BRIEF COMMUNICATION
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Low and High CD8 Positive T cells in Multiple Sclerosis Patients

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ABSTRACT

Cumulating evidence points to a key role for CD8+ T cells in the pathogenesis of multiple sclerosis.CD8 expression level was believed to be present constantly on the surface of human peripheral blood T cells. However, it was shown that peripheral blood lymphocytes may be divided by the level of CD8 expression, into CD8+^{high} and CD8+^{low} T cells. Now it is well established that the CD8^{low} population of CD8+ T cells demonstrates an activated effector phenotype while the CD8+^{high} T cells have been reported to have regulatory function.

In this report we used a flow cytometric assay to compare the frequency of these two subsets in multiple sclerosis patients (n=31) with healthy age- and gender-matched controls (n=18).

We found that CD8+ T cells and CD8+^{low} T cells significantly increased in secondary progressive (SP) and primary progressive multiple sclerosis (PPMS) patients in comparison to controls (p<0.0002 and p<0.004 respectively) and also RRMS (p<0.005 and p<0.017 respectively).

These results demonstrated the role of CD8^{low} T cells in progressive form of multiple sclerosis.

Keywords: CD8+ T cells; CD8+^{low} T cells; CD8+^{high} T cells; Multiple sclerosis

INTRODUCTION

Multiple Sclerosis (MS) is an autoimmune demyelinating and progressive degenerative disease of the central nervous system.[1,2] MS being twice as common in women and it typically develops in adults between the ages of 20 and 40 years.[3] Iran is also a high-risk area for MS disease, and the incidence has dramatically increased over the last 20 years.[4] The disease can lead to a wide variety of neurological deficits, depending on the specific regions of the CNS that become involved. Relapsing–remitting (RR) MS is the most frequent course of disease and as its name suggests, is characterized by the recurrent development of neurological deficits, followed by remissions. This form may develop into a secondary progressive (SP)

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phase, characterized by a progressive worsening of neurological function. Primary progressive (PP) MS, a form often seen in males, is characterized by an unremitting decline in neurological function.[5] Although the etiology of MS is unknown, but many findings indicate a central role for the immune system in the disease pathogenesis, and both genes and environmental factors (the most commonly proposed environmental factor is infection by a virus) influence the risk of developing disease.[6] Susceptibility to MS is associated with the MHC class II region, especially HLA – DR2, DR15 and DQ6.[7] As a result, it is widely believed that MS is a CD4+ Th1-mediated autoimmune disorder. Support for this view comes from a variety of studies involving both murine models and human beings.[8,9] While much emphasis had been placed on the role of CD4+ T cells in MS, many evidences have shown that CD8+ T lymphocytes might also have a critical role in this disease.[10,11] CD8+ T cells predominate over CD4+ T cells in both active acute and chronic MS brain lesions.[12,13]

CD8 expression is believed to be present constantly on the surface of human peripheral blood T cells. However, during flow cytometric analysis of lymphocyte populations, it was shown that peripheral blood lymphocytes may be divided by the level of CD8 expression, into $CD8^{high}$ and $CD8^{low}$ T cells.[14] Now, it is well established that circulating $CD8^{low}$ T cells resemble cytotoxic effector cells because they express cytolytic mediators and are cytotoxic. Therefore, the $CD8^{low}$ population represents a subset of activated CD8 effector T cells, resulting most probably from a continuous immune response to intracellular pathogens.[14] In this study, we examined the frequency of $CD8^{high}$ and $CD8^{low}$ T cells in MS patients with various clinical patterns and we observed the increased number of $CD8+^{low}$ T cells in SPMS and PPMS patients.

MATERIALS AND METHODS

Patients and Controls

Thirty-one MS patients (20 females, 11 males; mean age: 38.3±8.4) with clinically definite MS, according to the McDonald's criteria (McDonald et al., 2001) (19 RRMS, 6 PPMS and 6 SPMS) were studied. All patients met our predefined inclusion criteria of not being treated with any kind of IFN-β, receiving no corticosteroid for at least 3 prior months, with no previous history of hypersensitivity or recently acquired infectious diseases (e.g. respiratory and gastrointestinal infections) and were at acute phase. The RRMS patients had history of at least 2 relapses at the time of initial sampling. PPMS and SPMS patients had active progression. Exacerbation and progression were approved by the treating experienced neurologists. For each patient, the type of MS was determined according to the Lublin–Rheingold classification.[15] Disability was assessed by an experienced neurologist using Expanded Disability Status Scale (EDSS).[16]

The study was approved by ethics committee of our university and written informed consent was obtained from all patients prior to the study. All of the patients were referred to Iranian Center of Neurological Research in Emam-Khomeini General Hospital, Tehran University of Medical Sciences, Tehran, Iran.

