Expression of Chemokine Receptors on Th1/Th2 $CD4^+$ Lymphocytes in Patients with Multiple Sclerosis

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ABSTRACT

Background: Th1 cells preferentially express CXCR3, CCR5 and CCR6, while CCR3 and CCR4 are predominantly expressed by Th2 cell subsets. Multiple Sclerosis (MS) is a Th1 cell-dependant chronic inflammatory disease of the central nervous system, and immunomudolatory cytokines could alter the chemokine expression pattern of these lymphocyte subsets. **Objective:** This study was performed to measure chemokine receptor expression on CD4 T cells for evaluation of Th1/Th2 dominantly in IFN-β treated patients. **Methods:** flowcytometry was used to detect chemokine receptor expression on CD4 T cell population in PBMCs obtained from MS and healthy control groups. Twenty six MS patients participated in this study before and after IFN-β therapy and the same number of healthy individuals were included. **Results:** The percentage of lymphocytes was 41.28% ± 10.30 in the blood of MS group compared with 36.88% ± 5.51% in the control group (p=0.017). The $CD4^+CXCR3^+$ cells were 18.86% ± 8.46% in healthy group, 30.78% ± 9.8% in pre-treated MS patients and 21.06% ± 9.23% in post-treated group (p<0.001). The $CD4^+CCR4^+$ cell subsets were 27.35% ± 10.15% in healthy group; 28.17% ± 8.9% in pre-treated group and 34.20% ± 8.96% in the post-IFN-β treatment group. The subset of $CD4^+CCR4^+$ was found to be dominant after IFN-β therapy in comparison with the control group (p<0.001). $CD4^+CCR5^+$ percentage was 1.24% ± 0.92% in the healthy people, 1.23% ± 0.71% in the MS patients and 0.76% ± 0.49% in post-treatment status (p=0.003). $CD4^+CCR3^+$ cell subsets were 0.62% ± 0.67% in control group, 0.28% ± 0.26% in the MS patients (p=0.022) and 0.39% ± 0.54% in IFN-β treated patients (p=0.334). An association was found for CXCR3 expression in pre- and post- treatment status (r=0.840, p<0.001) as well as for $CCR4^+$ expression (r=0.712, p<0.001) in the same groups. The Th1 response was dominant in pre-treatment states, and then it shifted to a Th2 dominant state after IFN-β treatment. **Conclusion:** We suggest that the chemokine receptor expression of Th1/Th2 cell subsets could be used for monitoring and the evaluation of the MS disease status.

Keyword: Chemokine, Receptor, Multiple Sclerosis, IFN-β, Th1, Th2

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INTRODUCTION

One of the key pathological features of multiple sclerosis (MS) is inflammatory response in the central nervous system. Chemoattractant cytokines are critical molecules for cell trafficking and selective recruitment of inflammatory cells to the inflammation sites (1,2). Chemokines and their receptors play important roles in T cell activation and immune surveillance (3). Moreover, chemokine receptors are differentially expressed on T cells based on their function. The pattern of cytokines production is defined to explain how T lymphocytes acquire these functions. For example Th1 effector cells produce IL-2 and IFN-γ dominantly, while T cells which produce IL-4 and/or IL-5, IL-10, IL-13 are named Th2 type (4-6). Naïve and activated T cells express chemokines and chemokine receptors, but with different patterns. For instance naïve T cells express CXCR4 and CCR7 but activated T cells express CXCR3 as well as CXCR5. Chemokines may mediate T cell energy or may disable their ability to function. Reports show that CXCR3 and CCR1 CCR5, CCR6 are preferentially expressed by Th1 cells, while CCR3 and CCR4 are predominantly expressed by a subset of Th2 cells (7-9).

