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Decreased Expression of the CD57 Molecule in T Lymphocytes of Patients with Chronic Fatigue Syndrome

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Abstract

The chronic fatigue syndrome (CFS) is characterized by a prolonged incapacitating fatigue, headaches, sleep disturbances, and decreases in cognition, besides alterations in other physiological functions. At present, no specific biological markers have been described in this pathology. In the present study, we analyzed in lymphocytes the CD57 expression for the diagnosis of CFS, evaluating both the percentage of blood lymphocytes expressing CD57 and the average amount of the molecule expressed per cell. The study demonstrated a marked and significant decrease in the expression of CD57 in lymphocytes of CFS patients regarding healthy controls. In T lymphocytes, the decrease was significant both in the percentage of cells expressing CD57 (7.5 ± 1.2 vs 13.3 ± 1.6, $p = 0.024$) and in a more relevant way in the amount of CD57 molecule expressed per cell (331 ± 59 vs 1003 ± 104, $p \leq 0.0001$). In non-T lymphocytes, the decrease was significant only in the amount of CD57 expressed per cell (379 ± 114 vs 691 ± 95, $p = 0.007$). The study of CD57 antigen in blood lymphocytes is a useful marker that could cooperate in the diagnosis of CFS patients. Its decrease in T lymphocytes provides most valuable results than the results in other lymphocyte subpopulations.

Keywords Chronic fatigue syndrome · CD57

Abbreviations

CFS	Chronic fatigue syndrome
LD	Lyme disease

Introduction

The chronic fatigue syndrome (CFS) is a heterogeneous disorder of unknown etiology characterized by a prolonged disabling fatigue, headaches, sleep disturbance, and decreases in cognition [1, 2]. CFS could affect over 2.5 million Americans, and of these it is estimated that 90% have not yet been diagnosed among other causes because nowadays, no biomarker associated with the disease is available. The true prevalence of CFS is unknown [3]. In the UK, during the period 2001–2013, the annual incidence of CSF registered cases was 14.8 per 100,000 inhabitants [4].

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The main hypothesis on the onset of CFS is that it can take place after a viral infection or a period of stress. The hypothesis includes an altered central nervous system functioning resulting from an abnormal immune response against common antigens [5]. To date, no specific biological markers have been described for this pathology [6, 7]. CSF diagnosis is complicated many times since the patients present symptoms shared with in many other illnesses.

CD57 is an antigen with a sulfated glycan carbohydrate epitope in the terminal region. Its expression in T lymphocytes has been recognized as a marker of in vitro replicative senescence suffering the cells that express it, a reduced proliferative capacity and altered functional properties [8]. Until now CD57 has utility as a marker to measure functional immune deficiency in patients with autoimmune disease, infectious diseases, and some cancers [8]. In the same way, in patients with chronic Lyme disease (LD), they have been shown a decrease in the number of CD57+ natural killer (NK) cells without a specific T cell marker expression. But nevertheless in the acute Lyme, the patients maintain normal levels of these cells [9, 10]. There is a relationship between chronic Lyme disease and chronic fatigue syndrome. It has been described a group of patients with an "alternative diagnosis" of chronic Lyme syndrome, that is based in non-referenced methods, that

may produce false-positive results for more than 50% of people without Lyme disease [11, 12]. These patients with alternative diagnosis have a similar clinical phenotype to that of CFS patients, and many of them meet the diagnostic criteria of CFS [13].

Given the clinical similarity between patients with an "alternative diagnosis" of Lyme and patients with CFS, we propose to assess the utility of measurement of the CD57 molecule in the diagnosis of CFS.

Material and Methods

Patients

Twenty-two people, which sex distribution was an 80% of female and 20% of male, were diagnosed with CFS according to the Canadian criteria of 2003 [14], and subsequently they were reviewed with the WHO clinical criteria of February 2011 [15].

