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Th17 cells and CD4⁺ multifunctional T cells in patients with systemic lupus erythematosus



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ABSTRACT

Introduction/Objective: Recent evidence suggests that abnormalities involving Th17 lymphocytes are associated with the pathophysiology of systemic lupus erythematosus (SLE). In addition, multifunctional T cells (MFT), i.e., those producing multiple cytokines simultaneously, are present in the inflammatory milieu and may be implicated in the autoimmune process observed in SLE. In the present study, we aimed to characterize the functional status of CD4⁺ T cells in SLE by simultaneously determining the concentration of IL-2, IFN- γ and IL-17 in lymphocyte cultures under exogenous and self-antigenic stimuli.

Patients and methods: Eighteen patients with active disease, 18 with inactive disease, and 14 healthy controls had functional status of CD4⁺ T cells analyzed.

Results: We found that SLE patients presented a decreased number of total CD4⁺ cells, an increased number of activated T cells, and an increased frequency of Th17 cells compared to healthy controls (HC). MFT cells had increased frequency in SLE patients and there was an increased frequency of tri-functional MFT in patients with active SLE compared with those with inactive SLE. Interestingly, MTF cells produced larger amounts of IFN γ than mono-functional T cells in patients and controls.

Conclusion: Taken together these data indicate the participation of recently activated Th17 cells and MTF cells in the SLE pathophysiology.

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Linfócitos Th17 e linfócitos T CD4⁺ multifuncionais em pacientes com lúpus eritematoso sistêmico

RESUMO

Introdução/Objetivo: Evidências recentes sugerem que anormalidades que envolvem os linfócitos Th17 estão associadas à fisiopatologia do lúpus eritematoso sistêmico (LES). Além disso, os linfócitos T multifuncionais (LTM), ou seja, aqueles que produzem múltiplas

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Th17
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citocinas simultaneamente, estão presentes no meio inflamatório e podem estar implicados no processo autoimune observado no LES. No presente estudo, objetiva-se caracterizar o estado funcional dos linfócitos T CD4⁺ no LES e determinar simultaneamente a concentração de IL-2, IFN- γ e IL-17 em culturas de linfócitos sob estímulos exógenos e autoantigênicos.

Pacientes e métodos: Dezoito pacientes com doença ativa, 18 com doença inativa e 14 controles saudáveis foram submetidos à análise do estado funcional dos linfócitos T CD4⁺.

Resultados: Encontrou-se que os pacientes com LES apresentaram uma diminuição na quantidade total de células CD4⁺, um aumento na quantidade de linfócitos T ativados e um aumento na frequência de linfócitos Th17 em comparação com controles saudáveis (HC). As células LTM tinha frequência aumentada em pacientes com LES e houve um aumento na frequência de LTM trifuncionais em pacientes com LES ativo em comparação com aqueles com LES inativo. Curiosamente, as células MTF produziram quantidades maiores de IFN- γ do que os linfócitos T monofuncionais em pacientes e controles.

Conclusão: Analisados em conjunto, esses dados indicam a participação dos linfócitos Th17 recentemente ativados e células MTF na fisiopatologia do LES.

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Introduction

Effector CD4⁺ T cells have been initially categorized into two subsets based on the cytokines they produce.¹⁻³ Th17 cells are part of this new T cell scenario and display an activated CD4⁺ T cell phenotype characterized by the production of high amounts of IL-17.⁴⁻⁶ Th17 cells seem to play a crucial role in the development of a wide range of autoimmune and chronic inflammatory disorders.⁷⁻⁹ In fact, it has been suggested that inappropriate regulation of Th17 cells may be a key event in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus (SLE).¹⁰⁻¹⁴ Several studies reported significantly higher serum levels of IL-17 and higher frequency of IL-17-producing peripheral blood mononuclear cells (PBMC) in SLE patients as compared to normal individuals.^{13,15-18} It was also shown that Th17 response is correlated with disease activity in SLE patients.¹⁷

A novel strategy for the evaluation of T cell functionality is based on the simultaneous determination of several cytokines expressed by subtypes of cells. According to this approach T cells that produce multiple cytokines simultaneously are termed multifunctional T cells. Multifunctional T cell responses have been documented in HIV-1¹⁹ and in the immune response to vaccination against Hepatitis B virus²⁰ and HIV.^{19,20} This novel approach has been largely possible due to technological advances in flow cytometry that nowadays allows for the simultaneous detection of numerous functional, phenotypic, and lineage markers on T cells.²¹

In the present study, we sought to further characterize the functional status of CD4⁺ T cells in SLE patients with active and inactive diseases by simultaneously determining the concentration of several cytokines in lymphocyte cultures under different antigenic stimuli. We also quantified the proportion of Th17 and multifunctional T cells and correlated them with SLEDAI (Systemic Lupus Erythematosus Disease Activity Index), and with the frequency of activated T cells and T_{REG} cells.

