RESEARCH LETTER

Dysregulated interferon-γ and interleukin-2 synthesis in peripheral blood T cells in quiescent systemic lupus erythematosus is dependent on the affected T-cell receptor ζ chain expression

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Introduction Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease characterized by aberrant function of T cells and hyperreactive state of B cells. It is associated with the generation of autoantibodies and immune complex deposition inducing organ failure.[1] The disrupted T-cell functions might result from defective signaling through the T-cell receptor (TCR). The TCR ζ chain (TCRζ; CD247) is the main component of the TCR–CD3 complex and is critical for transducing the signal into the nucleus. TCRζ-mediated signaling results in cell proliferation and differentiation.[1]

A proportion of circulating T cells in SLE, characterized by diminished expression of TCRζ, is known as TCRζ^{dim} cells. In vitro studies have shown that a microenvironment rich in proinflammatory cytokines promotes the impairment of TCRζ expression and an increase in the percentage of TCRζ^{dim} cells.[2] The presence of TCRζ^{dim} lymphocytes in peripheral blood (PB) has been confirmed in various chronic inflammatory, neoplastic, and infectious diseases, and this is accompanied by the presence of biochemical inflammatory markers.[1] In addition, a correlation has been found between a frequency of TCRζ^{dim} cells and autoreactive CD4$^+$CD28$^-$ cells present in PB of SLE patients.[3] Although many reports demonstrated the lower TCRζ expression and mechanisms of its downregulation in SLE,[4,5] little is known about functional significance of TCRζ^{dim} cells in autoimmune disorders.

The main aim of the present study was to define the frequency of autoreactive TCRζ^{dim} lymphocytes in the blood of patients with low-activity SLE and to provide a functional characterization of TCRζ^{dim} and TCRζ^{bright} T cells based on the assessment of their capacity to synthesize certain cytokines secreted by type 1 helper T cells: interferon γ (IFN-γ) and interleukin 2 (IL-2) involved in SLE pathogenesis and to compare these results with values in healthy controls.

Patients and methods The study group consisted of 20 patients (18 women and 2 men) fulfilling the American College of Rheumatology classification criteria for SLE,[6] who showed quiescent disease and impaired expression of TCRζ in T cells, based on a preliminary estimation of the mean fluorescence intensity (MFI) as compared with the normal range in healthy individuals. The mean Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) in the patient group was lower than 8. The control group consisted of 20 healthy individuals matched for age and sex.

Patient characteristics, including age, sex, disease duration and activity (measured by SLEDAI), laboratory parameters (complement C3 and C4, lymphopenia, positivity for anti–dsDNA antibodies, erythrocyte sedimentation rate, C-reactive protein), organ involvement and treatment, as well as reagents used, were presented in detail in Supplementary material (*Table S1*).

Cell preparation and culture stimulation were performed according to the recently described methods.[7] Surface staining of T cells was performed by a standard method. For intracellular staining of the TCRζ and cytokines, cells were fixed with 2% formaldehyde and permeabilized in buffer containing 0.5% saponin according to a protocol described previously.[8] The frequency of IFN-γ and IL-2–expressing TCRζ^{dim} and TCRζ^{bright}