Initially, nine of them had a clinical suspicion of Lyme disease, either from living in rural areas or from suspicion of a tick bite. Everyone was tested for IgG and IgM serology (Borrelia Virclia®-Vircell) and a quantitative multiplex PCR for the detection of Borrelia sp. (Applied Biosystems). All the tests were carried out before the start of treatment with oral antibiotics. In all patients, the results were negative for both tests.

All patients were informed verbally about the study and given information sheets with written informed consent. As a control group, we choose 25 healthy people, 6 men and 19 women (76%), between 20 and 55 years, with an average age of 35 ± 11 years old.

Assay

The blood was collected from the antecubital vein of participants into EDTA collection tubes. Blood samples were analyzed within 4 h after the blood extraction.

We evaluate the sample of all participants by flow cytometry, using two monoclonal antibodies, PE conjugated anti-CD3 and FITC conjugated and anti-CD57 according to the cytometry protocol described by Cabrera et al. [16]. We analyzed the percent of cells with or without expression of CD57, as well as the mean amount of CD57 expressed per cell, quantified by the median value of the fluorescence (IF) detected in the CD57+ cell population.

Data Analysis

Statistical analyses were performed using IBM® SPSS® Statistics software (SPSS Inc., Chicago, USA). All data presented in this study are reported as means ± standard error of the mean (SEM). Comparative among CFS patients and healthy controls were performed using the non-parametric test U of Mann-Whitney. To assess the diagnostic capacity of CD57, a study of ROC curve was carried out. All statistically significant results had p value less than or equal to 0.05.

Results

CFS patients had an age between 20 and 68, with a mean age of 32 ± 3 years old. Inside the group of the patients, nine of the 21 patients had an initial suspicion of chronic LD, although they did not fulfill the diagnosis criteria of LD. All they had were an initial negative test for Borrelia IgG and IgM serology and negative PCR for the detection of Borrelia. The antibiotic treatments administered did not cause any clinical improvement. Five of them, however, showed a positive result for non-referenced tests for diagnosis of Lyme disease done in an external laboratory.

We compared between patients with CFS and healthy controls, on the one hand, the percentage of cells with CD57 expression and, on the other hand, the average expression per cell of CD57 molecule from the average fluorescence emitted (IF). The studies were carried out on T lymphocytes (CD3+) and on non-T lymphocytes. As shown in Fig. 1, in T cells, the percent of cells expressing CD57 were fewer in CFS patients than healthy controls (7.5 ± 1.2 vs 13.3 ± 1.6, $p = 0.024$). Conversely, in non-T lymphocytes, no differences were found (6.0 ± 1.1 vs 6.0 ± 0.6, $p = 0.41$). The IF analysis showed a marked and significant decrease on the mean expression per cell of CD57 both in CD3+ cells and in non-CD3 cells of patients with CFS with respect to the healthy controls (331 ± 59.3 vs 1003 ± 104, $p < 0.0001$ and 379.9 ± 114.1 vs 691 ± 95, $p = 0.007$ respectively). As shown in Fig. 1, the decrease was more pronounced in T cells.

With these data, we used the ROC curve analysis to value the ability of CD57 molecule to discriminate CFS patients from healthy individuals. The area under the curve in the ROC analysis was bigger in the IF results on T cells (0.874 ± 0.053: $p < 0.0001$) than on the non-T cells (0.737 ± 0.76; $p = 0.06$), and these results were greater than the results obtained on the percentage of T cells with expression of CD57 (0.726 ± 0.75; $p = 0.09$).

From these findings, together with the previous data that in patients with LD it has been proven a decrease in NK CD57+ cells, we compared the results between patients with an initial suspicion of LD and patients without initial clinical suspicion of LD. As shown in Fig. 2, no difference was observed either in the percentage of cells with CD57 expression or in the mean cell expression of CD57 between both groups.

