Journal of Neuroimmunology 276 (2014) 195–201

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Immunological effects of methylprednisolone pulse treatment in progressive multiple sclerosis

R. Ratzer *, J. Romme Christensen, B. Romme Nielsen, P.S. Sørensen, L. Börnsen, F. Sellebjerg

Danish Multiple Sclerosis Center, Rigshospitalet, University of Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 8 August 2014
Accepted 21 August 2014

Keywords:
Progressive multiple sclerosis
Methylprednisolone
CCR2
CD38
CD80
CD70
CD27

ABSTRACT

Objective: To investigate the effect of monthly oral methylprednisolone pulse treatment in progressive MS.
Methods: 30 progressive MS patients were treated with oral methylprednisolone every month. Peripheral blood mononuclear cells were analyzed by flow cytometry.
Results: Out of 102 leukocyte phenotypes investigated, 25 changed at nominal significance from baseline to week 12 ($p < 0.05$). After correction for multiple comparisons, we found 5 subpopulations that changed compared to baseline. No pattern were suggesting modulation of Th17 or TFH cells.
Conclusion: Methylprednisolone pulse treatment has some effects on circulating immune cells but does not modulate markers of Th17 and TFH cell activity in progressive MS.

1. Introduction

Multiple sclerosis (MS) is considered an autoimmune disease in which inflammation in the central nervous system (CNS) is mediated by autoreactive immune cells, and treatments targeting immune cells are efficacious in relapsing–remitting MS (RRMS). T cells are thought to play a pivotal role, but B cells and innate immune cells are also involved in the MS-pathogenesis (Sospedra and Martin, 2005; Weiner, 2008; Korn et al., 2009; Lassmann, 2013).

Untreated, the majority of patients with RRMS will develop a secondary progressive disease course (SPMS) with steady accumulation of neurological disability, while approximately 15% of patients have a primary progressive disease course (PPMS) from onset (Confavreux et al., 2000). The extent to which systemic immune activation and CNS inflammation are involved in the pathogenesis of PPMS and SPMS is a matter of debate. Some argue that neurodegenerative processes independent of inflammation in the CNS are crucial (Stys et al., 2012), but pathology studies in MS show that there is an ongoing inflammation even in the CNS of progressive MS patients (Frischer et al., 2009). A recent study from our group suggested that systemic immune activation involves activated follicular helper T cells (T_{FH}) and B cells that may play a central role in the immune pathogenesis of SPMS, and might be related to disease progression (Romme Christensen et al., 2013). In addition we found that patients with progressive MS in general showed evidence of increased activation of circulating T helper type 17 (Th17) cells. Other studies indicated increased systemic immune activation patients with progressive MS, including changes in cytokine expression and changes in the phenotype of circulating immune cells in progressive MS (Balashov et al., 1997; Sorensen and Sellebjerg, 2001; Karni et al., 2006; Ukkonen et al., 2007). Moreover, we found that treatment with natalizumab, which blocks recruitment of circulating lymphocytes to the CNS, reduces biomarkers of inflammation in the cerebrospinal fluid (CSF) and reduces biomarkers of tissue damage in patients with progressive MS (Romme Christensen et al., 2014).

Methylprednisolone is widely used to treat relapses in RRMS (Sellebjerg et al., 2005), and monthly pulse therapy with methylprednisolone used as add-on to interferon-beta treatment reduces the attack rate in RRMS patients, and may also be efficacious as monotherapy (Zivadinov et al., 2001; Sorensen et al., 2009; Ravnborg et al., 2010). Methylprednisolone has a wide range of effects on the immune system that may be important in MS, e.g. inhibiting T cell activation, dampening inflammatory cytokines, decreasing the extravasation of immune cells into the CNS or facilitating apoptosis of activated cells of the immune system (Sloka and Stefanelli, 2005).

* Corresponding author at: Danish Multiple Sclerosis Center, Department of Neurology, Section 2082, Rigshospitalet, DK-2100 Copenhagen, Denmark. Tel.: +45 20720504; fax: +45 35452626.
E-mail address: rikke.ratzer@rh.regionh.dk (R. Ratzer).

http://dx.doi.org/10.1016/j.jneuroim.2014.08.623
0165-5728/

We hypothesized that pathogenic systemic immune activation in patients with progressive MS could be influenced by monthly oral pulsed methylprednisolone. We used 8 color flow cytometry to study peripheral blood mononuclear cells (PBMCs) before and after twelve weeks of treatment with oral pulsed methylprednisolone in patients with PPMS and SPMS who participated in a phase 2A proof-of-concept clinical trial.

2. Material and methods

2.1. Patient material

Patients were recruited from August 2011 to May 2012 and 30 progressive MS patients (15 with SPMS and 15 with PPMS) were assigned to treatment with monthly oral methylprednisolone 500 mg daily for 3 days every fourth week. Inclusion criteria were patients diagnosed with progressive MS following the Lublin and Reingold criteria (Lublin and Reingold, 1996). Patients were aged 18–65 years and had an Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) score of 6.5 or less and had progressed at least 1 EDSS point the last 2 years before inclusion (0.5 if EDSS was higher than 5.5) or had progressed at least 2 functional system (FS) points (either 1 point in 2 independent FS scores or 2 points in 1 FS system). Two of the SPMS patients had a relapse in the year preceding inclusion in the study. None of the patients had received immunomodulatory or immunosuppressive drugs for at least 6 months prior to baseline (Supplementary Table 1 for detailed inclusion and exclusion criteria). The study was designed as a single center study and approved by the local ethics committee (protocol number H-4-2011-006) was registered at clinicaltrials.gov (NCT 01305837), and written informed consent was obtained from all study participants. Table 1 shows patient demography and clinical characteristics of the patients included in the study.

