephalitogenic T cells must be preactivated in the periphery before they are able to penetrate into the CNS parenchyma.[53,54] Then, expansion of inflammatory processes within the CNS is triggered by pro-inflammatory cytokines and chemokines secreted by infiltrating autoreactive T cells after recognizing self-antigens in a major histocompatibility complex (MHC) class II-restricted manner in the CNS. Encephalitogenic T cells that generate the development of MS can be composed of both Th1 and Th17 cells, and the relative contributions of either of these distinct helper T cell populations might help explain the diversity of clinical and pathological manifestations, as well as the varying responses to therapy.[4] However, little is known about the helper T cell population responsible for the development of MS partly because of a lack of appropriate methodology to discriminate those encephalitogenic T cells. For example, although RORγt is a good marker to identify Th17 cells, the aforementioned complex behavior of RORγt expression in T cells, in addition to the fact that RORγt is also involved in lymphoid organogenesis, means that it is necessary to find new specific marker(s) that enable identification of pathogenic T cells in MS. Through comprehensive gene expression profiling analysis of RR-MS patients in remission, we previously showed that NR4A2 is selectively upregulated in peripheral blood T cells of MS patients.[12] Quantitative reverse transcription polymerase chain reaction analysis further revealed that NR4A2 expression in T cells from MS patients showed an approximately fivefold increase compared with healthy donors. We then applied an animal model of MS to further assess the role of the novel orphan nuclear receptor gene on T cell function.

NR4A2 in EAE

In EAE induced in C57BL/6 mice by immunization with a MOG_{35-55} peptide, NR4A2 was selectively upregulated in T cells of the peripheral circulation and those infiltrating into the CNS, but NR4A2 expression was not observed in T cells from secondary lymphoid organs, such as the spleen or draining lymph nodes.[13,14] In a kinetic analysis using reverse transcription polymerase chain reaction, we observed that NR4A2 expression in peripheral circulating T cells reached a maximum value 21 days after EAE induction, and the entire expression pattern of NR4A2 in peripheral blood T cells was well correlated with the clinical severity of EAE. Meanwhile, significant expression of NR4A2 was observed in the CNS-infiltrating T cells from day 9, when early signs of EAE become evident. These results suggest that NR4A2 expression was induced in T cells on induction of EAE, but the kinetics of expression significantly differs between peripheral blood T cells and CNS-infiltrating T cells. Recent studies have shown that autoimmune Th17 cells producing IL-17 play a central role in causing autoimmune inflammation,[55] and analysis of fate-reporter animals showed that those Th17 cells have a tendency to change their phenotype to become IFN-γ producing T cells in the CNS milieu of EAE mice.[23] Therefore, T cells accumulating in the CNS are characterized by massive production of those inflammatory cytokines along with significant expression of NR4A2. Accordingly, retroviral transduction of NR4A2 cDNA into splenic CD4+ T cells *in vitro* augmented production of IL-17 and IFN-γ on restimulation. Furthermore, NR4A2-expressing T cells in the CNS of EAE animals were accumulated in those producing IL-17 regardless of their secretion of IFN-γ (Fig. 2).[14] Therefore, NR4A2 might be considered as a useful marker to identify pathogenic Th17 cells in target organs or peripheral circulation. In addition, T cell expression of NR4A2 seems to have a strong link with exposure to a certain autoantigen, as *in vivo* induction of NR4A2 in T cells is observed only when immunized with self-peptide and not with a peptide of exogenous origin, such as ovalbumin. Interestingly, forced subcutaneous inflammation by intradermal injection of IL-23 causes upregulation of NR4A2 in peripheral blood T cells.[14] Therefore, T cell upregulation of NR4A2 emerges only after recognition of self-antigen that induces autoreactive IL-17-producing cells *in situ*.