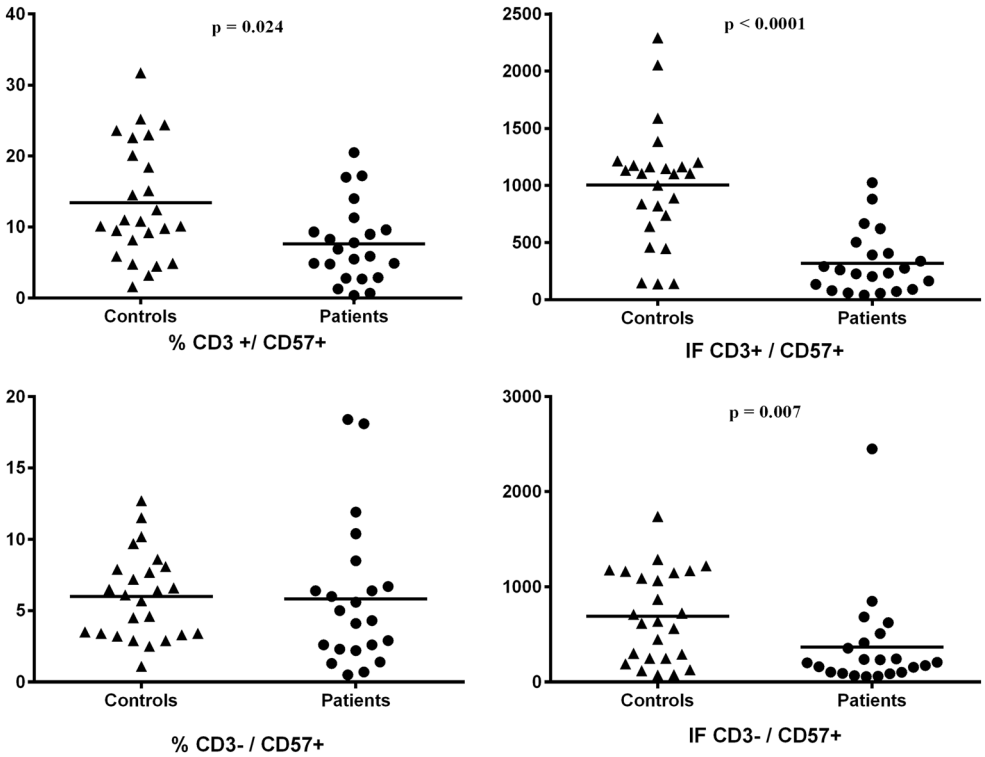

Fig. 1 Expression of CD57 molecule in T and non-T lymphocytes of patients with chronic fatigue syndrome. In the upper part, the results obtained in T lymphocytes (CD3+) are shown, while the lower part shows results of non-T cells (CD3 −). The left part shows the cell percentage with CD57 expression (%), while the right part shows results about the amount of CD57 expressed per cell quantified by the fluorescence median (IF). The mean expression of CD57 per cell is significantly lower in patients with CFS, and this effect is more prominent in T lymphocytes

Discussion

The CFS is a heterogeneous disease with unknown etiology for which until now, it has not been described any specific biological markers. This absence of one objective biochemical and/or cellular diagnostic markers has resulted to be a noteworthy problem in diagnosing the CFS, since the patients present symptoms which may appear in many other illnesses. In our work, we showed in patients with a diagnosis of CFS a decrease in the expression of the CD57 surface antigen,