Multiple sclerosis (MS) is a T cell-dependent chronic inflammatory disease of the central nervous system with a likely autoimmune etiology. It is known that activated T cells can cross the blood-brain barrier, but the mechanisms by which activated T cells are recruited and remain in the brain, and whether chemokines or chemokine receptors are involved in the pathogenesis of MS are subjects of many studies (10,11). Researchers show that T cell production of a cytokine such as IFN-γ would precede clinical attacks of MS in patients, and injection of recombinant IFN-γ to MS patients would also induce exacerbations of the disease (10,12,13). Moreover, MS involves different stages, usually beginning with a relapsing-remitting phase (RRMS) and later, a progressive form. However, Immune factors associated with different stages of the disease are not well understood (10). In addition to autoimmune responsivness, the influx of activated autoreactive T cells and macrophages across the disrupted blood brain barrier (BBB) into the CNS is a crucial step in the development and pathogenesis of MS (14,15). Chemokines and their receptors may change the lymphocytes attraction to the site of MS lesion in CNS (16). The cytokines including TNF-α, IFN-γ, IL-2, IL-12 released by the Th1 cell type and then macrophages trigger a chain of immunopathological events, resulting in the formation of demyelinated plaques and the damage of axons (17,18). The possibility of blocking T cell migration into CNS could be practical by utilizing chemokine receptor antagonists or anti-chemokine antibodies (19). IFN-β1a and IFN-β1b are immunomodulators widely used in RRMS, but their mechanisms of action in MS are still not fully understood (15). It has been stated that IFN-β inhibits the proliferation of auto-reactive T cells as well as the expression of MHC-II molecules on the antigen presenting cells. It also reduces the production of pro-inflammatory cytokines by the immune cells (15,20,21).

It was found that in vitro addition of IFN-β1b to peripheral blood mononuclear cell cultures significantly reduced the production of several chemokines, such as inflammatory chemokines (15,22). Moreover, treatment of RRMS patients with IFN-β 1a altered the serum levels of a few chemokines and cytokines in CNS and in circulation (15,23,24). A recent study shows that IFN-β treatment responses of MS patients were associated with elevated IL-10 and TGF-β, and decreased CXCL10,

IL-18, IFN-γ as well as TNF-α transcription (25). However, Shapiro et al. reported that IFN-β in multiple sclerosis patients may block endogenous IFN-β function and alter the chemokine/cytokine microenvironment within the CNS (26). Therefore, it could be speculated that the profile and course of the local inflammatory response might have altered by administration of cytokines or chemokines. Moreover, the study by Satoh et al. showed that IFN-β administration to MS patients altered the pattern of cytokine/chemokine gene expression in immune cells (27). Evaluation of Th balance by chemokine receptors was reported by Krakaur who indicated that CCR4, CCR5, CCR7 and CXCR3 as well as plasma chemokine levels would be altered during the treatment of MS patients with IFN-β resulting in the normalization of Th2 related chemokine expression in MS patients (28). Hence we have attempted to evaluate the Th1 and Th2 status in MS patients upon considering chemokine receptors, and speculated that the frequency of Th-type dominant cells in the peripheral blood of MS patients was associated with the expression of several chemokine receptors (Th1, Th2 subsets) on CD4 T cells. So the benefit of urgent evaluation of Th-types which might be used in monitoring and follow up of MS progression as well as identifying the appropriate treatment time period for drug administration. In addition, considering the ratio of CD4/CD8 and the pattern of chemokine expression of T cell subsets after treatment with IFN-β was another aim of the present study.

MATERIALS AND METHODS

Experimental groups. Twenty six patients with RRMS (Table 1) according to the McDonald criteria entered IFN-β treatment protocol. 16 patients received Avonex, 30 μg in 1 ml, by intramuscular route once weekly, 7 patients were received Cinnovex 30 μg in 2 ml by the intramuscular route, once weekly and 3 patients received Rebif, 22 μg in 1 ml, by subcutaneous route, 3 times/week. Twenty six healthy controls were also chosen the study.

Table 1. characteristics of the patients and the controls.