Eighteen ethnically matched individuals (12 females, 6 males; aged: 37±7.4), who had no history of MS or other inflammatory diseases in their families, were used as healthy controls. All patients and controls were of Iranian Caucasian origin.

Flow Cytometry of T Cell Population

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from heparinized venous blood by density gradient centrifugation over Lymphodex (inno-Train, Diagnostic Gmbh, Germany) and stained with anti-CD8-PE-CY5 and anti-CD3-FITC (eBioscience San Diego, CA). Samples were analyzed using a FACSalibur flow cytometer (BD Bioscience).

Statistical Analysis

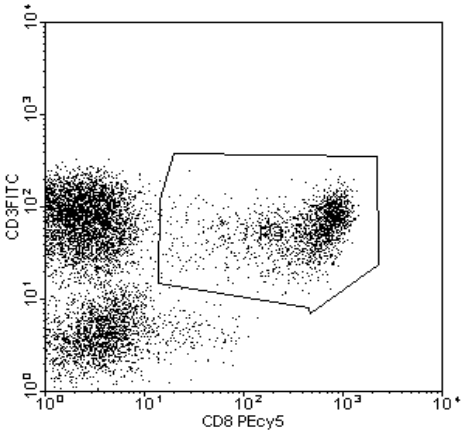
Data were analyzed in SPSS software (version 18.0; SPSS Inc., Chicago, USA). Variables were compared using student's t-test, or one-way Anova, followed by Scheffe post hoc comparisons. Results with $p<0.05$ were considered statistically significant.

RESULTS

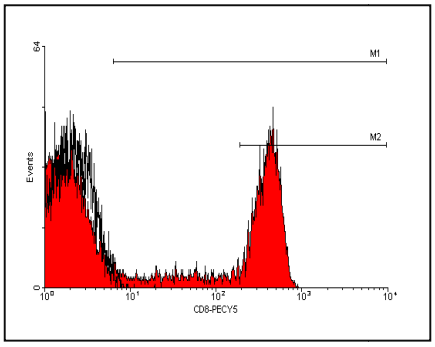
$CD8+$ T Cells and $CD8^{low}$ T Cells Significantly Increased in SPMS and PPMS Patients but not in RRMS Patients

We analyzed CD8+, $CD8^{high}$ and $CD8^{low}$ T cells using flowcytometry in 31 MS patients. CD3+ CD8+ T cells were determined on Lymphocytes gated on a forward vs. side scatter dot Plot. Then we analyzed

CD8+, CD8^{high} and CD8^{low} T cells in histogram on CD3+ CD8+ T cells gated cells. Figure 1A shows that cells expressing low levels of CD8 were CD3 positive. The strategy for quantification of the CD8^{low} and CD8^{high} T cell populations is shown in Fig. 1B. We observed a significant increase in the percentages of CD8+ T cells and CD8^{low} T cells amongst the patients with SPMS and PPMS (p<0.0002 and p<0.004, respectively) in comparison to controls (Figure 2A). The frequency of CD8^{high} T cells were also compared between SPMS/PPMS and healthy controls, however no difference was observed (Figure 2A). Analysis of CD8^{low} and CD8^{high} T cell populations in groups of patients with RRMS and healthy controls showed no significant differences (Figure 2B).



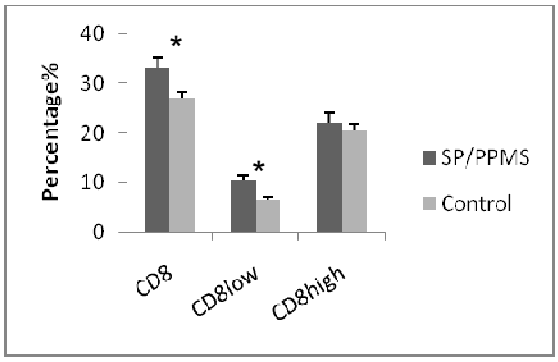
(A)



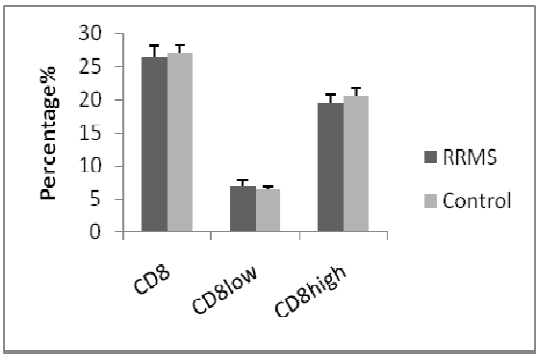
(B)