It is noteworthy to point out that despite an apparent requirement for NR4A2 in Th17 differentiation in target organs, little NR4A2 expression was detected in secondary lymphoid tissues that are the proposed sites for T cell priming on encounter with self-antigens and the subsequent acquisition of a Th17 phenotype.[14] In addition, NR4A2 expression by CD4+ T cells was first observed in the target organ, much earlier than in peripheral blood T cells. Intriguingly, when autoreactive cells were induced by immunization with self-antigens in the absence of pertussis toxin administration, NR4A2 expression was not upregulated, possibly because of impaired access to target organs. Thus, the target organs, the CNS in the case of EAE, might represent the site of NR4A2 upregulation where pathogenic Th17 differentiation occurs *in vivo*, rather than during initial

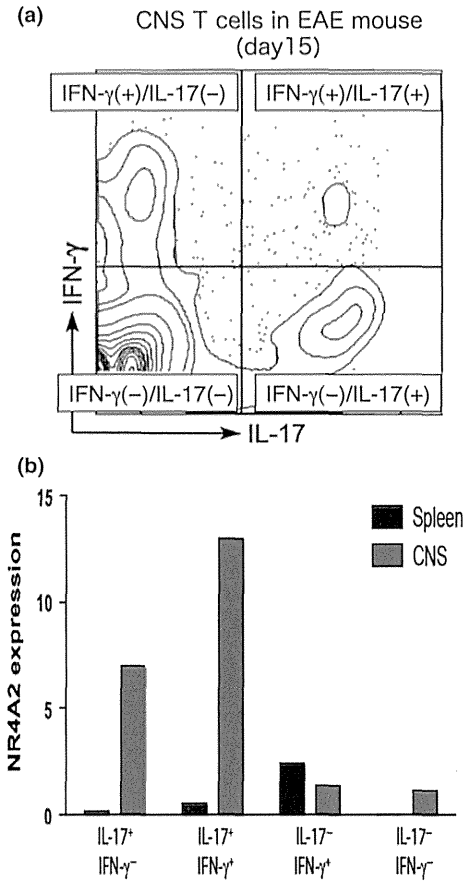

Figure 2 Distribution of cytokine-producing T cells in the central nervous system (CNS) of experimental autoimmune encephalomyelitis (EAE) mice and their expression of NR4A2. (a) There are distinct subsets of T cells accumulated in the CNS of EAE mice, composed of interleukin (IL)-17-producing T cells, interferon (IFN)-γ-producing T cells and double producers. (b) NR4A2 expression by subsets of cytokine-producing CD4+ T cells was analyzed using quantitative polymerase chain reaction at day 15 post-EAE induction for spleen and CNS-infiltrating cells.

T cell priming in the secondary lymphoid tissues. Cognate antigen interactions *in situ* are required to permit primed T cells moving from peripheral circulation to the CNS parenchyma.[53,54] Accordingly, primed encephalitogenic CD4+ effector T cells transferred into naive animals rapidly infiltrated CNS tissue, requiring MHC class II-dependent antigen presentation, whereas effector T cells specific for an irrelevant antigen did not enter CNS lesions.[54] Therefore, our data could suggest that active infiltration of encephalitogenic T cells primed in the periphery is not sufficient for generation of CNS autoimmunity despite the inflammatory potential of autoimmune responses and the potential T cell pathogenicity, and instead suggests that clinical induction of autoimmune disease is dependent on local reactivation of infiltrating T cells.[56] Furthermore, the differentiation processes of pathogenic T cells in the target organ under autoimmune conditions might enable the upregulation of NR4A2 in CD4+ T cells after reactivation by interactions with target organ antigen-presenting cells expressing CNS antigens, and the NR4A2-expressing T cells could fully represent activated pathogenic Th17 cells. Indeed, when NR4A2 expression is prevented by administration of NR4A2-specific siRNA *in vivo*, CNS-infiltrating T cells are still observed, albeit at lower numbers, but the Th17 responses in the target organ are markedly reduced with a reduction in clinical EAE. Again, although the importance of the activation of particular local responses has been previously suggested, our data shows that pathogenic Th17 responses in EAE result from a critical differentiation in the target organ.[11] The subsequent appearance of NR4A2 expression in the peripheral blood might represent T cells trafficking from, rather than to, the target organ and perhaps it is these T cells that are later reactivated after returning to the target organ and triggering disease relapses. Thus, NR4A2 might provide a cell marker identifying T cells, both in the target organ and circulatory systems, which have been reactivated during pathogenic inflammatory responses in the CNS. In addition, measurement and manipulation of NR4A2 could prove to be useful in clinical settings. As NR4A2 expression by peripheral blood CD4+ T cells is only observed after the initiation of inflammatory Th17 responses in the target organ, the presence of NR4A2 expression in the blood might be used to indicate when such responses have developed or that they are ongoing. It is conceivable that the status of immune activation in a target organ might be determined by measuring NR4A2 expression in a patient's blood. Thus, the use of NR4A2 as a biomarker for MS could indicate whether T cell infiltration into the target organ has been recently established, giving valuable insight into disease status.