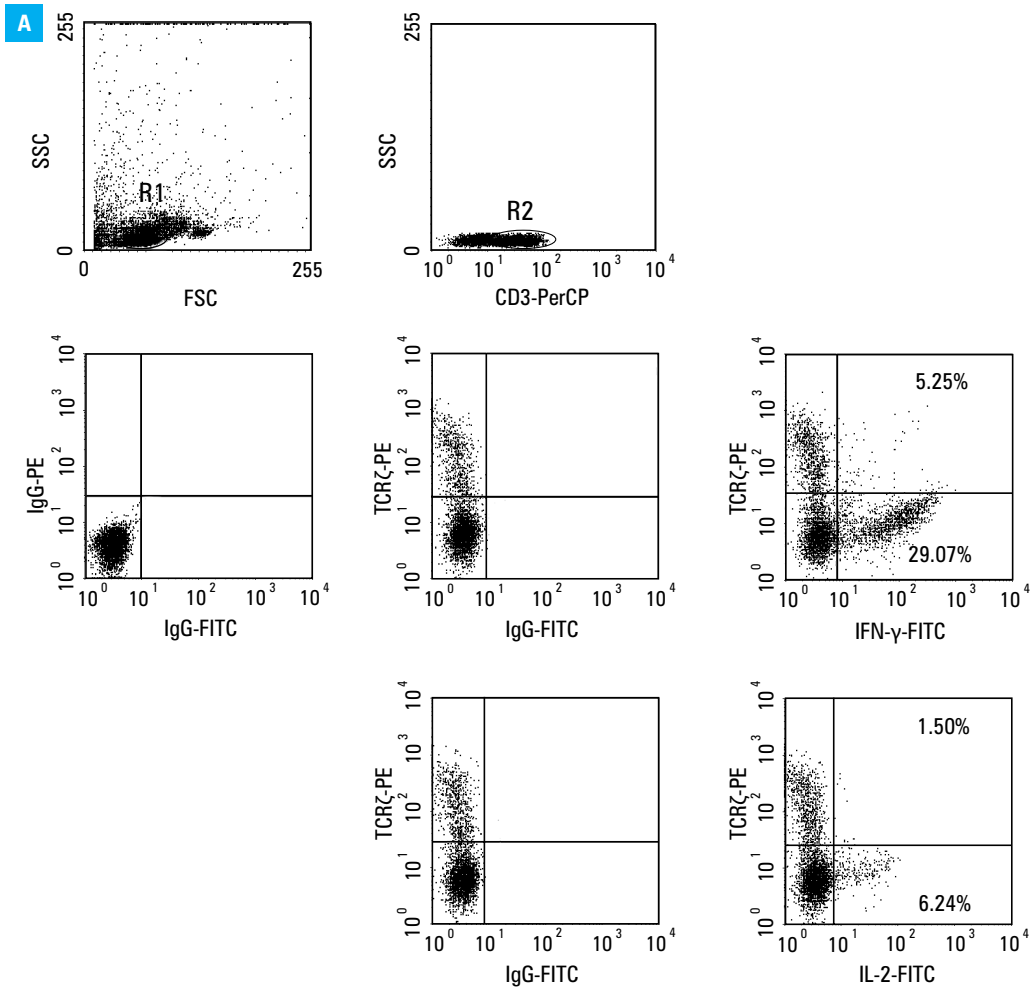

FIGURE 1 **A** – interferon-γ (IFN-γ) and interleukin-2 (IL-2) expression in T cell–receptor ζ^{bright} (TCRζ^{bright}) and T cell––receptor ζ^{dim} (TCRζ^{dim}) cells in T lymphocytes from patients with quiescent systemic lupus erythematosus. The dot plots show data illustrating the analysis method for identification of TCRζ^{bright} and TCRζ^{dim} cells synthesizing IFN-γ or IL-2 following three-color staining. The dot plots show the forward scatter/side scatter (FSC/SSC) distribution, and the gate (region R1) was used to select lymphocytes for analysis. The R1-gated events were then analyzed for CD3--PerCP staining, and CD3-positive cells were gated (as region R2). The final dot plots show the frequency of IFN-γ or IL-2–expressing TCRζ^{bright} (right-upper quadrant) and TCRζ^{dim} cells (right-bottom quadrant). Isotype-matched control monoclonal antibodies were used to confirm expression specificity.
Abbreviations: FITC, fluorescein isothiocyanate; PE, phycoerythrin

T cells was determined by flow cytometry using a FACSCalibur (Becton Dickinson, San Diego, California, United States). Representative flow cytometry data are presented in FIGURE 1.

Statistical analysis A *P* value of less than 0.05 was considered significant. If relevant assumptions were met, the groups were compared with the parametric *t* test. Otherwise, the nonparametric Mann–Whitney test was used. For dependent variables, the nonparametric Wilcoxon test or *t* test was used.

Results The numeric data are summarized in Supplementary material (*Table S2*). The analysis of the MFI of the TCRζ in T cells revealed its semiquantitative defect in SLE ($P = 0.004$). We found that in PB of SLE patients, the CD3$^+$TCRζ^{dim} count was higher than in controls ($P < 0.001$). In unstimulated and stimulated cultures, we observed a decrease in the percentages of TCRζ^{dim} T cells in all studied individuals ($P \leq 0.04$); however, the values were higher in SLE. Accordingly, an upregulated mean percentage of TCRζ^{dim} T cells was seen in both unstimulated ($P = 0.03$) and stimulated cultures ($P < 0.001$) in patients with SLE, as compared with controls.

A comparative functional analysis revealed the predominance of IFN-γ–producing TCRζ^{dim} T cells over TCRζ^{bright} T cells ($P = 0.01$). In contrast, the production of IFN-γ in controls was mainly seen in TCRζ^{bright} T cells ($P = 0.05$). In the SLE group, the frequency of TCRζ^{bright}IFN-γ$^+$ T cells was lower than in controls ($P < 0.001$). In contrast, the mean proportion of TCRζ^{dim}IFNγ$^+$ T cells was higher in patients with SLE ($P = 0.02$), resulting in a tendency to generate an increase in the proportions of total T cells producing IFN-γ ($P = 0.08$) (FIGURE 1).