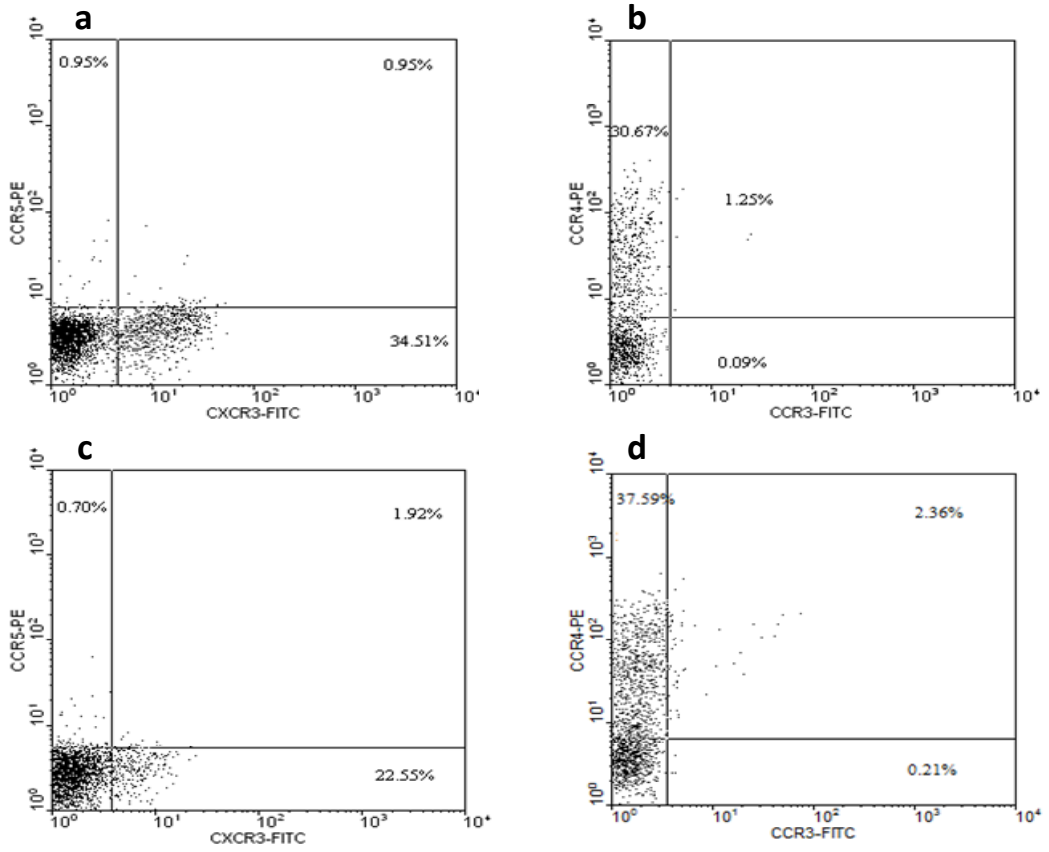
	MS patients	Healthy group
Mean age in years (min-max)	32.69 ± 9.4 (22-54)	28 ± 6.9 (18-45)
Female:male	17:9	15:11
Mean EDSS (min-max)	2.84 ± 1.6 (1-6)	-
Mean disease duration in months (min-max)	57.2 ± 47 (1-180)	-

None of the patients or controls was on any immunomodulatory therapy prior to taking the samples, except those who were recruited for post-IFN-β treatment sampling after 2-3 months of therapy. The control group was matched for age and sex. All the patients had records in the University hospital confirming their diagnoses as MS. This study was approved by the ethical committee of Isfahan University of Medical Sciences (Isfahan, Iran). Informed consent was obtained from all patients. Routine automated hematology device was used for WBC and lymphocyte counting.

Whole blood was collected in sterile test tubes containing EDTA as anti-coagulant. Peripheral blood mononuclear cells (PBMC) were separated by centrifugation over Ficoll-Hypaque (Nyco med Oslo, Norway) density gradient and were resuspended (10^6 cells per ml) in Hank's balanced salt solution (10). The cell count was performed using a Neubauer Zahlkammer hemocytometer. 100 µl of cell suspension containing 1×10^5 cells were transferred into each flow-cytometer tube. The tubes containing the cells were immediately stained in duplicate using 10 µl of anti γ1/γ2 isotype control PE/FITC conjugated (R&D, USA) for isotype control in the dark for 30 minutes. In addition, unstained tubes containing 100 µl of the cell suspension were kept as negative controls.

Antibodies. Anti-human CCR4-phycoerythrin-conjugated mouse monoclonal antibody (isotype, IgG2B clone # 205410), anti-human-CCR3-carboxy fluorescein-conjugated monoclonal antibody (isotype, rat IgG2a, clone # 61828.111), Anti-human CXCR3-carboxyfluorescein-conjugated monoclonal antybody (isotype, mouse IgG1, colne # 49801), Anti-human CCR5-phycoerythrin-conjugated monoclonal (mouse IgG2B, clone # 4553), and Anti-CD8 (FITC). All of the antibodies were obtained from R&D system, USA. Monoclonal anti-human-CD4 PE/Cy5 (mouse isotype IgG2b, clone # OKT4, Biolegend), Carboxyfluoresciein (CFS) conjugated mouse IgG1 antibody control isotype (clone 11711) were used as negative controls.

Figure 1. Representative dot plots from PBMC of a MS patient. The percentage of CD4 positive cells expressing chemokine receptors CCR5, CXCR3, CCR4 and CCR5 in pre-treatment (a and b) and post-treatment status (c and d).