Regulation of IL-21 by NR4A2 in autoimmunity

IL-21 is a pleiotropic cytokine primarily produced by activated T cells.[57] IL-21 plays a pivotal role in CD4+ T cell differentiation, the survival of both CD4+ and CD8+ T cells, and the effector function of cytotoxic T cells.[58–68] In addition, IL-21 is crucial for B cell survival and differentiation, leading to proper development of antibody-producing cells secreting mature immunoglobulins.[61] Intriguingly, IL-21 is shown to have a strong link to inflammation and autoimmune

diseases,[62] such as patients with systemic lupus erythematosus (SLE),[63,64] inflammatory bowel diseases (IBD)[65,66] and type 1 diabetes (T1D),[67,68] or in animal models for SLE,[69] IBD,[66] T1D[70] and rheumatoid arthritis (RA).[71] Although follicular helper T cells are known as professional IL-21-producing CD4+ T cells, IL-21 is also produced by Th17 cells.[72] The primary role for IL-21 in mouse Th17 cells is the expansion of developing Th17 cells.[17] IL-6 can induce the production of IL-21 by Th17 cells, and in turn, IL-21 acts in an autocrine manner to induce the expression of IL-23R on Th17 cells and further stabilize the Th17 phenotype.[73] The requirement of IL-21 for Th17 cell development *in vivo* is still controversial, as some reports show that Th17 cells can develop in the absence of IL-21,[74,75] whereas other reports show that the generation of Th17 cells is impaired in the absence of IL-21.[73,76,77] This is partly due to the redundant role of IL-6 and IL-21, in which Th17 cell differentiation *in vivo* under a strong inflammatory milieu with massive IL-6 production would conceal the effects of IL-21.[73,77] Interestingly, the amount of IL-21 production induced by Th17 differentiation is strongly reduced by transfection of NR4A2-specific siRNA into differentiating Th17 cells *in vitro*. We further showed that the sequential upregulation of IL-21 and c-Maf, followed by the induction of IL-23R and IL-17 transcripts during Th17 differentiation, was abolished when NR4A2 expression was prevented by siRNA treatment. Therefore, one consequence of preventing NR4A2 upregulation during Th17 differentiation *in vitro* is an absence of IL-21 secretion. The essential role of NR4A2 expression in Th17 differentiation through regulation of IL-21 is shown by the fact that the addition of exogenous IL-21 led to IL-23R upregulation, subsequently yielding normal IL-17 secretion. The precise mechanisms of the NR4A2-mediated regulation of IL-21 in Th17 cell development *in vivo* have not yet been elucidated, but it is postulated that NR4A2 might regulate T cell production of IL-17 *in vivo* by controlling signaling pathways intrinsic for effective Th17 cell differentiation (Fig. 3).