Materials and methods

Thirty-six adult patients (33 women and 3 men, aged 40 \pm 7.2 years) with a diagnosis of SLE based on the American College of Rheumatology criteria²² were consecutively enrolled in the study after providing informed consent. All patients were referred from the outpatient clinic at the Division of Rheumatology of the Universidade Federal de São Paulo (Federal University of São Paulo). Patients were divided into two groups based on their SLE Disease Activity Index (SLEDAI) score.^{23,24} The active SLE group (A-SLE) comprised 18 patients (SLEDAI score ≥ 6 ; 17 women and 1 man, aged 36.7 \pm 10.2 years) and the inactive SLE group (I-SLE) comprised 18 patients (SLEDAI score = 0; 16 women and 2 men, aged 39.2 \pm 13.9 years). **Table 1** depicts the demographic and clinical characteristics of all patients enrolled in the study. As a control, 14 healthy laboratory workers were enrolled in the study (13 women and 1 man, aged 33.9 \pm 10.4 years) after giving informed consent. Patients and healthy controls underwent a structured questionnaire and donated 60 mL of venous blood. The study protocol was reviewed and approved by the institution's research ethics committee.

Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient sedimentation over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) and washed twice in Hank's balanced salt solution (Gibco, Grand Island, NY). For cryopreservation, cells were slowly frozen in 90% of fetal bovine serum (FBS; HyClone Laboratories, Logan, UT), and stored in liquid nitrogen. At the time of the assay, PBMC were rapidly thawed in a 37 °C water bath and washed in pre-heated RPMI 1640 supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μ g/mL streptomycin and 20 mM glutamine (R10). Cells were counted, checked for viability, and suspended in R10 at a concentration of 1 \times 10⁶ viable cells/mL.

Thawed PBMC were incubated in 96-well plates (200 μ L/well) (Becton Dickinson, San Jose, CA) in the presence of 100 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma) and 500 ng/mL ionomycin or with 100 ng/mL HEP-2 extract for 16 h. After stimulation, cells were centrifuged at

Table 1 – Demographic characteristics of controls and patients with systemic lupus erythematosus.

	Controls (n = 14)	A-SLE (n = 18)	I-SLE (n = 18)	p Value
Age	33.9 ± 10.4	36.7 ± 10.2	39.2 ± 13.9	0.624
Female (%)	92.8%	94.4%	88.9%	0.987

SD, standard deviation; A-SLE, active systemic lupus erythematosus; I-SLE, inactive systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; ns, not significant.

1500 g for 5 min, suspended in Macs buffer and transferred into V-bottom 96-well plates (Nunc, Roskilde, Denmark) in 100 μ L of staining buffer [phosphate-buffered saline (PBS) supplemented with 0.1% sodium azide (Sigma) and 1% FBS, pH 7.4] with the panel of surface monoclonal antibodies (CD4-PerCP, CD3-APC-CY7 and CD69-PE-Cy7). Cells were incubated at 4 °C in the dark for 30 min, washed twice with PBS and then suspended in 100 μ L of fixation buffer [1% paraformaldehyde (Polysciences, Warrington, PA) in PBS, pH 7.4]. For intracellular staining, cells already labeled for surface markers were incubated with 100 μ L of 4% fixation buffer and washed with permeabilization buffer (PBS supplemented with 0.1% sodium azide, 1% FBS and 0.1% saponin; Sigma). Each sample was suspended in 100 μ L of permeabilization buffer, incubated for 15 min at room temperature in the dark, washed with 100 μ L of staining buffer and incubated for 30 min at 4 °C in the dark with either no antibody (unstained tube) or with a pool of anti-IL-2-PE, anti-IFN- γ -APC, and anti-IL-17-FITC monoclonal antibodies in 50 μ L of staining buffer.²⁵ Cells were washed with 200 μ L of staining buffer and suspended in 100 μ L of freshly prepared 1% paraformaldehyde (PFA) pH 7.4.