Staining. Appropriate amounts of the cells and the antibodies were used in test tubes according to manufacturer's instruction. After appropriate incubation time, the stained cells were washed twice with phosphate buffered saline (PBS). In order to minimize the in vitro alteration of chemokine receptors, the staining process was performed immediately and the control samples were run in parallel with the patient samples in each experiment. Ten thousand cells were counted in each sample by gating using forward scatter versus side scatter, and gated cells were analysed with CD45/CD14 to ascertain that cells were of lymphoid origin. Isotype matched mouse IgG1 (PE)/IgG2a (FITC) (IQ products, Netherland) antibody was used for background staining. The percentages of $CD4^+$ lymphocytes expressing both Th1/Th2 chemokine receptors (PE/FITC) were calculated using triple labeling (PE/FITC/PE-cy5) following compensation (Figure 1). Three-color flow cytometer Facs-Calibur system (Becton-Dickinson, USA; Facs-sort and cell-quest program) was applied, and the data obtained were recorded for further statistical analysis.

Statistical analysis. Independent sample student t-test was applied for comparing the control with patient groups. Data were expressed as mean ± standard deviation. Paired t-test was used for comparing between the samples taken in before and after the immunomodulating therapy. Pearson correlation analysis was used for chemokine expression on the cells in different occasion experiments. The SPSS 15 software was used for all the analysis and p value ≤ 0.05 was considered statistically significant.

RESULTS

As shown in Table 1, the lymphocyte count in PBMCs of the control group was 2362 ± 851 and that of the MS patient group was calculated to be 2511 ± 885. While the total number of lymphocytes in MS group seemed to be higher than the control group, there was no statistically significant difference between the two groups (p=0.521).

Table 2. Ferquencies of lymphocyte subsets in MS patients before and after treatment with IFN-β compared with the healthy controls.

	Control group	Pre-treatment	Post-treatment	P value
Lymphocyte Number	2362 ± 851	2511 ± 885	2442 ± 860	0.52
$CD4^+$ T cell count	1015 ± 388	1171 ± 461	1133 ± 399	0.2
Lymphocyte (%)	36.88 ± 5.51	41.88 ± 10.30	40.53 ± 9.30	0.02
$CD4^+$ T cell (%)	45.89 ± 7.01	45.4 ± 6.6	45.39 ± 5.7	0.77
$CD8^+$ T cell (%)	19.47 ± 4.5	19.68 ± 6.1	20.13 ± 5.6	0.36
CD4/CD8 ratio	2.35	2.30	2.25	>0.05

However, the percentage of lymphocytes was 36.9% ± 5.5% in the control group and 41.9% ± 10.3% in the MS group (p=0.017). The percentage of $CD4^+$ T cells was similar in all the groups (p=0.769), (Table 2). Analysis did not show any association between chemokine receptor expression with the duration of the disease or the sex of the patients (data is not shown). There were no notable significant differences between RRMS and secondary progressive MS for all the analyses performed. The analysis did not show any significant difference in the brands of IFN medication used.

The CD4 positive T cells expressing chemokine receptors were analysed and the following results were obtained. CXCR3 expression subsets belonging to Th1 type and CCR4 expression subsets from Th2 type have had an adequate amount of expression molecules for statistical evaluation on the Th1/Th2 in the PBMC samples. However, in our study due to the low expression of CCR5 and CCR3 on the lymphocyte cells, it was not logical to consider them for Th1/Th2 status in the PBMC samples for all the cases. The data obtained indicate that subsets of CXCR3 cell expression increased (up to 63%) in the pre-treatment MS patients in comparison with the healthy control group ($p<0.001$). Then, IFN-β treatment reduced the CXCR3 cell subsets to near normal ($p<0.001$, Table 2 and Figure 2a).