One obvious mechanism by which IL-21 drives autoimmunity is by supporting the expansion, promotion and survival of pathogenic helper T cell subsets. Accordingly, it is well known that autoimmune-prone mice produce more IL-21 compared with resistant strains,[59,68] and that the level of IL-21 production is well correlated to the progression of autoimmune diseases.[60] As aforementioned, IL-21 has a strong link to inflammation and organ-specific or systemic autoimmune diseases including SLE, IBD, T1D, and RA. Regarding the possible link of the

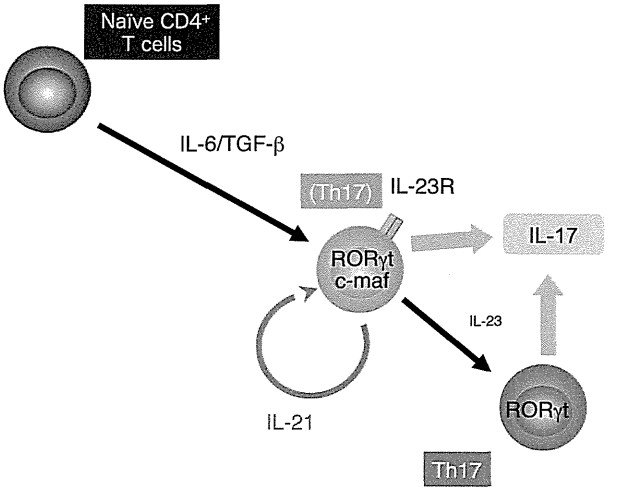

Figure 3 Multistep differentiation processes of T helper 17 (Th17) cells. T cell receptor stimulation of naive T cells in the presence of transforming growth factor (TGF)-β and interleukin (IL)-6 triggers initial Th17 cell differentiation. Th17 cells acquiring *c-Maf* expression produce IL-21, which augments Th17 cell amplification in an autocrine manner. IL-21 induces expression of the IL-23 receptor on the surface of differentiating Th17 cells that renders them responsive to IL-23. Exogenous IL-23 stabilizes the Th17 phenotype to secrete IL-17 and confers its effector function. RORγt, retinoic acid-related orphan receptor γt.

IL-21–Th17 axis to human CNS autoimmunity, such as MS, the proportion of memory Th17 cells and the IL-17 level are both shown to be much higher in patients with MS and neuromyelitis optica (NMO).[78] Accordingly, CNS-infiltrating cells expressing IL-21 were observed in both acute and chronic active white matter MS lesions in which IL-21 expression was restricted to CD4+ helper T cells.[6] Furthermore, therapeutic treatment with alemtuzumab causes secondary autoimmunity in a subset of MS patients who selectively show higher levels of serum IL-21, possibly through excessive T cells apoptosis and cell cycling after alemtuzumab-mediated lymphocyte depletion.[79] Interestingly, there are a couple of reports suggesting a significant correlation between IL-21 and NMO. First, production of IL-6 and IL-21 by CD4+ T cells *ex vivo* is shown to be directly associated with neurological disability in NMO patients.[80] In addition, higher concentrations of serum IL-21 were observed in NMO patients,[81] and concentrations of IL-21 protein in cerebrospinal fluid were significantly elevated in NMO patients, suggesting a positive correlation with humoral immunity.[82] Therefore, regulation of the IL-21–Th17 cell axis through NR4A2-mediated intervention holds considerable significance not only for MS, but also for NMO and other related neuroimmunological diseases.

