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T Helper Cell Subgroups and Their Integrins in the Peripheral Blood as Indicators of Relapse in Multiple Sclerosis

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Research Article

Keywords: Relapsing-Remitting Multiple Sclerosis, T Lymphocyte subgroups, integrin, peripheral blood

Posted Date: April 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9073317/v1

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Additional Declarations: No competing interests reported.

Abstract

Objective and design: Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system. This study aims to determine whether T lymphocyte subgroups and the integrin levels expressed on their surfaces can be used to differentiate between the relapse and remission phases in patients with relapsing-remitting MS (RR-MS).

Subjects: The study included 20 RR-MS patients in remission, 20 relapsed patients, and 20 healthy controls. Included patients had been diagnosed using the 2017 McDonald criteria and were not using immunomodulatory or immunosuppressive therapies.

Treatment: Not applicable.

Methods: We analyzed peripheral blood samples for T lymphocyte subgroups and integrin expression on the surfaces of these cells using flow cytometry. The expression levels of αLβ2, αMβ2, α4, and β3 integrin were evaluated as percentages.

Results: The percentage of Th17.1 cells was significantly higher in the relapse patients than in the healthy controls (p = 0.042). The Th2:Th17.1 ratio was lower in the relapse group than in the control group (p = 0.003). The expression of αMβ2 integrin in Th17.1 cells was lower in both the relapse and remission groups than in the control group (p = 0.012 and p = 0.046, respectively).

Conclusion: Our results suggest that the peripheral blood Th2:Th17.1 ratio can be considered a potential non-invasive biomarker for monitoring disease activity in MS patients. However, integrin expression cannot be used for this purpose.

Introduction

The etiopathogenesis of multiple sclerosis (MS) is multifactorial, involving complex interactions between genetic predisposition, environmental factors, and immune system dysfunction [1]. Current evidence suggests that T lymphocyte-mediated immune responses play a central role [2].

When peripheral immune tolerance is impaired, the autoimmune process activates $CD4^+$ T helper (Th) cells and $CD8^+$ cytotoxic T lymphocytes. These then cross the blood-brain barrier (BBB) and infiltrate the central nervous system. These cells are known to be the main effectors of abnormal immune responses to myelin antigens. Specifically, the Th1 and Th17 cell subgroups trigger microglial activation, oligodendrocyte damage, and demyelination through the release of proinflammatory cytokines, including interferon-γ (IFN-γ), interleukin-17 (IL-17), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [3]. Th1 cells primarily promote cellular immune activity by increasing macrophage activation, whereas Th17 cells increase CBC permeability, facilitating the passage of inflammatory cells into the central nervous system. In experimental autoimmune encephalomyelitis (EAE) models, Th17 cells have been shown to play a critical role in disease onset and progression [5]. In patients with MS, functional deficiencies have been found in regulatory T cells (Tregs), which normally suppress the autoimmune

response [4]. The secretion of cytokines such as IL-4, IL-5, and IL-10 by Th2 cells has been associated with reduced inflammation and improvement of symptoms in MS [6]. Furthermore, Th17.1 cells have recently been identified as a novel subgroup of T helper cells that produce both IFN-γ and IL-17. They are sometimes referred to as “Th1-like Th17” cells because of their production of these interleukins. These Th cells involved in neuroinflammation have the capacity to cross the BBB and accumulate in the central nervous system (CNS) in both EAE and MS. They have also been observed in the brain tissue of MS patients and found at high levels in both the peripheral blood and cerebrospinal fluid (CSF) of relapsed MS patients [7].

The integrins on the surfaces of T cells mediate the passage of the cells across the BBB. The interaction of α4β1 integrins with vascular cell adhesion molecule-1 (VCAM-1) on the endothelium of the inflamed area in the CNS causes leukocytes to adhere to it [8, 9]. This information led to the development of natalizumab, a monoclonal antibody against α4β1, which is approved for the treatment of MS [10]. Another integrin that mediates leukocyte adhesion within the CNS is lymphocyte function-associated antigen-1 (LFA-1), also known as alpha-L beta-2 (αLβ2) integrin. This adheres to the endothelium via intercellular adhesion molecule-1 (ICAM-1) [11]. Alpha-M beta-2 (αMβ2) integrin, also known as MAC-1, is an adhesion molecule expressed on the leukocyte surface. It plays a critical role in the regulation of innate immune responses [12], but there has been little research into its role in MS. Alpha-V beta-3 (αVβ3) integrin is closely associated with autoimmune diseases, including MS [13]. It is most highly expressed in T helper cell 17 (Th17). Its suppression has been shown to reduce the severity of EAE in mice [14].