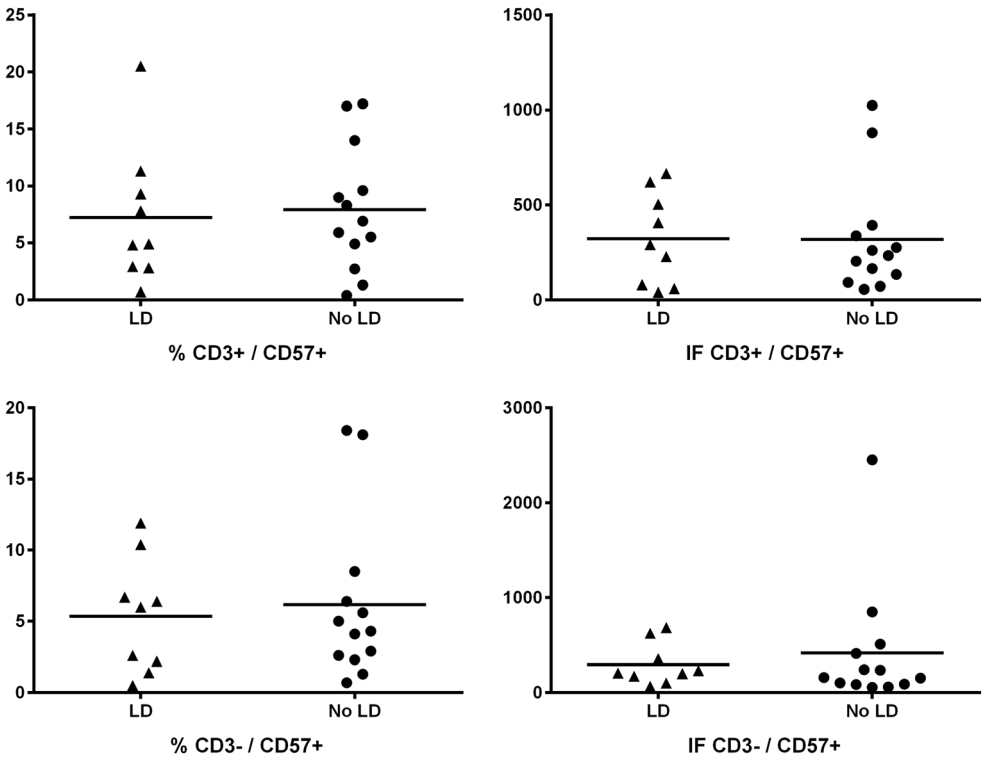

Fig. 2 Expression of CD57 in patients with initial suspicion of chronic Lyme disease (LD) or without an initial suspicion of chronic Lyme disease (not LD). In the upper part of the figure, the results of T lymphocytes (CD3+) are shown, while the lower part shows results of non-T cells (CD3 −). The left part shows the cell percentage with CD57 expression (%), while the right part shows results of the fluorescence mean (IF). There is no difference between the two groups

especially in T lymphocytes. Patients who have a termed "alternative diagnosis" of chronic Lyme syndrome have a similar clinical phenotype to that of CFS patients, and in one recent work between both types of patients did not find differences in the percentage of CD57 expressing cells [13]. From the aforementioned data, a possible overlap between both pathologies can be deduced, and it is possible that both belong to the same entity. With our results, we cannot rule out the presence of Borrelia as a potential CFS trigger agent whose infection has been resolved subsequently. Instead, patients with demonstrated chronic LD showed a significant decrease in the expression NK cells CD57+, while the acute LD patients sustain normal levels for the described cells [9]. Patients with CFS appear to have a variety of abnormalities in their immune cells that support the presence of an underlying immunological problem [6]. A large study on the role of cytokines in CSF demonstrated elevated levels of the cytokine TGFβ of immunosuppressive nature in the blood of patients [17]. These alterations are important too in T cells and NK, showing a lower cytotoxic activity and an increase in Treg lymphocytes [18]. CD57 molecule is related to T CD8 lymphocytes associated with a high rate of cytotoxicity and high ability to migrate to tissues [19]. A decrease of CD57 cellular expression could be related to a reduced ability to eradicate infections especially of intracellular nature. The CD57 decrease expression in T lymphocytes may be related to an insufficient maturation of the cells and consequently a functional immunodeficiency.

In this study, we show that CD57 antigen could be a useful marker for the diagnosis of patients suffering from the CFS. Additionally, the expressed average of CD57 protein per cell is a better marker than the percentage of cells expressing CD57, and its decreased expression in T lymphocytes is a better diagnostic marker than in non-T lymphocytes.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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