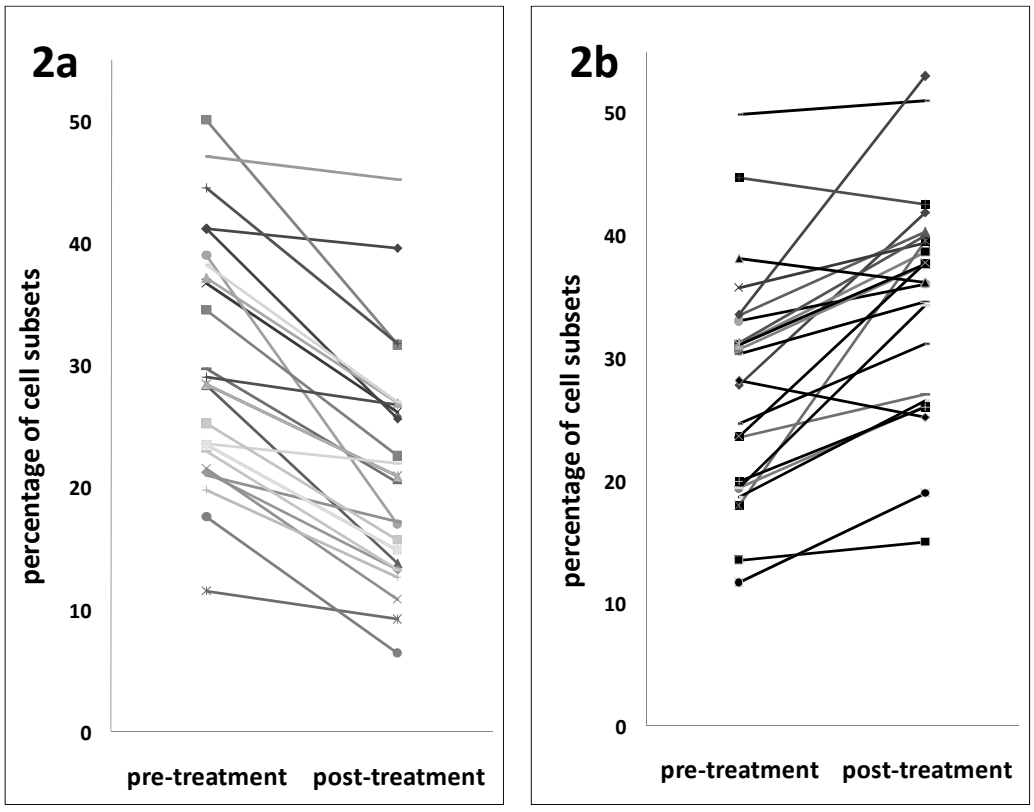


Figure 2. Pre- and post-treatment chemokine expression levels. (a) $CD4^+CXCR3^+$ expression on PBMCs and (b) $CD4^+CCR4^+$ expression on PBMCs in MS patients.

In addition, the mean percentage of $CCR4^+$ cell subset in the control group was 27.35% ± 10.15% which was approximately similar to the untreated MS patients (with 28.17%

± 8.9%), but IFN-β treatment increased the Th2 cells (CCR4 expression) (Table 3 and Figure 2b), (34.20% ± 8.96%). This increase was statistically significant ($p<0.001$) compared with the control and the pre-treatment groups. IFN-β could reduce CXCR3 expression in Th1 cells by 52%, and increase CCR4 expression in Th2 cells by 23% in the MS patient group. Pearson correlation analysis between the pre- and post-treatment status showed an $r=0.840$ with $p<0.001$ for CXCR3 expression on Th1 cells and an $r=0.712$ with $p<0.001$ for CCR4 expression on Th2 cells (Table 3).

Table 3. Chemokine expression of $CD4^+$ cell subsets in MS patients and the healthy control group as analysed by flow-cytometry.

	Chemokine expression	Control	P value	Pre-treatment	Post-treatment	P value	Correlation Coefficient
Th1	$CD4^+/CXCR3^+$	18.86 ± 8.46	< 0.001	30.78 ± 9.8	21.06 ± 9.23	< 0.001	r=0.840 p< 0.001
	Alteration (%)	100	-	163	111	-	
Th2	$CD4^+/CCR4^+$	27.35 ± 10.15	0.758	28.17 ± 8.9	34.20 ± 8.96	< 0.001	r=0.712 p< 0.001
	Alteration (%)	100	-	102	125	-	
Th1	$CD4^+/CCR5^+$	1.24 ± 0.92	0.960	1.23 ± 0.71	0.76 ± 0.49	0.003	r=0.335 p=0.094
	Alteration (%)	100	-	99.2	61	-	
Th2	$CD4^+/CCR3^+$	0.62 ± 0.67	0.022	0.28 ± 0.26	0.39 ± 0.54	0.334	r=0.105 p=0.611
	Alteration (%)	100	-	45	62	-	

In addition, there were significant correlations between the expression of CXCR3/ CCR4 on the CD4 lymphocyte subsets in all the groups tested (Table 4). Similar trend was found for the association between CCR5 and CCR3 on the cells with the exception of the pre-treatment group ($r=0.184$ with $p=0.368$) (Table 4).