Figure 1. Human CD8^{low} and CD8^{high} T cells form distinct populations. (A) Dot plot of CD3-FITC fluorescence (y-axis) versus CD8-PE-CY5 fluorescence (x-axis) of T cells gated on the lymphocyte population. (B) CD8^{low} and CD8^{high} T cells within the indicated gates were determined using the Flowing software. M1and M2 show total CD8+ T cells and CD8^{high} T cells respectively.

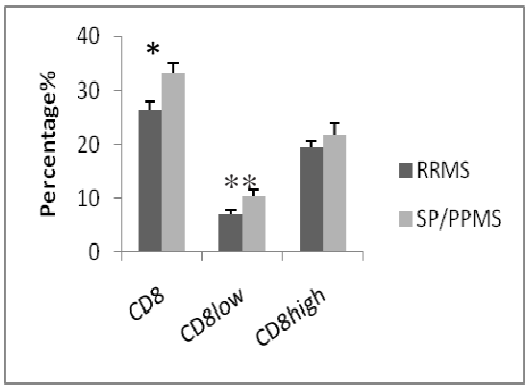
Comparison between SPMS/PPMS and RRMS patients also showed that the percentages of CD8+ T cells and CD8^{low} T cells significantly increased in SPMS and PPMS patients (p<0.005 and p<0.017, respectively) (Figure 2C).



(A)



(B)



(C)

Figure 2. Graphic representation of T cell population among different types of MS patients and healthy group. (A) Frequency of CD8 ^{low} T cells in SPM/PPMS and HC. (B) Frequency of CD8^{low} T cells in RRMS and HC. (C) Comparison of CD8 T cell subsets between SPMS/PPMS and RRMS. *p<0.001; **p<0.05

DISCUSSION

Cumulating evidence points to a key role for CD8 T cells in MS.[11] The goal of our study was to find out differences of CD8 + T cell populations between different types of patients with MS and then compare them with healthy subjects. Now it is well established circulating CD8+ T cells may be divided by the level of CD8 expression, into $CD8^{high}$ and $CD8^{low}$ T cells. $CD8^{low}$ T cells display a distinct flow cytometric pattern and form a small distinct population. The $CD8^{low}$ population of CD8+ T cells display an elevated expression of CD25, CD45RA, and CD95L, and low levels of CD28, CD62L and CD45RO and it represents activated effector CD8 T cells comprising a limited number of TCR Vβ.[14] According to pervious study, this distinct subset of CD8+ T cells show abundant perforin and granzyme B, pointing to their role as cytotoxic/effector T cells.[14] Increased $CD8^{low}$ T cells in patients with SPMS and PPMS in our study suggested this subset might have a unique role in the degeneration of myelin sheets of CNS.

$CD8^{low}$ T cells also produce significant amounts of the immunoregulatory cytokines IL-4 and IL-10 and it is probable that they may also play a role in immune regulation.[14,17] Utilizing a flow cytometric based approach, in this report, we found that total CD8+ T cells and $CD8^{low}$ T cell population significantly increased in SP and PPMS patients compared to healthy subjects and RRMS patients, but there was no significant difference between RRMS and controls. Inverse trend to our data was observed by another group, showing reduced frequency of $CD8^{low}$ cells in untreated RRMS subjects.[18] In particular, it is interesting that the same $CD8^{low}$ cell population was found to be increased in frequency after treatment with daclizumab (anti-IL2Ra) in a recent Phase 2 trial.[19] In addition a high frequency of myelin-reactive $CD8^{hi}$ $CD28^{-}CD57^{+}$ T cells was observed in MS patients.[20] Since it is well determined that $CD3^{+}$ $CD8^{low}$ T cells are generated from intracellular pathogens like EBV,[14] CMV and HIV,[21] our finding demonstrate the role of $CD8^{low}$ T cells as activated effector cells in progressive form of MS. These results may refer to the role of different kinds of viruses in the pathogenic process of MS which needs to be investigated more in the future. Our study showed the importance of different subsets of CD8+ T cells in MS patients but further research may be useful to disclose the function and kinetic of $CD8^{low}$ and $CD8^{high}$ T cell populations more precisely and their relationship with viral infections in MS patients compared to healthy controls.

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