The pathogenesis of MS can be traced to complex immunological processes. The activation, differentiation, and effector functions of T lymphocytes in the CNS play key roles in the initiation and progression of the disease. In this study, we aim to determine whether the levels of T lymphocyte subgroups and their integrins in the peripheral blood can be used as markers of MS relapse.

Materials and Methods

Participants

The participants in this study included 20 cases of relapsing-remitting MS (RR-MS) in remission, 20 in relapse, and 20 healthy controls. All MS patients were diagnosed using the 2017 McDonald criteria at the Neurology Outpatient Clinic of Afyonkarahisar Health Sciences University Health Application and Research Center between February 1, 2025, and September 1, 2025. They had received no immunosuppressive or immunomodulatory drugs in the last year and had no chronic or acute diseases other than MS. The remission group comprised patients who had not relapses in the last 6 months. The control group had no known acute or chronic diseases (Table 1).

Determination of the T lymphocyte subgroups

The identification and quantification of T lymphocyte subgroups were achieved using flow cytometry. Participant blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and then transferred to appropriate polypropylene or polystyrene tubes in specific amounts. Fluorochrome-labeled antibodies specific to the antigens of the target cells were added to these tubes and incubated for a specific period. These antibodies directly bind to the cell surface, so they can be used to stain cell surface antigens. Before staining, the cells were fixed and permeabilized to facilitate the examination of intracellular antigens. Following incubation and washing, the tubes containing the prepared samples were loaded into the flow cytometry device (Navios EX Flow Cytometry Analyzer; Beckman Coulter Co., Marseille, France). The fluorescent signals obtained by laser stimulation were analyzed using Kaluza, v. 2.1 software. First, a viable cell population was gated on a forward scatter–side scatter (FSC–SSC) dot plot, excluding cellular artifacts and debris. Lymphocytes were identified within this population. Among the cells obtained from the lymphocyte gate, those showing double-positive expression of CD3 and CD4 were gated to determine the percentage of Th lymphocytes. The Th lymphocytes showing double-positive expression of FOXP3 and CD25 were identified as the Treg subgroup. The rest of the Th subgroups were identified by examining the expression of CD183 and CD196. CD183+- CD196– cells were identified as Th1 lymphocytes, CD183–- CD196– cells as Th2 lymphocytes, CD183–-CD196+ cells as Th17 lymphocytes, and CD183+- CD196+ cells as Th17.1 lymphocytes (Fig. 1).

Identification of integrins in the T lymphocyte subgroups

In each of the Th subgroups, we measured the expression of αLβ2 integrin by looking at the percentage of CD11a expression, the expression of αMβ2 integrin by looking at the percentage of CD11b expression, the expression of α4 integrin by looking at the percentage of CD49d expression, and the expression of β3 integrin by looking at the percentage of CD61 expression.

Statistics

Data were described as mean (standard deviation). The normality of the distributions was evaluated using the Shapiro–Wilk test. When comparing the means of three independent groups, we used one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test when parametric conditions were met. When parametric conditions were not met, the Kruskal–Wallis test was used, followed by the Mann–Whitney U test with Bonferroni correction. If homogeneity was not achieved, Welch's ANOVA was applied, followed by the Games–Howell test. Chi-square tests were used to compare the percentage distributions of categorical data between groups. Statistical analyses were performed using SPSS for Windows (IBM Corp., Armonk, NY, USA) software. *P*-values <0.05 were considered statistically significant.

Results

The mean lymphocyte percentage in the peripheral blood of patients in the relapse group was significantly lower than in the control group ($p = 0.007$), while the mean percentage of Th cells in the lymphocyte population of the remission group was higher than in the control group ($p = 0.038$) (Table 2).

The mean percentages of Treg, Th1, Th2, and Th17 cells within the Th cells did not differ between the control group and either the relapse or remission group. However, the percentage of Th17.1 cells was higher in the relapse patients than in the control group (p = 0.042) (Table 2).