Table 4. The association and the ratio of the chemokine expression in CD4 T cell subsets in all experimental groups.

	Th1/Th2	Control	Pre-treatment	Post-treatment
Chemokine Expression (Correlation)	$CXCR3^+/CCR4^+$	r=0.374 p<0.001	r=0.596 p<0.001	r=0.676 p<0.001
	$CCR5^+/CCR3^+$	r=0.433 p=0.027	r=0.184 p=0.368	r=0.489 p=0.011
Chemokine Expression (ratio)	$CXCR3^+/CCR4^+$ $CCR5^+/CCR3^+$	0.66 2	1.07 4.4	0.61 1.94

The ratio of Th1/Th2 cells (using the expression of CXCR3/CCR4) was 0.66 in the healthy control group, 1.07 in the pre- and 0.61 in the post-IFN-β-treatment groups, respectively. A similar trend was found for Th1/Th2 ratio (using the expression of CCR5/CCR3) as indicated in Table 3. As it can be seen; the ratio returned to normal in both cases after IFN-β treatment. The ratio of CD4/CD8 was calculated and the statistical analysis did not show any significant difference in these T cell subsets of all groups ($p<0.05$).

DISCUSSION

In the present study, we have tried to consider several Th1 and Th2-related chemokine receptors on circulating T cells in MS patients. Since $CD4^+CXCR3^+$ cells are Th1-related and $CD4^+CCR4^+$ cells are Th2-related (7,8), $CD4^+CXCR3^+/CD4+CCR4^+$ ratios are representative of Th1/Th2 ratio. There were differences in the percentage of $CXCR3^+$ cells between the MS patients and healthy control group. In our study, Th1/Th2 ratios are compared between MS groups before and after immunomodulation and the healthy groups. We speculated if we could evaluate the Th-type status in MS patients by a blood test as fast as it possible using flow cytometry technique, an appropriate estimation would be available for monitoring and the follow up of MS progression. Assessment of chemokine receptors expression by flow-cytometer might be a suitable device for the evaluation of an immunomodulator cytokine treatment.

Nakajima reported that the percentage of $CD4^+CXCR3^+$ cells among all T cells tested in peripheral blood was higher than the other subsets in MS patients ($p=0.010$) (29). In addition, Balashov's study shows that $CD3^+CXCR3^+$ cells were elevated in progressive MS patients ($p<0.005$), and the percentage of $CXCR3^+$ T cells increased in RRMS compared with their controls (10). Our data, in which MS patients had increased CD4/CXCR3 subsets, ($p<0.001$) support these studies. Similar results obtained by other researchers reporting the expression of CXCR3 on CD4 cells, showed significantly reduced expression after IFN-β treatment (6,30).

Our data do not show any significant alteration in the ratio of CD4/CD8 in MS peripheral bloods in comparison with control group. However, shifting of $CD8^+$ to $CD4^+$ percentage was reported by Nakajima in MS patients with evaluation the chemokine receptor expression (29).

$CCR4^+$ expression on $CD3^+$ cells (Th2) in Balashov study was 9.0% ± 1.1% which was not statistically different from MS patient in chronic progressive or relapsing-remitting conditions (10). However, our data illustrate that $CD4^+$CCR4 expressing cells were 27.35% ± 10.15% in healthy group, which were not different from MS patients ($p=0.758$), but after IFN-β treatment, it was augmented by 25% in the treated group. The difference in the percentage of expression of this chemokine could be due to the distinctive clone of antibody used in these two different experiments or should be clarified further. Moreover, similar to our study, the data obtained by Krakauer shows an increase in $CD4^+$CCR4 cells after three months of treatment with IFN-β (28), which means a downregulation of Th2 related CD4 T cell subsets in patients with progressive MS occurs due to the treatment. Further study by Karni made it clear that increase in the

percentage of $CCR4^+$ T cells produce IL-4 (Th2 cytokine) which reverses the effect of IFN-γ T cell activity (Th1 cytokine) (30).