T_{REG} cells and effector CD25⁺ T cells were identified according to a previously established protocol.²⁶ Briefly, PBMCs were washed in PBS and 0.5 \times 10⁶ viable cells were incubated with fluorescein isothiocyanate (FITC)-labeled anti-CD127, allophycocyanin-Cy3 (APC-Cy3)-labeled anti-CD3, PerCP-labeled anti-CD4, and phycoerythrin (PE)-Cy7-labeled anti-CD25 (Becton Dickinson, San Jose, CA, USA) antibodies, according to the manufacturer's instructions. After 30 min of incubation at 4 °C in the dark, cells were washed with Macs buffer, fixed and permeabilized with FoxP3 fixation/permeabilization buffer (eBioscience, San Diego, CA, USA) and then processed for FoxP3 staining using the FoxP3 staining kit and APC-labeled anti-FoxP3 (eBioscience) according to the manufacturer's instructions.

Samples were processed on a FACSCanto flow cytometer, using FACSDIVA software (BD Biosciences), and the acquired data were analyzed with FLOWJO software (Tree Star, San Carlo, CA). Fluorescence voltages were determined using matched unstained cells. Compensation was carried out using CompBeads (BD Biosciences) single stained with CD3-PerCP, CD4-FITC, CD8-APC-CY7, CD4-PE-CY7, CD3-PE or CD3-APC, respectively. Samples were acquired until at least 500,000 events in a live cell gate were obtained.

Data were reported as median and interquartile range (IQR). Comparisons among groups were carried out using the Kruskal-Wallis non-parametric test, followed by inter-group comparisons by the Dunnett test. Correlations were performed using the Spearman's non-parametric method. Statistical inference level was established at 5% ($p < 0.05$).

Results

Decreased frequency of CD4⁺ T cells and increased frequency of newly activated cells in SLE

The relative frequency of CD4⁺ cells over total CD3⁺ cells in PBMC cultures stimulated with HEP-2 cell extract was significantly lower in samples from patients with A-SLE (18.4 ± 8.9) and I-SLE (17.3 ± 7.6) as compared to controls (24.0 ± 8.2) (Fig. 1A). Likewise, PBMC cultures stimulated with PMA/Io presented lower relative frequency of CD4⁺ cells over total CD3⁺ cells in samples from patients with A-SLE (16.6 ± 8.4) and I-SLE (19.6 ± 7.6) as compared to controls (25.3 ± 6.4) (Fig. 1B). The same was observed for non-stimulated PBMC cultures (data not shown). In contrast, when we evaluated newly activated cells, characterized by the expression of the CD69 molecule, higher relative frequency of CD4⁺CD69⁺ cells over total CD4⁺ cells was observed in patients with A-SLE (5.1 ± 6.6) and I-SLE (4.9 ± 5.2) when compared to controls (2.8 ± 1.9) in cultures stimulated with HEP-2 cell extract (Fig. 1C) as well as with PMA/Io: A-SLE (6.6 ± 8.3), I-SLE (5.9 ± 5.5), and controls (3.5 ± 2.0) (Fig. 1D). Again, a similar behavior was observed in non-stimulated PBMC cultures (data not shown).

Multifunctional profile of CD4⁺ T cells in SLE

Next, we investigated the functional profile of CD4⁺ cells after auto-antigen-specific (HEP-2 extract) and non-specific (PMA/Io) stimuli. This was accomplished by simultaneously determining intracellular IL-2, IL-17 and INF- γ by flow cytometry after in vitro stimulation with HEP-2 extract and PMA/Io, respectively. Fig. 2A shows the gating strategy and the multiparametric characterization of mono-, bi-, and tri-functional response patterns in a representative patient with active SLE. The frequency of these T cell functional subsets was then explored in healthy controls and in patients with active and inactive SLE.

There was increased relative frequency of cells that produced at least one of the three cytokines analyzed in patients with A-SLE and I-SLE compared to controls, either when stimulated with HEP-2 (Fig. 2B) or PMA/Io (Fig. 2C), but not in non-stimulated cultures (data not shown). There was no difference in the relative frequency of tri-functional cells among SLE patients and controls in cultures stimulated with HEP-2 cell extract or PMA/Io (Fig. 2D, Table 2). In addition, there was no difference in the relative frequency of mono- or bi-functional cells in cultures from the three groups stimulated with extract of HEP-2 cell extract or PMA/Io (Fig. 2D, Table 2). Most bi-functional and mono-functional T cells had similar