We also evaluated the ratio of anti-inflammatory Th2 to inflammatory Th17.1 cell percentages (Th2/Th17.1). We found this ratio to be lower in relapse patients than in healthy controls (p = 0.003) (Table 2). Our examination of integrin on the Th cell surfaces found no differences between the three groups in the levels of αLβ2 (LFA-1) integrin, α4 integrin, or β3 integrin (Fig. 2). However, we found αMβ2 (MAC-1) integrin levels to be significantly higher in the Th17.1 cells of the control group than both the relapse and remission groups (p = 0.012 and p = 0.046, respectively) (Fig. 2). The integrin levels expressed by Th cell subgroups are given in Table 3.

Discussion

In this study, we aimed to determine whether the relapse phase of RR-MS can be non-invasively indicated through evaluation of the T lymphocyte subgroups and their integrin expression levels in the peripheral blood of patients with RR-MS.

We found that the percentage of lymphocytes in the peripheral blood was lower in patients in the relapse phase compared to healthy controls. This can be explained by the increased permeability of the CNS during relapses and the migration of immune cells, including lymphocytes, to the CNS. This finding is consistent with those of previous studies [15–17].

Although both B and T lymphocytes contribute to MS pathogenesis, recent evidence has highlighted the role of autoreactive Th cells in the onset and progression of the disease [18]. We found higher percentages of Th cells in lymphocytes in the peripheral blood of patients in remission than in the healthy controls. Similarly, Mikulkova et al. found higher levels of memory Th cells in the peripheral blood of patients with RR-MS in remission than in that of healthy individuals [19]. Canto-Gomes et al. compared total Th, immature Th, and memory Th cell levels and the immature/ memory Th cell ratios in newly diagnosed RR-MS patients and healthy individuals. Like us, they found no significant difference [20]. Taken together, these results suggest that immune activity in RR-MS is not limited to the relapse period. Some level of T cell activity and immune response continues in the peripheral blood. Our investigation of the Th subgroups showed no significant differences in the percentages of Treg, Th1, Th2, and Th17 cells between the three groups. Tregs have been extensively studied in MS, and findings regarding the ratio, number, and function of the cells have varied [19–24]. However, recent work increasingly suggests that impaired Treg cell functionality in MS may contribute to the development and exacerbation of the disease [25–30]. While we did not evaluate the function of Treg cells, we determined their percentages within T lymphocytes. We observed no differences from healthy individuals in either the relapse or remission group. Similar results have previously been reported in the literature [19, 20].

Th1 cells are known to be implicated in MS pathogenesis. Their secretion of IFN-γ leads to macrophage-induced demyelination [31]. Th17 cells are also thought to play an important role, both by targeting

resident astrocytes and microglia in the CNS and through the secretion of IL-17A-F, IL-21, and IL-22, which causes neuroinflammation leading to impaired ENT [32, 33]. However, other studies have produced conflicting results. While some have found no differences in the proportions of inflammatory TH1 and Th17 cells in patients with RR-MS compared to healthy individuals [20, 34, 35], others report significantly higher levels in RR-MS [36-38]. This may be due to differences between studies in the biomarkers used to identify Th1 and Th17 cells.

Th17.1 cells, which produce both IFN-γ and IL-17, can accumulate in the CNSs of MS patients and cross the BBB [7]. Higher proportions of these cells have been observed in patients with RR-MS during exacerbations, with high levels of myelin-specific Th17.1 cells found in both the peripheral blood and CSF of patients with MS. Furthermore, high levels of IFN-γ, GM-CSF, and very late antigen-4 (VLA-4), and low levels of IL-17 in Th17.1 cells have been associated with disease activity in RR-MS [7]. We found significantly higher percentages of Th17.1 cells in relapsed RR-MS patients than in healthy controls. This demonstrates the critical role of these cells in the neuroinflammatory process. Their ability to produce both IFN-γ and IL-17 imbues Th17.1 cells with strong proinflammatory properties. Previous studies have shown accumulation of Th17.1 in both EAE models and the brain tissue of MS patients. Thus, Th17.1 cells may be important effector cells potentially responsible for triggering and maintaining MS relapses.

A key finding of our study was the lower Th2/Th17.1 ratio in our relapse group than in our control group. This indicates a disruption in the balance between pro- and anti-inflammatory T cells during MS relapses. Th2 cells are known to suppress the immune response by releasing anti-inflammatory cytokines such as IL-4, IL-5, and IL-10. Therefore, a decrease in the Th2/Th17.1 ratio is likely to contribute to an increased inflammatory load. Among our results, we found decreases in the Th2/Th17.1 ratio to be the best indicator of relapse.