Our investigation indicates that CCR5 expressing $CD4^+$ cells (Th1 cells) had extremely low percentage in the blood samples of both MS (1.23% ± 0.71%) and the control group (1.24% ± 0.92%). However, there is a 61% reduction after IFN-β treatment. Sellebjerg et al. speculated that CCR5 expressing cells might have an important role in MS patients but they found only 7% of monocytes in the patients that express $CCR5^+$ in comparison with 5% observed for healthy control. They did not show any significant difference in $CD4^+$ and $CD8^+$ cells between MS patients treated with IFN-β and the healthy controls (31). Moreover, in an independent study it was shown that CCR5 expression on CD3 T cells was 7.9 ± 0.8% in the control group, 9.9 ± 1.3% in RRMS and 13.1% ± 1.4% in progressive MS group (10). However, another study shows a low expression of CCR5 with no difference in all studied groups (29) which is similar to our present data. In addition, Kivisakk's investigation indicates that IFN-β has no effect on CCR5 expressing T cells in the circulation (32). Therefore, it appears that more studies are needed for the clarification of this point.

Our data shows an extremely low expression percentage of CCR3 on CD4 T cell subsets. However the calculation indicates that there is a lower number of CCR3 cells in MS patient groups compared with the normal group (Table 2). Nakajima et al. recommended more research in the evaluation of CCR3 expression in immunopathology of MS disease (29). Similar to our data, they also reported a few CCR3 expressing cells in the PBMC of both MS and healthy group with no correlation to the stage of MS activity (29). In addition, Karni et al. also pointed out that CCR3, CCR4 and CCR8 are expressed on Th2 cells, but they concluded that the role of CCR3 in the pathogenesis of MS could not be clearly understand (30). Interestingly, it has been reported that there was no alteration in CCR3 expression in PBMC of MS patients even after IFN-β therapy (6). Similar to our study, Suzuki's study shows that the percentage of CCR3 expression on CD4 T cells of Myasthenia gravis patients were less than 2% before and after therapy, as well as in healthy individuals (8). Because of low expression percentages of these receptors, the statistical evaluation would not be practical for this data, so it could be recommended that CCR3 expression must be amplified with second or a third antibody staining for intensifying CCR3 expression. Conversely, it could be ignored in Th2 cells for clinical purpose especially in MS patients.

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REFERENCES

1 Fox RJ, Kivisakk P, Lee JC, Tucky B, Lucchinetti C, Rudick RA, *et al.* Chemokine receptors as biomarkers in multiple sclerosis. Dis Markers. 2006; 22:227-33.