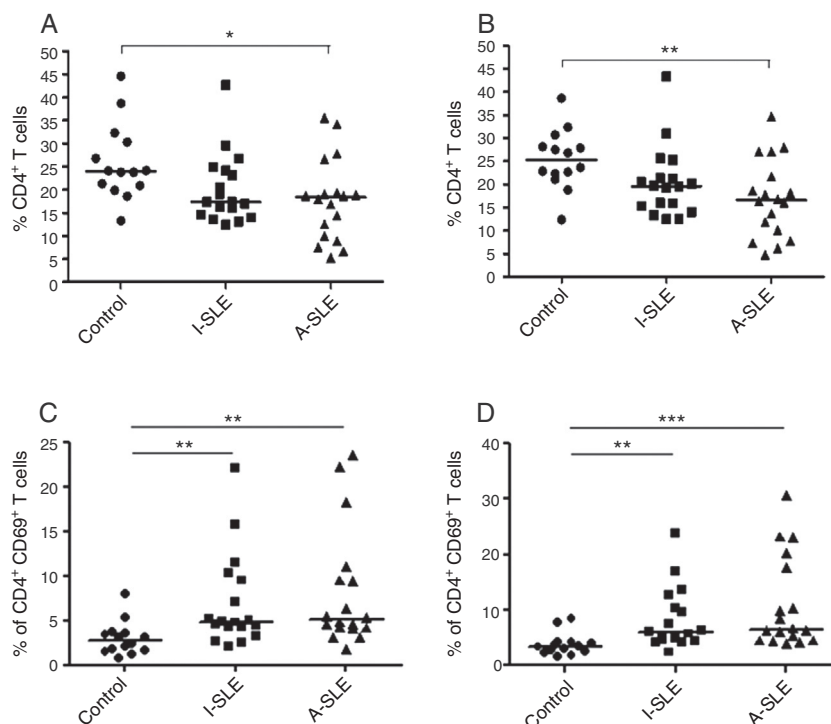


Fig. 1 – Relative frequency of CD4⁺ T cells in patients with active SLE, inactive SLE and controls (A) and relative frequency of CD4⁺CD69⁺ T cells in patients with active SLE, inactive SLE and controls in stimulation with HEp-2 (A, C) and stimulated with PMA/Io (B, D). The bars represent the median for each group. * $p < 0.05$; ** $p < 0.01$; * $p < 0.001$.**

frequency in samples from SLE patients and controls. However, samples from patients with A-SLE and I-SLE presented higher frequency of IL-17 producing cells in cultures stimulated with PMA/Io but not in those stimulated with HEp-2 cell extract (Fig. 2D and Table 2). In addition, bi-functional cells producing IL-17 and INF- γ showed a strong trend for higher frequency in PMA-stimulated cultures from A-SLE and I-SLE patients as compared with those from normal controls (Fig. 2D and Table 2). As can be appreciated in Fig. 2D, there was a broad variation in the frequency of mono-functional cells in stimulated cultures from SLE patients, with several patients presenting very high frequency of cells expressing IL-17 and INF- γ .

Bi-functional IL-2⁺/INF- γ ⁺ CD4⁺ T cells produce more INF γ than mono-functional INF- γ ⁺ CD4⁺ T cells

Next we asked if the amount of cytokines produced by multifunctional and mono-functional T cells was equivalent. To evaluate this parameter we assessed the mean fluorescence intensity (MFI) for each of the three cytokines evaluated. As can be seen in experiments with PMA/Io-stimulated samples from controls, IFN- γ MFI in bi-functional IL-2⁺/INF- γ ⁺ cells was significantly higher than in other T cell subpopulations, even those that produce only INF- γ (Fig. 3A and B). This feature was equally observed in samples from SLE patients and controls (Fig. 3C-E). This phenomenon was not observed in those cultures without stimulation or stimulated with HEp-2 cells extracts (data not shown). This behavior was observed in all 36 SLE patients and 14 healthy volunteers examined.

Such a behavior was not observed for IL-17 and IL-2 in CD4⁺ T cells in any of the three groups of individuals (data not shown).