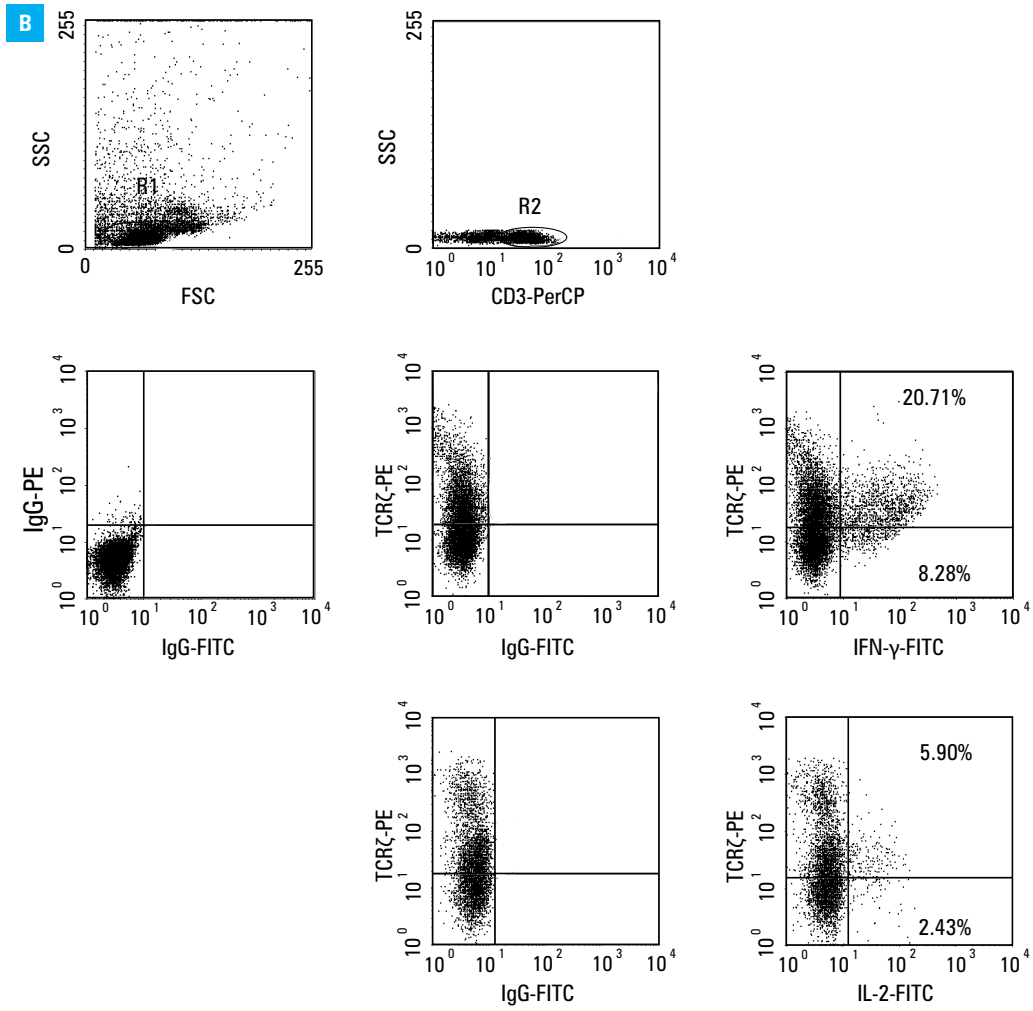

FIGURE 1 B – interferon-γ (IFN-γ) and interleukin-2 (IL-2) expression in T cell–receptor ζ^{bright} (TCRζ^{bright}) and T cell–receptor ζ^{dim} (TCRζ^{dim}) cells in T lymphocytes from healthy controls. See FIGURE 1A legend on the previous page for a detailed description.

We also observed a defect in the mean proportion of IL-2–producing T cells in SLE (P = 0.008). In controls, the frequency of TCRζ^{bright}IL-2^{+} T cells was higher than the percentages of TCRζ^{dim}IL-2^{+} T cells (P <0.001). Similar situation was observed in SLE; however, the difference had lower significance (P = 0.02). We found no differences between the frequencies of TCRζ^{dim}IL-2^{+} T cells from SLE and controls. On the contrary, the proportions of TCRζ^{bright}IL-2^{+} T cells in patients were lower than those observed in controls (P = 0.002).

Among the studied immune and clinical parameters, we found a strong negative correlation between the MFI of IL-2 in CD3^{+}TCRζ^{dim} cells and SLEDAI (r = –0.79, P = 0.02). Also, the percentages of TCRζ^{dim} T cells were higher in patients with anti–dsDNA antibodies in sera compared with those without the antibodies (P = 0.007). We found no significant correlations with other clinical parameters, probably due to the small number of individuals and low SLE activity.