Our investigation into integrin expression in the T lymphocyte subgroups found no between-group differences in the levels of αLβ2 (LFA-1), α4, or β3 integrin. This suggests that the basal expression levels of these molecules in the peripheral blood are independent of MS disease activity. As we did not evaluate integrin activity, we could not fully ascertain their role in RR-MS attacks. Although α4 integrin is targeted in MS treatment, the observed lack of significant differences between our groups suggests that the functional activation status or expression of integrin in the CNS may be more informative than integrin levels in the peripheral blood. A striking finding of the study was the significantly lower expression of αMβ2 (MAC-1) integrin in Th17.1 cells in both the relapse and remission groups than in the control group. While αMβ2 integrin is more strongly associated with innate immune cells, its expression in lymphocytes is known to contribute to cell adhesion and migration and regulation of the immune response. Its decreased expression in Th17.1 cells suggests that either these cells migrate to the CNS using alternative adhesion mechanisms, or that they acquire different functional properties in the inflammatory microenvironment. The role of αMβ2 integrin in MS pathogenesis requires further investigation. LFA-1 is an integrin molecule that participates in rolling circulating leukocytes along vessel walls. However, its primary duty is to ensure the tight attachment of these cells to the vascular bed of lymphoid organs and areas of inflammation. It achieves this through its interaction with ligands such as

intercellular adhesion molecule-1 (ICAM-1) [39]. The limited number of studies of LFA-1 in the Th cells of MS patients have shown that Th17 and Treg cells pass into the CNS via LFA-1 [40–42]. Eikelenboom et al. examined the expression of LFA-1 integrin on the surface of Th cells in different MS subtypes. In $CD4^+$ T cells, they found significantly higher LFA-1 expression in patients with secondary progressive multiple sclerosis (SPMS) than in those with RR-MS. This finding can be explained by the longer disease duration of SPMS, which results in a more advanced level of immune activation [43]. We found higher LFA-1 expression in Th1 and Th17.1 cells than in other Th subgroups. However, there was no significant difference in the LFA-1 levels of Th subpopulations between the three groups. Given the established importance of LFA-1 to α4-independent cell migration (e.g., Th17 and Treg cells), the lack of expression level differences suggests that the expression of ICAM-1, which is the ligand of this cell in the CNS, may be primarily responsible for Th cell migration.

MAC-1 integrin is expressed by myeloid cell lines and, to a lesser extent, in some T lymphocyte subpopulations [44]. This integrin moves along the vascular lumen by following adhered leukocytes and interacting with ICAM-1. It has been suggested that the LFA-1/ICAM-1 interaction is the dominant factor in the binding of LFA-1 and MAC-1 integrins with ICAM-1. Therefore, LFA-1 and MAC-1 may compete for binding [45]. Since MAC-1 expression on Th cell surfaces is low, there have been few studies on the role of this integrin in MS. However, an EAE model study found that MAC-1-deficient T cells cannot develop EAE. This suggests that, in T cells, MAC-1 may play a role in inflammatory activation and pathogenic migration processes [46]. However, another study argues that the pathogenic role of MAC-1 is unclear. The study found that, while it may play a role in the differentiation of pathogenic T cells, MAC-1 does not appear to contribute to their migration to the CNS [47]. In the present study, we found that MAC-1 is expressed by Th1 and Th17.1 cells. The expression levels in Th17.1 cells in the relapse group were significantly lower than those in the control group. This suggests that Th17.1 cells migrate to the CNS, but the role of MAC-1 in this process appears to be minor.

VLA-4 is an important integrin. It is involved in lymphocyte differentiation and tissue-specific migration during inflammation. Previous studies have found no difference in the expression of α4 in Th1, Th17, and Treg cells in the peripheral blood of MS patients and healthy controls [48, 49]. However, α4 expression in Th17 cells in the CSF was higher in the MS group [49]. This would explain the decrease in α4 expression in Th17 cells in the peripheral blood during relapse. We postulate that the Th17 cells with increased α4 expression may have migrated from the periphery to the CNS.