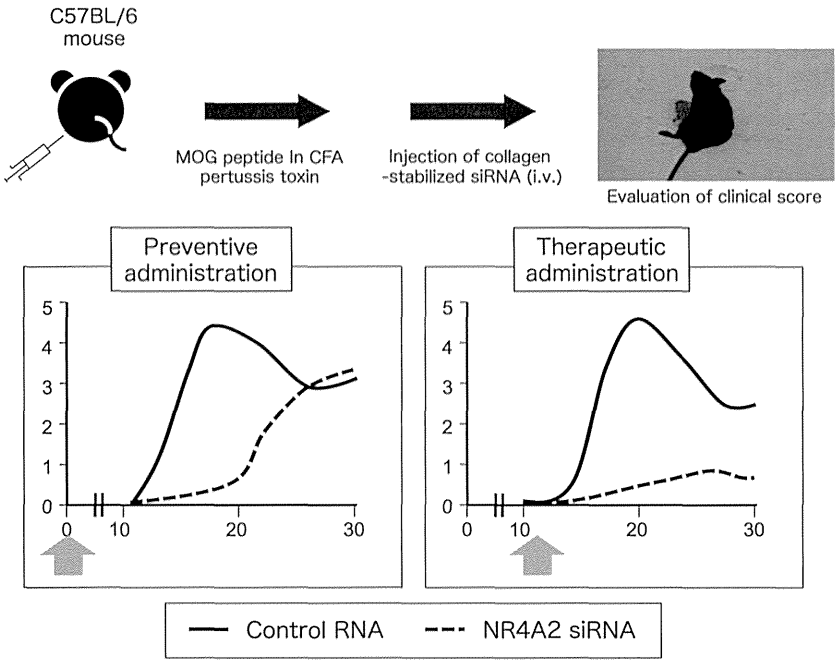

Figure 4 Effect of systemic administration of NR4A2-specific small interfering RNA (siRNA) on experimental autoimmune encephalomyelitis (EAE). NR4A2-specific or control siRNA was stabilized in a collagen matrix and administered intravenously to EAE mice either at the time of (preventive administration) or 10 days after (therapeutic administration) myelin oligodendrocyte glycoprotein (MOG) immunization. CFA, complete Freund's adjuvant.

NR4A2 as a possible target for MS therapy

NR4A2 expression is associated with the generation of autoimmune Th17 responses, and NR4A2 appears to control Th17 differentiation. We then aimed to prevent NR4A2 upregulation *in vivo* by systemically administering NR4A2-specific siRNA stabilized in a collagen matrix.[14] Both clinical EAE and NR4A2 expression in T cells were significantly reduced, with peak disease delayed by 10 days (Fig. 4). In addition, IL-17 production, but not IFN-γ production by CD4+ T cells infiltrating the CNS, was also reduced. NR4A2 siRNA-treatment prevented the accumulation of CD4+ T cells, particularly those secreting IL-17 into the CNS. Furthermore, equivalent numbers of IL-17-producing T cells in the CNS were observed between the control and the NR4A2 siRNA-treated mice at the delayed onset of clinical EAE. The late onset of EAE could be attributed to degradation of the siRNA, as administration of the siRNA at the onset of EAE significantly prevented the induction of clinical EAE. These findings suggest that the absence of NR4A2 expression during active autoimmune disease reduces clinical symptoms of EAE and Th17 responses. Therefore, NR4A2 might prove to be a potent therapeutic target for the treatment of MS and other Th17-mediated autoimmune diseases.

It is well known that methotrexate significantly suppresses expression of NR4A2 in patients with active psoriatic arthritis.[83] Accordingly, the expression level of NR4A2 after treatment with methotrexate is well-correlated to the disease activity score. Therefore, intervention of NR4A2 activity with chemical compounds might provide a potential strategy for future treatment of MS. In addition, the fact that NR4A2 mutations are associated with familial Parkinson's disease has led to significant interest in the identification of selective low-molecular-weight modulators that are helpful for analyzing the mode of action of the NR4A subfamily.[43] A growing number of NR4A2 modulators with unique chemical structures have also been described.[84–87] Furthermore, the antineoplastic and anti-inflammatory drug, 6-mercaptopurine, has been shown to activate NR4A2 through modulation of the cellular content of purine nucleotides.[88] A number of typical and atypical antipsychotic drugs, such as haloperidol, chlorpromazine, clozapine and so on, induce the transcription of NR4A2, even though they are all developed to augment NR4A2 activity.[89,90] Therefore, therapeutic application of NR4A2 inhibitors or NR4A2 modifiers converted from those NR4A2 activators through modification of chemical structure might be considered for possible future treatment of RR-MS.