Discussion Our present study demonstrated the presence of TCRζ^{dim} T cells in SLE patients despite very low disease activity. Our observation that TCRζ^{dim} cells are more prevalent in patients with high serum levels of anti–dsDNA antibodies confirms former studies indicating the association of this defect with disease development and clinical course. It is currently believed that TCRζ^{dim} lymphocytes involve some of autoreactive T cells, and as such they are gaining increasing attention in studies on the pathogenesis of autoimmune disorders.

Molecular causes of TCRζ defect in SLE are complex. Recent findings linked the lower TCRζ abundance with its gene polymorphisms or abnormal mRNA splicing.[4,5] The regulation of TCRζ expression may also occur at a protein level, and includes internalization, degradation, and intracellular recycling of TCRζ. It is tightly controlled at different stages of cell activation, and TCRζ early re-expression into the cell membrane occurs shortly after the termination of stimulation.[9] TCRζ impairment in SLE may be caused by chronic stimulation of TCR with factors present in PB, which may disturb degradation of TCRζ. Some of them may affect the activity of the TCRζ gene promoter and include tumor necrosis factor α and

immunoglobulin G autoantibodies found at high serum levels in patients with low disease activity.[2] The maintenance of TCRζ^{dim} cells at higher values in unstimulated culture confirms the presence of long-lasting exogenous factors inhibiting TCRζ expression in SLE.

We also compared the ability of CD3$^+$TCRζ^{dim} and CD3$^+$TCRζ^{bright} cells to produce IFN-γ and IL-2 involved in the pathogenesis of SLE. IFN-γ induces the differentiation of B lymphocytes secreting autoantibodies. In contrast, IL-2 is involved in the maintenance of T-cell homeostasis and immunological tolerance including normal regulatory T-cell function and number. We confirmed that T lymphocytes in SLE patients, despite a lower TCRζ expression, showed increased capacity to synthesize IFN-γ and diminished ability to produce IL-2, even in patients with inactive disease.[10] The immunological defects in patients with quiescent autoimmune disorders have been described previously.[7] Consistently, in vitro stimulation of T lymphocytes in SLE may lead to IFN-γ overproduction associated with the diminished expression of TCRζ.[10] Our finding of the predominance of TCRζ^{dim}IFNγ$^+$ T cells over TCRζ^{bright}IFN-γ$^+$ population only in SLE patients corresponds with the previous observations.[10] The percentage of TCRζ^{dim}IFN-γ$^+$ T lymphocytes in SLE was also higher compared with healthy controls. It strongly indicates SLE-mediated dysregulation of the signal transduction pathway in TCRζ^{dim} lymphocytes. This phenomenon, reported only in patients with SLE, has been attributed to the functional and structural substitution of TCRζ by the γ-chain subunit of the Fc receptor (FcRγ).[11] TCRζ^{dim} cells account for a substantial proportion of IFN-γ–producing T cells, since we also noted a trend towards enhancement of the total IFN-γ$^+$ T-cell population in quiescent SLE.

In contrast to the impact on IFN-γ, the FcRγ-dependent activation of T cells in SLE does not result in an increased IL-2 synthesis. T lymphocytes in SLE are actually weak producers of IL-2.[1] In this study, we showed diminished capacity to synthesize IL-2 in T lymphocytes despite the low activity of the disease in SLE patients. Contrary to the IFN-γ synthesis, we observed that the presence of TCRζ favors the production of IL-2 primarily in controls. However, the TCRζ^{bright}IL-2$^+$ cell population in SLE remains significantly lower than in healthy controls probably due to the quantitative defect of TCRζ. In fact, the association between TCRζ and IL-2 expression occurs at the level of transcription regulation under the same stimulating conditions.[10] The opposite effect of TCRζ downregulation on IFN-γ and IL-2 synthesis shown recently in SLE seems to be in accordance with the current report. The in vitro restoration of TCRζ in SLE normalizes signal transduction and restores normal IL-2 and IFN-γ synthesis.[12]

Our report indicates that SLE patients show immune abnormalities despite no signs of active disease; dysregulated IFN-γ and IL-2 synthesis may be linked to the higher abundance of TCRζ^{dim} cells within peripheral T lymphocytes. We also showed that TCRζ^{dim} T cells are prevalent primarily in patients positive for anti–dsDNA antibodies, which are closely linked to the clinical course of SLE, thus emphasizing a pathogenic role for TCRζ^{dim} T cells in this autoimmune disorder.

The limitation of the present study is the enrollment only of patients with a decreased TCRζ expression. It remains to be clarified if there are any differences in clinical treatment, laboratory parameters, or T-cell function between SLE patients with impaired TCRζ expression and those with the cells within the normal range. If so, further studies are needed to verify whether the higher frequency of TCRζ^{dim} cells in PB might predict SLE flares. Therefore, our results should be considered as preliminary data and conclusions should be drawn with caution due to the lack of a comparative analysis.

SUPPLEMENTARY MATERIAL Supplementary material is available with the article at www.pamw.pl.

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