2 Mahad DJ, Trebst C, Kivisakk P, Staugaitis SM, Tucky B, Wei T, *et al.* Expression of chemokine receptors CCR1 and CCR5 reflects differential activation of mononuclear phagocytes in pattern II and pattern III multiple sclerosis lesions. J Neuropathol Exp Neurol. 2004; 63:262-73.
3 Yoshie O, Imai T, Nomiyama H. Chemokines in immunity. Adv Immunol. 2001; 78:57-110.
4 Raman D, Baugher PJ, Thu YM, Richmond A. Role of chemokines in tumor growth. Cancer Lett. 2007; 256:137-65
5 Sorensen TL, Tani M, Jensen J, Pierce V, Lucchinetti C, Folcik VA, *et al.* Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. J Clin Invest. 1999; 103:807-15.
6 Sorensen TL, Sellebjerg F. Selective suppression of chemokine receptor CXCR3 expression by interferon-β1a in multiple sclerosis. Mult Scler. 2002; 8:104-7.
7 Sallusto F, Lanzavecchia A, Mackay CR. Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. Immunol Today. 1998; 19:568-74.
8 Suzuki Y, Onodera H, Tago H, Saito R, Ohuchi M, Shimizu M, *et al.*. Altered expression of Th1-type chemokine receptor CXCR3 on CD4+ T cells in myasthenia gravis patients. J Neuroimmunol. 2006; 172:166-74.
9 Viola A, Contento RL, Molon B. T cells and their partners: The chemokine dating agency. Trends Immunol. 2006; 27:421-7.
10 Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci U S A. 1999; 96:6873-8.
11 Tischner D, Weishaupt A, van den BJ, Muller N, Beyersdorf N, Ip CW, et al. Polyclonal expansion of regulatory T cells interferes with effector cell migration in a model of multiple sclerosis. Brain. 2006; 129:2635-47.
12 Adorini L. Immunotherapeutic approaches in multiple sclerosis. J Neurol Sci. 2004; 223:13-24.
13 Panitch HS, Hirsch RL, Haley AS, Johnson KP. Exacerbations of multiple sclerosis in patients treated with gamma interferon. Lancet. 1987; 1:893-5.
14 Ransohoff RM. Mechanisms of inflammation in MS tissue: adhesion molecules and chemokines. J Neuroimmunol. 1999; 98:57-68.
15 Szczucinski A, Losy J. Chemokines and chemokine receptors in multiple sclerosis. Potential targets for new therapies. Acta Neurol Scand. 2007; 115:137-46.
16 Sellebjerg F, Sorensen TL. Chemokines and matrix metalloproteinase-9 in leukocyte recruitment to the central nervous system. Brain Res Bull. 2003; 61:347-55.
17 Lassmann H, Ransohoff RM. The CD4-Th1 model for multiple sclerosis: a critical [correction of crucial] re-appraisal. Trends Immunol. 2004; 25:132-7.
18 Segal BM. CNS chemokines, cytokines, and dendritic cells in autoimmune demyelination. J Neurol Sci. 2005; 228:210-4.
19 Kivisakk P, Trebst C, Eckstein DJ, Kerza-Kwiatecki AP, Ransohoff RM. Chemokine-based therapies for MS: how do we get there from here? Trends Immunol. 2001; 22:591-3.
20 Hemmer B, Nessler S, Zhou D, Kieseier B, Hartung HP. Immunopathogenesis and immunotherapy of multiple sclerosis. Nat Clin Pract Neurol. 2006; 2:201-11.
21 Szczucinski A, Kalinowska A, Losy J. CXCL11 (Interferon-inducible T-cell alpha chemoattractant) and interleukin-18 in relapsing-remitting multiple sclerosis patients treated with methylprednisolone. Eur Neurol. 2007;58:228-32.
22 Comabella M, Imitola J, Weiner HL, Khoury SJ. Interferon-β treatment alters peripheral blood monocytes chemokine production in MS patients. J Neuroimmunol. 2002; 126:205-12.
23 Bartosik-Psujek H, Stelmasiak Z. The levels of chemokines CXCL8, CCL2 and CCL5 in multiple sclerosis patients are linked to the activity of the disease. Eur J Neurol. 2005; 12:49-54.
24 Bartosik-Psujek H, Stelmasiak Z. Correlations between IL-4, IL-12 levels and CCL2, CCL5 levels in serum and cerebrospinal fluid of multiple sclerosis patients. J Neural Transm. 2005; 112:797-803.
25 Cucci A, Barbero P, Clerico M, Ferrero B, Versino E, Contessa G, et al. Pro-inflammatory cytokine and chemokine mRNA blood level in multiple sclerosis is related to treatment response and interferon-β dose. J Neuroimmunol. 2010; 226:150-7.
26 Shapiro AM, Jack CS, Lapierre Y, Arbour N, Bar-Or A, Antel JP. Potential for interferon β-induced serum antibodies in multiple sclerosis to inhibit endogenous interferon-regulated chemokine/cytokine responses within the central nervous system. Arch Neurol. 2006 ;63:1296-9.
27 Satoh J, Nanri Y, Tabunoki H, Yamamura T. Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNβ-responsive genes in peripheral blood lymphocytes in vitro: an implication for IFNβ-related adverse effects in multiple sclerosis. BMC Neurol. 2006; 6:18.
28 Krakauer M, Sorensen PS, Khademi M, Olsson T, Sellebjerg F. Dynamic T-lymphocyte chemokine receptor expression induced by interferon-β therapy in multiple sclerosis. Scand J Immunol. 2006; 64:155-63.
29 Nakajima H, Fukuda K, Doi Y, Sugino M, Kimura F, Hanafusa T, et al. Expression of TH1/TH2-related chemokine receptors on peripheral T cells and correlation with clinical disease activity in patients with multiple sclerosis. Eur Neurol. 2004; 52:162-8.
30 Karni A, Balashov K, Hancock WW, Bharanidharan P, Abraham M, Khoury SJ, et al. Cyclophosphamide modulates CD4+ T cells into a T helper type 2 phenotype and reverses increased IFN-gamma production of CD8+ T cells in secondary progressive multiple sclerosis. J Neuroimmunol. 2004; 146:189-98.
31 Sellebjerg F, Kristiansen TB, Wittenhagen P, Garred P, Eugen-Olsen J et al. Chemokine receptor CCR5 in interferon-treated multiple sclerosis. Acta Neurol Scand. 2007; 115:413-8.
32 Kivisakk P, Cotleur AC, Lee JC, Rudick RA, Ransohoff RM. Interferon-β 1a does not reduce expression of CCR5 and CXCR3 on circulating T cells. J Neuroimmunol. 2003; 141:150-4.