Flow cytometric analysis of CD4+ T cell receptor zeta chain deficiency in patients with systemic lupus erythematosus

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Abstract

Purpose: It has been reported that a dysfunction of T lymphocytes can be responsible for alteration in immune system in patients with systemic lupus erythematosus (SLE).

Material and methods: Using flow cytometric analysis, we determined the abnormalities of T cell receptor zeta (TCR ζ) chain contents in $CD4^+$ T cells of SLE patients.

Results: We observed a decrease in mean fluorescence intensity of TCR ζ in $CD4^+$ T cells of patients with SLE. The multiple analysis did not show a correlation between gender, age, disease specific manifestation, treatment, duration and TCR ζ mean fluorescence intensity in $CD4^+$ T cells.

Conclusions: High prevalence of TCR ζ chain deficiency in $CD4^+$ T cells confirms the significance of this signaling molecule in SLE pathogenesis.

Key words: SLE, TCR ζ, flow cytometry.

Introduction

The etiology of systemic lupus erythematosus (SLE) is still elusive. The abundant production of autoantibodies result in immune complexes formation. These immune complexes can be deposited in different tissues causing abnormal function of organs and clinical manifestation of SLE [1-3].

The T cells stimulation is triggered by direct interaction of the T cell receptor/CD3 complex (TCR/CD3) with an antigen bound to the major histocompatibility complex on antigen-presenting cells [4,5]. The TCR ζ is a component of CD3 complex and plays a pivotal role in the signaling cascade, which triggers biochemical events including second messenger and, transcription factors activation [5].

It has been reported that a dysfunction of T lymphocytes can be responsible for alteration in immune system in patients with SLE [6]. SLE T cells exhibit signaling defects, which result in enhancement of TCR/CD3 complex responses. These include disease-specific increase of intracytoplasmic calcium concentration and high-level phosphorylation of various intracellular proteins tyrosyl residue [1]. These receptor-mediated signaling aberrations are associated with deficient TCR ζ chain expression in patients with SLE [7]. The defective expression of the TCR ζ chain in SLE T cells has been determined by Western blotting technique [7].

We decided to evaluate the usefulness of flow cytometry analysis for evaluation of TCR ζ chain deficiency in peripheral $CD4^+$ T cells of patients with SLE.

Material and methods

Thirty patients (*Tab. 1*) tested in this study were diagnosed as having SLE according to the 1982 revised criteria of American College of Rheumatology [3]. Control samples (20 women and 2 men) were obtained from age and sex matched healthy individuals. The protocol of the study was approved by the Local Ethical Committee of Karol Marcinkowski University of Medical Sciences, Poznan. Written informed consent was obtained from all participating subjects. Patients who were on prednisone were asked not to take this medication for at least 24 h before drawing the blood. All participating patients were in active stages of the disease, which were determined by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (*Tab. 1*).

Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Ficoll-Hypaque ($d=1.077$ g/cm^3) [8]. For flow cytometry analysis, the PBMC were incubated for 30 min

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Table 1. **Demographic, organ involvement and immunological findings of SLE patients**

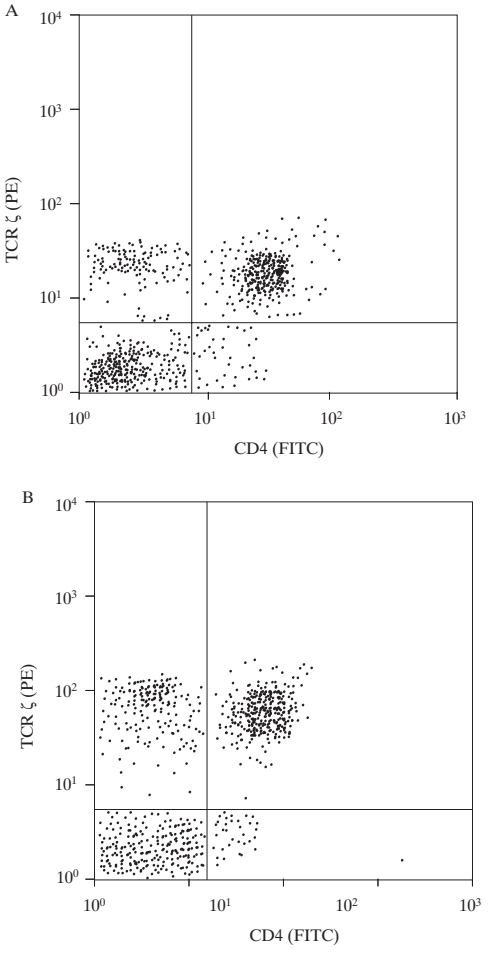
Features	
Number	30
Sex (Female/Male)	27/3
Median of age at diagnosis (years)	30 (range 19-58)
Median of disease duration (years)	6 (range 1-12)
Central nervous system*	5/30
Vascular*	3/30
Renal*	11/30
Musculoskeletal*	14/30
Serosal*	6/30
Dermal*	13/30
Immunologic*	6/30
Constitutional (fever)*	2/30
Hematologic*	10/30
Prednisone treated patients[b]	25/30
Hydroxychloroquine treated patients	6/30
No medication treaded patients	5/30
Median of SLEDAI scores	7 (range1-28)