Correlation between the frequency of Th17 cells and newly activated cells in patients with inactive SLE and with CD4⁺ T effector cells in active SLE

There was a positive correlation between the frequency of Th17 cells and recently activated cells in PBMC samples from patients with I-SLE in non-stimulated cultures (Fig. 4A) or under stimulus with HEp-2 cells extract (Fig. 4B), but not when cells were stimulated with PMA/Io (Fig. 4C). There was also a positive correlation between the frequency of Th17 lymphocytes and CD4⁺CD25⁺Foxp3^{lo} cells in samples from patients with active SLE either without or under different stimuli (Fig. 4D-F). In control samples, there was also a positive correlation between the frequency of Th17 and CD25⁺Foxp3^{lo} cells when stimulated with HEp-2 cell extract and in non-stimulated cultures, but not in those stimulated with PMA/Io (data not shown). There was no such correlation in cultures of samples from patients with inactive SLE (data not shown). There was no correlation between the relative frequency of Th17 cells and TREG cells (CD25^{high}CD127^{lo}Foxp3⁺) in samples from controls and patients with SLE independent of the existence of culture stimulus (data not shown). Finally, there was no correlation between SLE activity measured by SLEDAI and the relative frequency of Th17 cells or any other investigated T lymphocyte subset in stimulated and non-stimulated cultures.