αVβ3 integrin plays critical roles in various biological processes, including cell adhesion, migration, and signal transduction [50]. To date, there has been almost no research on the expression of αvβ3 integrin in the Th cells of patients with MS. In EAE model studies, Th17-mediated EAE was significantly suppressed in rats deficient in this integrin. Conversely, it had no effect on Th1-mediated EAE and could not infiltrate the CNS. Therefore, αvβ3 integrin may be required for the pathogenic migration of Th17 cells to the CNS in EAE [14, 51]. We found the highest expression of αVβ3 integrin in Th17 cells. We detected similar levels of β3 expression in the peripheral blood Th cells of the relapse, remission, and

control groups. This suggests that αVβ3 integrin contributes to tissue-specific or functional mechanisms in MS pathogenesis, rather than having any peripheral involvement.

This study had some limitations. These include its cross-sectional design and the lack of longitudinal observation of the relapse and remission phases of the patients with MS. Peripheral blood samples obtained from the same individuals at different time points would allow more accurate mapping of immune cell dynamics to disease progression. Furthermore, the integrins were evaluated only for their expression levels; we did not assess their functional activation status or ligand binding capacities.

In conclusion, this study revealed an increase in Th17.1 cells and a proinflammatory imbalance in the Th2/Th17.1 ratios in the peripheral blood of patients with RR-MS during periods of relapse. We also observed a decrease in αMβ2 integrin expression in Th17.1 cells. This suggests the need for new research directions concerned with the migration and functional properties of these cells. We tentatively conclude that the Th2/Th17.1 ratio in peripheral blood is a potential biomarker of disease activity in patients with MS. However, future studies with larger patient groups are needed to confirm this.

Declarations

Funding Declaration

The study was supported by the Afyonkarahisar Health Sciences University Scientific Research Projects Coordination Unit (project no. 24.TUS.004).

***Author contributions*:** All authors contributed to the study conception and design. Material preparation, and analysis were performed by Muammer Üzüm. Data collection was performed by Hayri Demirbaş and Abdullah Güzel. The first draft of the manuscript was written by Tülay Köken, revised and edited by Hayri Demirbaş and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

***Research involving human participants*:** This study was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. It was approved by the Kütahya Health Sciences University Rectorate Non-Interventional Clinical Research Ethics Committee (07.05.2024, decision no. 2024/06-28).

***Informed consent*:** All participants gave written informed consent to participation.

Data availability: Data are available upon reasonable request.

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Tables

Table 1. Demographic characteristics of the participants in each study group

	Healthy Controls (Mean ± SD)	Attack Phase patients (Mean ± SD)	Remission Phase Patients (Mean ± SD)
n	20	20	20
Age	27,70 ± 8,18	31,40 ± 11,13	40,15 ± 10,12
Gender (female/male)	14/6	16/4	13/7

SD, standard deviation

Table 2. Percentages of T helper subgroup cells within all Th cells in healthy controls and multiple sclerosis patients relapsed or in remission

	Healthy Controls (Mean ± SD)	Attack Phase patients (Mean ± SD)	Remission Phase Patients (Mean ± SD)
Total lymphocytes (%)	33,05 ± 8,74	25,73 ± 7,35*	29,84 ± 5,63
Th in all lymphocytes (%)	42,85 ± 6,22	44,82 ± 8,63	49,30 ± 9,09*
Treg (%)	5,08 ± 1,52	5,68 ± 2,58	5,00 ± 2,26
Th1 (%)	21,51 ± 5,39	22,55 ± 3,83	19,23 ± 5,01
Th2 (%)	42,65 ± 5,81	37,73 ± 5,89	40,80 ± 8,94
Th17 (%)	21,68 ± 4,92	22,25 ± 3,82	24,66 ± 4,75
Th17.1 (%)	14,16 ± 3,24	17,47 ± 3,06*	15,30 ± 5,25
Th2/Th17.1	3,41 ± 1,02	2,16 ± 0,53*	3,03 ± 1,51

*p <0.05 when compared with the control group

SD, standard deviation; Th, T helper cells; Treg, regulatory T cells

Table 3. Percentages of integrin expression on the surface of T helper subgroup cells in peripheral blood samples from in healthy controls and multiple sclerosis patients relapsed or in remission