* As defined by SLEDAI score index [6]; [b] Ten, seven, four, three and one patients were respectively receiving prednisone in dosage 10, 15, 20, 30 and 40 mg per day

with anti-CD4 Leu3A (Becton-Dickinson, Mountain View, CA) mouse monoclonal antibody (MmAb) conjugated with fluorescein isothiocyanate (FITC). After incubation, the cells were washed three times in phosphate buffered saline (PBS) supplemented with 2% FCS and 1% sodium azide (PBS/FCS). The cells were permeabilized with digitonin 10 μg/ml, fixed with 0.25% paraformaldehyde and washed three times with PBS/FCS. The cells were then stained with phycoerythrin (PE)-conjugated anti-TCR-ζ.

(6B10.2) MmAbs (Santa Cruz Biotechnology, Inc. CA) washed three times with PBS/FCS and immediately analysed in FACSCanto (Becton-Dickinson, Mountain View, CA). The TCR-ζ density on surface of cells was expressed as average of fluorescence intensity of analysed cells population (mean fluorescence intensity).

Figure 1. **Representative picture of flow cytometry analysis of TCR-ζ contents in CD4+ T cells of SLE patients (A) and healthy individuals (B).**
PBMC were incubated for 30 min with anti-CD4 Leu3A MmAb conjugated with FITC. After incubation, the cells were washed three times in PBS/FCS. The cells were permeabilized with digitonin 10 μg/ml, fixed with 0.25% paraformaldehyde and washed three times with PBS/FCS. The cells were then stained with phycoerythrin (PE)-conjugated anti-TCR-ζ (6B10.2) MmAbs washed three times with PBS/FCS and immediately analysed in FACSCanto (Becton-Dickinson, Mountain View, CA)



Results

Using flow cytometric analysis we observed a decrease in the expression of the TCR ζ chain in peripheral CD4+ T cells of SLE patients. The medians of TCR ζ mean fluorescence intensity were 44.0 (range 38.5-49.0) and 71.0 (range 48.5-74.5) in patients with SLE and healthy individuals (*Fig. 1*). The multiple analysis did not show correlation between gender, age, disease specific manifestations, treatment, duration and TCR ζ mean fluorescence intensity in CD4+ T cells. Our studies also showed the usefulness of flow cytometric analysis for determination of TCR ζ chain deficiency in CD4+ T cells. This analysis is easier and less time consuming than Western blotting technique. Using the flow cytometry analysis we confirmed the results of previous studies, which determined by Western blotting analysis the TCR ζ chain deficiency in total subset of SLE T cells [7].

Discussion

Present investigations of mechanism responsible for deficiency of TCR ζ chain in SLE T cells suggest abnormal expression of ζ chain at transcription and protein level [9,4]. Disregulation at transcription level encompasses defective expression of transcription factor Elf-1 as well as defective splicing of ζ heteronuclear RNA [8]. Recent investigations employing histone deacetylase inhibitor Trichostatin A indicates the possibility that abnormal chromatin structure can be also responsible for the decrease in ζ chain content in SLE T cells [10-12].

The TCR ζ can be replaced functionally by FcR gamma chain in SLE T cells [13]. The FcR gamma is a member of TCR ζ chain protein family and is also a component of the high-affinity IgE receptor [14]. The FcR gamma may mediate abnormal signal transduction through Syk kinase, which is more potent

than ZAP-70 kinase cooperating with TCR ζ [5]. The decrease of TCR ζ and increase of FcR gamma content in SLE T cells can be responsible for TCR/CD3-mediated signaling, which differs from the normal T cells [5]. To skew of TCR ζ and FcR gamma expressions, drugs or gene therapy can be used, resulting in correction of the defective signal transduction in SLE T cells [8].

High prevalence of TCR ζ chain deficiency in $CD4^+$ T cells of SLE patients indicates the significance of this signaling molecule in SLE pathogenesis. However, the effect of TCR ζ chain deficiency on mechanism of cytoplasmic proteins hyperphosphorylation and increase in SLE T cells profile calcium response, requires further investigation.

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