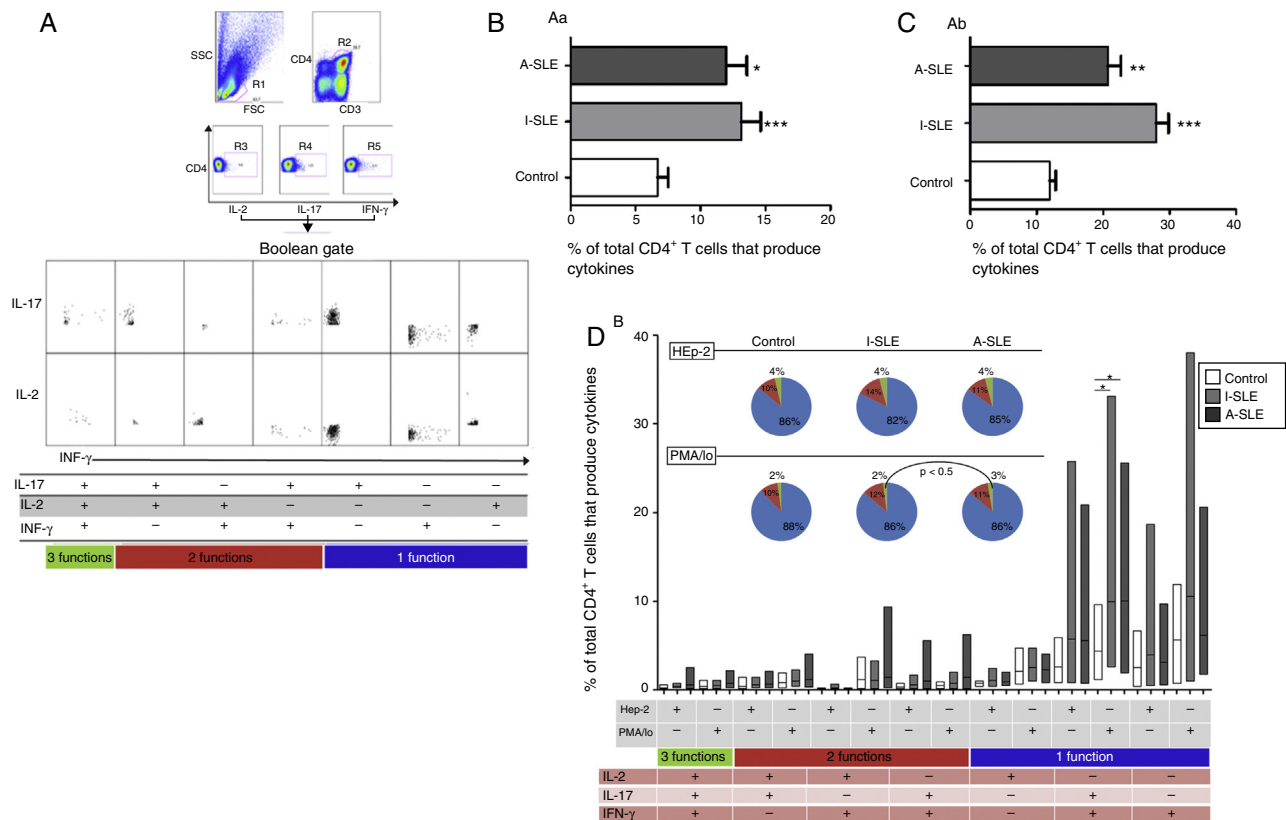


Fig. 2 – (A) Multi-parameter strategy of analysis to identify Th17 cells and polyfunctional cells in PBMC of HC stimulated with PMA/Io. Initially was established a gated in lymphocyte population (R1), then only the population CD3⁺CD4⁺ was selected (R2). From this population were obtained cells expressing IL-2 (R3) IL-17 (R4) and INF- γ (R5). Relative frequency of CD4⁺ T cells that produce cytokines (IL-2, IL-17 or INF- γ) by stimulation with HEP-2 (B) and PMA/Io (C). The bars represent the standard error for each group. (D) Diagram illustrating the behavior of functional subsets of CD4⁺ T cells from A-SLE, I-SLE and controls. The box plot graph represents the relative frequency of each CD4⁺ T functional subset in the three groups under the two stimulus conditions (extract of HEP-2 and PMA/Io, respectively). The response patterns are grouped and color-coded by number of positive functions and summarized in pie chart form where each pie slice represents the mean proportion of the total CD4⁺ T cell response contributed by response patterns that have all three (green) or any combination of two (red) or one (blue) of the measured functions. * $p < 0.05$; ** $p < 0.01$; * $p < 0.001$.**

Discussion

The purpose of the present study was to evaluate the immune system of patients with SLE, regarding effector pathways of CD4⁺ T cells producing IL-17 (Th17) and multifunctional CD4⁺ T cells. Therefore, we stimulated PBMC cultures with HEP-2 cell extract, which expresses most self-antigens of clinical importance in SLE, including Sm/RNP, SS-A/Ro, and SS-B/La polypeptides, as well as chromatin antigens. This stimulus allowed us to evaluate, among the pool of CD4⁺ T cells, those with self-reactive capacity, compared to non-stimulated cells and with cells stimulated with PMA/Io (polyclonal stimulation). These experiments showed that PBMC from SLE patients did respond to stimulation with auto-antigens. Furthermore, we observed that even in healthy individuals, there were CD4⁺ T cells sensitive to stimulation with HEP-2 cell-derived self-antigens, but in smaller proportions compared to patients with SLE.

In this context, we observed that cultures from patients with active SLE presented lower relative frequency of CD4⁺ T cells, as previously reported by Wouters et al.²⁷ On the other hand, samples from patients with active and inactive SLE had an increased frequency of newly activated T cells (CD4⁺CD69⁺). The reduction in CD4⁺ cell relative frequency may be related to the immunosuppressant therapy effects, since several of the studied patients were under immunosuppressant therapy. In contrast, the increased frequency of CD4⁺CD69⁺ T cells possibly corresponds to the fraction of recently activated T cells that may contribute to the maintenance of exacerbated autoimmune response and disease activity despite treatment.

There was an increased relative frequency of CD4⁺ T cells producing either IL-2, IL-17 or INF- γ in samples from patients with A-SLE and I-SLE after stimulus with PMA/Io or HEP-2 cell extract. The increased proportion of circulating cells able to assume a phenotype of cytokine secretion after stimulation indicates that the immune system in SLE patients presents an expressive pool of partially differentiated cells ready to