Methylprednisolone (Medrol, Pfizer, Denmark) was administered as 5 100 mg tablets once daily for three days every fourth week for 60 weeks (full length of the study). Concomitantly, the patients took vitamin D and calcium supplements (Calcichew-D3 Forte, Takeda Pharma, Denmark) containing 500 mg elemental calcium and 10 μg vitamin D3 to maintain bone mineral density, and the patients continued to take their previous medications.

2.2. Sample collection and flow cytometry

Blood was drawn at baseline and at week 12 in BD Vacutainer EDTA tubes (BD Biosciences, Denmark). At week 12 blood was drawn approximately 4 weeks after the latest administration of methylprednisolone. PBMCs were isolated by density gradient centrifugation of blood samples over Lymphoprep (Fresenius Kabi, Norway) and the interphase was collected and washed twice with phosphate buffered saline (PBS). PBMCs were incubated with fluorochrome-conjugated monoclonal antibodies as described in detail in Supplementary Table 2. We ran the samples on a FACSCanto II flow cytometer and the data analysis was done using FACSDiva Software (BD Biosciences, USA). A panel of CD4+ and CD8+ T cells, B cell, myeloid and plasmacytoid DC and monocyte populations also investigated in a previous study from our group were selected for the study (Romme Christensen et al., 2013).

Table 1
Patient demography and clinical characteristics. The values are given in mean (range).

MS type	SPMS	PPMS
Gender (women;men)	7;8	11;4
Age (years)	47 (33;56)	53 (44;61)
MS duration (years)	14.3 (4;36)	9.8 (3;24)
Duration of progression	6.1 (2;17)	9.8 (3;24)
MSSS	6.36 (3.29;9.08)	6.67 (3.29;9.08)
EDSS 2 years before inclusion	4.7 (2.5;6.5)	4.2 (3.0;6.5)
EDSS 1 year before inclusion	4.9 (3.0;6.5)	4.4 (3.0;6.5)
EDSS at baseline	5.4 (4.0;6.5)	5.2 (4.0;6.5)
MSFC at baseline	−0.175 (−1.188;0.852)	0.183 (−0.870;0.828)
MSIS at baseline	55 (28;90)	53 (27;109)

2.3. Clinical assessment

Clinical assessments at baseline and week 12 were done by the same neurologist (RR) who measured EDSS (Kurtzke, 1983), Multiple Sclerosis Impairment Scale (MSIS) (Ravnborg et al., 1997), and Multiple Sclerosis Functional Composite (MSFC) (Cutter et al., 1999; Fischer et al., 1999) including the subcomponents of the MSFC: timed 25 foot walk (T25FW), 9 hole peg test (9HPT) and Paced Auditory Serial Addition Test (PASAT). Multiple sclerosis severity scores (MSSS) were calculated from the EDSS and disease duration (Roxburgh et al., 2005).

2.4. Statistical analysis

Statistical analysis was performed using SPSS 19 software (IBM, USA) and SAS version 9.1.3 (SAS Institute Inc., USA). Differences were calculated as baseline values minus week 12 values. We used paired t-test to compare PBMC subsets at baseline and week 12 if differences were normal distributed and used Wilcoxon signed rank test when this was not the case. We used the false discovery rate (Benjamini and Hochberg, 1995) (FDR) to correct for multiple comparisons. The q-value indicates the percentage of variables below that specific value that are false positive findings. We considered a q-value below 0.05 to be significant.

3. Results

3.1. Flow cytometry studies

No difference was seen in total lymphocyte and granulocyte counts from baseline to week 12. Furthermore, we found no differences in percentages of CD4+ T cells, CD8+ T cells, B cells, monocytes, NK cells and dendritic cells (DCs) (mDCs and pDCs) from baseline to week 12 (Table 2).

We selected 102 PBMC subpopulations of interest and compared baseline to week 12 levels for these. These subpopulations were the same chosen for analysis in our previous study of immune activation in patients with progressive MS (Romme Christensen et al., 2013). Out of these, 25 subpopulations were found to change from baseline to week 12 at a nominal significance level ($p < 0.05$). Table 2 lists baseline levels of the 102 subpopulations and the difference from baseline to week 12.

After FDR correction for multiple comparisons we found that from baseline to week 12: the percentage of CCR2+ CD4+ T cells increased ($q < 0.03$); the percentage of CD80+ monocytes increased ($q < 0.03$); the percentage of CD70+ B cells decreased ($q < 0.03$) and the percentage of CD27+ B cells increased ($q < 0.03$); and the percentage of CD38+ mDCs decreased ($q < 0.03$). No difference between the PPMS and the SPMS group or between the sexes was found for these 5 variables (data not shown).

3.2. Correlation between clinical and immunological changes

We observed significant improvements in most clinical scores, except the PASAT, from baseline to week 12. Median EDSS scores improved from 5.0 at baseline (IQR 4.5–6.3) to 4.5 at week 12 (IQR 4.0;6.3) ($p < 0.006$). The MSIS improved with 6.5 points (IQR 3.5;10.5, $p < 0.0003$) and the MSSS changed with 0 points (IQR −0.6;0, $p < 0.002$). There was no significant change in the MSFC score from baseline to week 12, but 2 of the components (9HPT and T25FW) decreased significantly. The 9HPT decreased with 0.9 s (IQR −0.1;3.3) from 24.4 s (IQR 22.4 s; 35.2 s) ($p = 0.002$). The T25FW decreased with 0.6 s (IQR −0.2;2.2) from 8.9 s (6.5 s; 12.4 s) ($p < 0.018$). We