Integrin	Cell	Healthy Controls (Mean ± SD)	Attack Phase patients (Mean ± SD)	Remission Phase Patients (Mean ± SD)
αLβ2	Th (%)	58,00 ± 13,62	61,39 ± 20,62	59,90 ± 19,94
	Th1 (%)	85,86 ± 6,60	83,17 ± 7,08	87,00 ± 7,67
	Th2 (%)	40,02 ± 14,79	32,79 ± 11,96	42,71 ± 15,78
	Th17 (%)	38,44 ± 9,65	36,48 ± 9,03	39,68 ± 14,22
	Th17.1 (%)	80,45 ± 6,88	79,60 ± 7,94	84,15 ± 8,93
αMβ2	Th (%)	2,16 ± 1,79	1,12 ± 0,93	1,25 ± 1,14
	Th1 (%)	3,42 ± 1,43	2,30 ± 1,32	2,17 ± 1,57
	Th2 (%)	0,62 ± 0,48	0,40 ± 0,35	0,44 ± 0,37
	Th17 (%)	0,63 ± 0,43	0,41 ± 0,25	0,44 ± 0,34
	Th17.1 (%)	2,55 ± 1,50	1,36 ± 0,80*	1,47 ± 1,15*
α4	Th (%)	75,23 ± 8,55	70,74 ± 11,09	72,88 ± 9,58
	Th1 (%)	83,48 ± 4,78	77,56 ± 8,68*	80,73 ± 7,00
	Th2 (%)	72,65 ± 11,54	64,30 ± 11,60	69,02 ± 11,94
	Th17 (%)	69,04 ± 10,55	62,58 ± 10,44	67,20 ± 12,10
	Th17.1 (%)	82,07 ± 5,57	77,46 ± 8,69	80,15 ± 6,96
β3	Th (%)	13,38 ± 3,07	15,57 ± 5,53	14,02 ± 3,32
	Th1 (%)	10,46 ± 3,16	12,20 ± 4,99	10,71 ± 2,77
	Th2 (%)	14,97 ± 3,61	16,07 ± 5,35	15,21 ± 3,70
	Th17 (%)	16,36 ± 3,75	17,69 ± 5,57	16,44 ± 4,04
	Th17.1 (%)	10,47 ± 3,34	12,55 ± 5,10	11,08 ± 2,87

*p <0.05 when compared with the control group

SD, standard deviation, Th, T helper cells

Figures

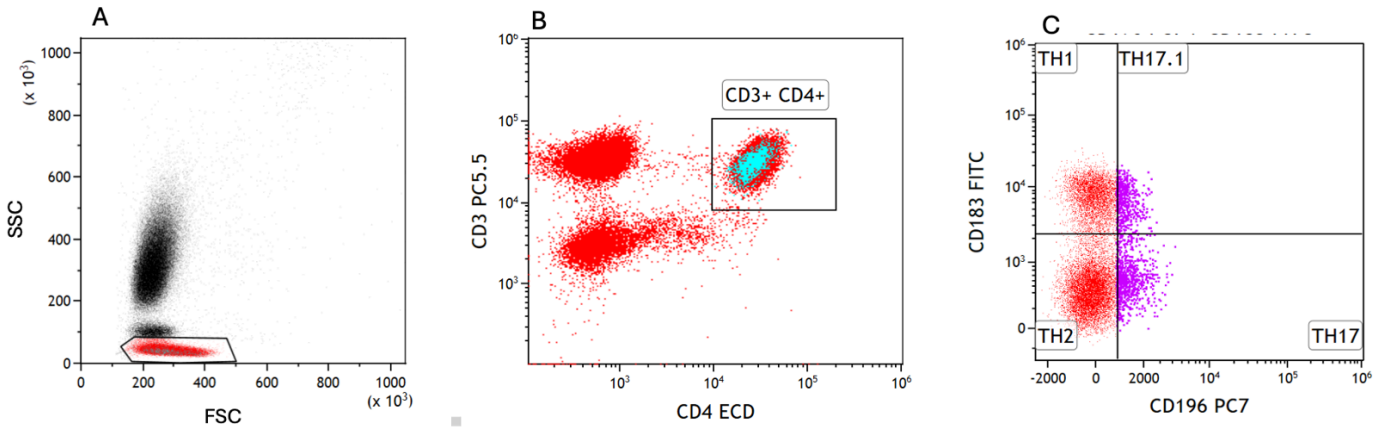


Figure 1

Quantification of Th cell subgroups in peripheral blood samples using flow cytometry. (A) Forward versus side scatter (FSC vs SSC) gating was used to identify lymphocytes; (**B**) The percentages of Th lymphocytes were determined by gating the cells from the lymphocyte gate showing double positive CD3 and CD4 expression; (**C**) Th subgroups were quantified by looking at CD183 and CD196 expression on Th lymphocytes