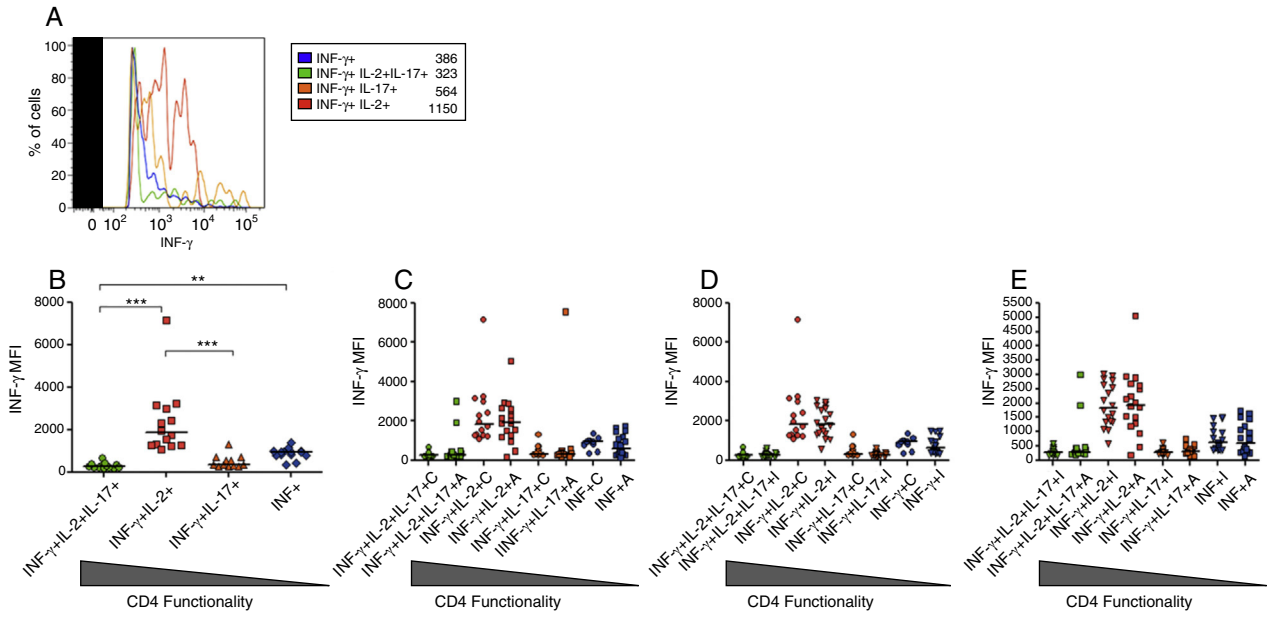


Fig. 3 – (A) Histogram representing the mean fluorescence intensity (MFI) of INF- γ after stimulation with PMA/Io in the different functional subpopulations of CD4⁺ T cells from a representative sample of normal control. The blue line represents the MFI of cells with one function, the green line three functions cells and rows of orange and red two functions cells to INF- γ + IL-17 and INF- γ + IL-2, respectively. (B) Graph representing the difference between the MFI of IFN- γ between different functional subpopulations of CD4⁺ T cells in controls by stimulation with PMA/Io. Comparison of the MFI of IFN- γ by different types of CD4+ cells after stimulation with PMA/Io among the control and A-SLE (C), control and I-SLE (D) and I-SLE and A-SLE (E). *p* < 0.01; ****p* < 0.001.**

SLE and opens perspectives to the development of alternative therapies for this disease. We believe that the immunological pattern observed in the present study occurs in SLE patients in general, but there is considerable heterogeneity in the group.

The present study did not aim to investigate the association of this phenomenon with the diverse manifestations of SLE. This is a relevant point to be investigated in a future study specially designed for this aim. It is conceivable that