Future perspective

EAE is a versatile experimental model useful for analyzing the immunopathological, neuropathological and therapeutic aspects of MS, including inflam-

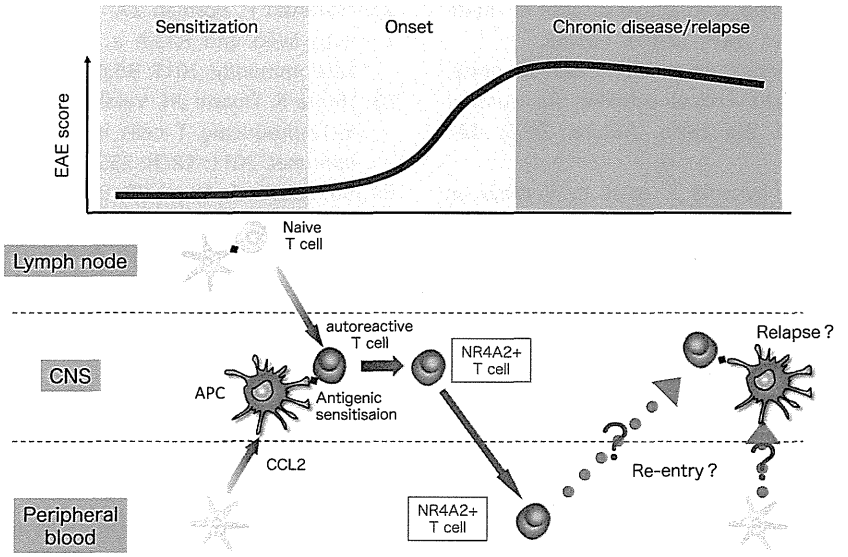

Figure 5 Possible behavior of NR4A2-expressing T helper 17 (Th17) cells during the course of multiple sclerosis/experimental autoimmune encephalomyelitis (EAE). On encountering the myelin antigen, naive T cells are primed to differentiate into effector T cells, such as Th17 cells in secondary lymphoid tissue. Then, those effector T cells are recruited to the central nervous system (CNS) and restimulated with antigen presented by local antigen presenting cells, resulting in upregulation of NR4A2 expression in pathogenic Th17 cells. Th17 cell-mediated local inflammation causes recruitment of inflammatory effector cells to the CNS, leading to immunopathogenic symptoms of multiple sclerosis/EAE. The subsequent appearance of NR4A2 expression in the peripheral blood could represent T cells trafficking from the target organ. At the later phase of EAE, T cells might egress the target organ to peripheral circulation and perhaps it is these T cells that are reactivated thereafter in the target organ triggering disease relapses. Therefore, NR4A2 could provide a good biomarker for identifying pathogenic T cells in both the target organ and in circulation. APC, antigen-presenting cells; CCL2, chemokine (C-C motif) ligand 2.

mation, demyelination, axonal damage, and after gliosis, the resolution of inflammation, remyelination and drug screening. Given the data showing that NR4A2 is selectively upregulated in the peripheral blood T cells of RR-MS patients, we have shown a strong link between NR4A2-expressing Th17 cells and their pathogenic role in CNS autoimmune inflammation through the analysis of EAE (Fig. 5), suggesting that NR4A2 represents a promising therapeutic target for MS and other autoimmune diseases. In addition, there are other inflammatory CNS diseases with distinct, but overlapping with the RR-MS, phenotype, such as NMO, progressive forms of MS and related demyelinating diseases.[91] Therefore, further analysis of RR-MS as an NR4A2-expressing Th17-mediated autoimmune disease will provide helpful clues for understanding the pathogenesis of CNS autoimmunity.

Acknowledgement

This work was supported by grants from the Ministry of Health, Labor, and Welfare of Japan (JSPS KAKENHI, Grant Number 24590497), and a research grant on "Super Special Consortia" for supporting the development of cutting-edge medical care from the Cabinet Office, Government of Japan.

Conflict of interest

None.

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