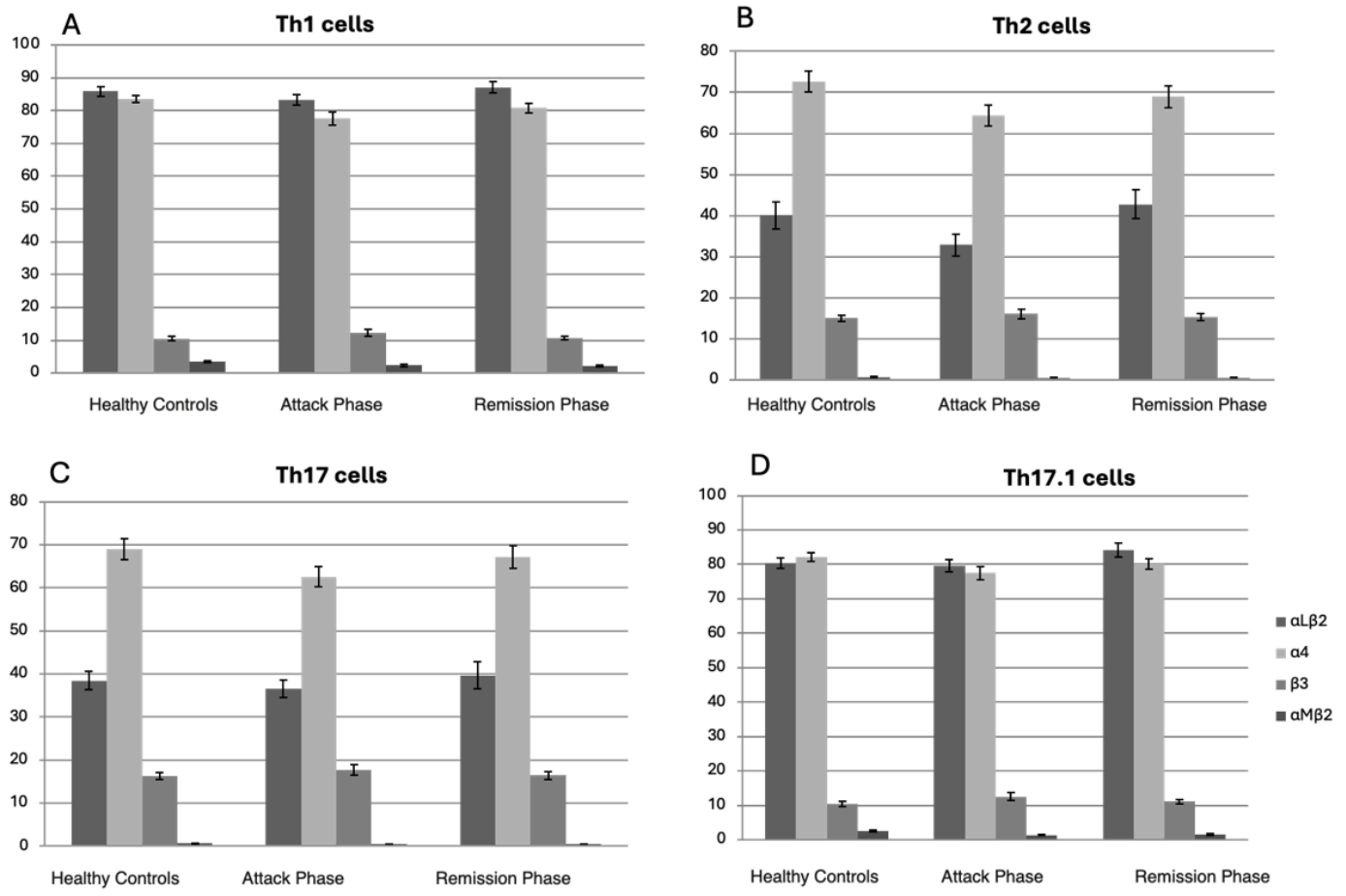


Figure 2

Percentages of αLβ2, αMβ2, α4, and β3 expressed in (**A**) Th1, (**B**) Th2, (**C**) Th17, and (**D**) Th17.1 cells from peripheral blood samples of patients with multiple sclerosis in remission, patients with relapsed multiple sclerosis, and healthy controls