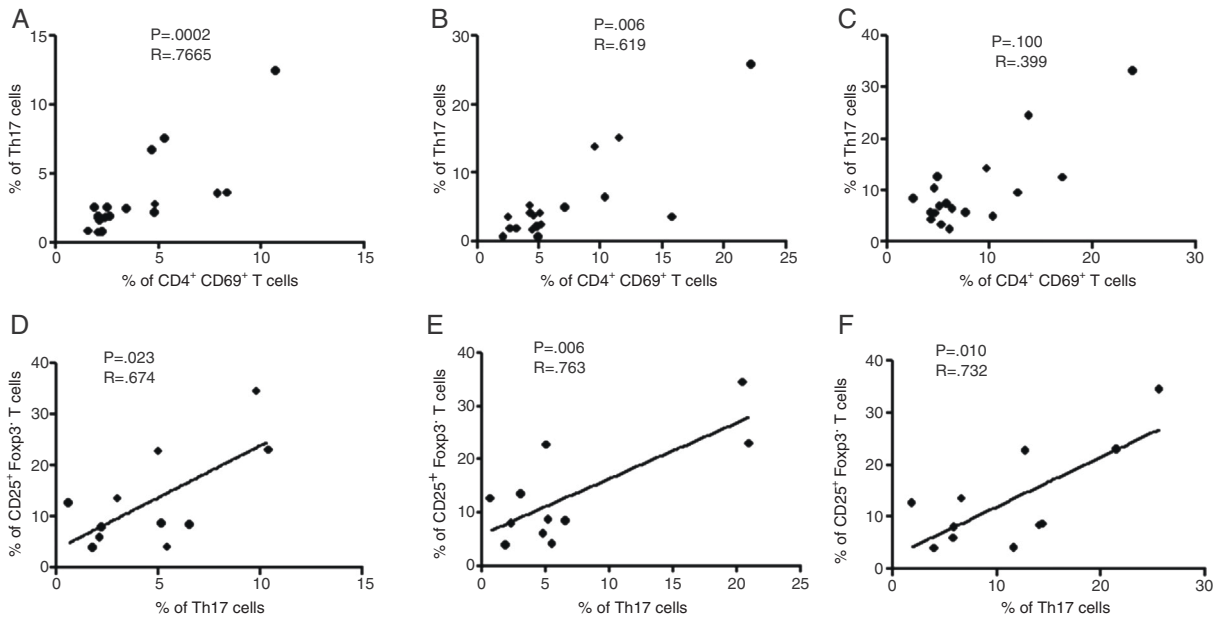


Fig. 4 – Correlation among the relative frequency of Th17 cells and CD4+CD69+ T cells in cultures of PBMC of patients with I-SLE without stimulation (A), culture stimulated with HEp-2 cells extract (B) and culture stimulated with PMA/Io (C) n = 18. Correlation among the relative frequency of Th17 cells and CD4+CD25+Foxp3– in cultures of PBMC of patients with A-SLE without stimulation (D), culture stimulated with extract of HEp-2 (E) and culture stimulated with PMA/Io (F) n = 11.

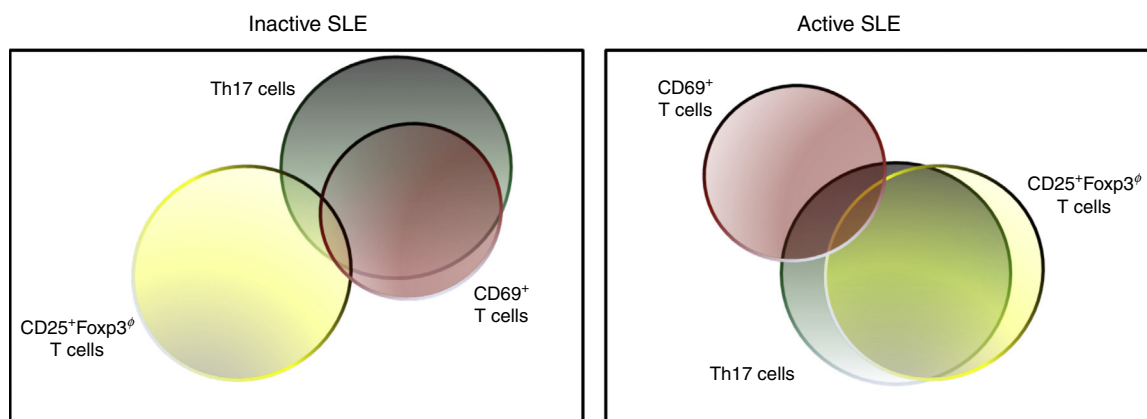


Fig. 5 – Proposed conception of correlation among the relative frequency of Th17 cells and CD4⁺CD69⁺ T cells in patients with I-SLE and correlation among the relative frequency of Th17 cells and CD4⁺CD25⁺Foxp3⁻ in patients with A-SLE. Showing that the majority of cells responsible for production of IL-17 are those newly activated in patients with I-SLE, while in patients with SLE-A, most of the IL-17 producing cells are those chronically activated.

personalized cytokine blocking therapy specifically designed according to the predominant profile of multifunctional effector T cells will be effective in helping restore the immunologic balance in each patient. IL-17-producing T lymphocytes seem to play a prominent role in SLE pathophysiology and may represent a potential target for therapy. In fact, it is possible that cytokine-targeted and personalized therapy may contribute to improving the balance of effector immune response, avoiding or minimizing the damage caused by the autoimmune response.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

- Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol.* 1986;136:2348-57.
- Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol.* 1989;7:145-73.
- Nakayama S, Takahashi H, Kanno Y, O'Shea JJ. Helper T cell diversity and plasticity. *Curr Opin Immunol.* 2012;24:297-302.
- Infante-Duarte C, Horton HF, Byrne MC, Kamradt T. Microbial lipopeptides induce the production of IL-17 in Th cells. *J Immunol.* 2000;165:6107-15.
- Shin MS, Lee N, Kang I. Effector T-cell subsets in systemic lupus erythematosus: update focusing on Th17 cells. *Curr Opin Rheumatol.* 2011;23:444-8.
- Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4⁺ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol.* 2005;6:1123-32.
- Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol.* 2005;6:1133-41.
- Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev.* 2008;223:87-113.
- Crispín JC, Tsokos GC. Interleukin-17-producing T cells in lupus. *Curr Opin Rheumatol.* 2010;22:499-503.
- Garrett-Sinha LA, John S, Gaffen SL. IL-17 and the Th17 lineage in systemic lupus erythematosus. *Curr Opin Rheumatol.* 2008;20:519-25.
- Lubberts E. IL-17/Th17 targeting: on the road to prevent chronic destructive arthritis? *Cytokine.* 2008;41:84-91.
- Ma J, Yu J, Tao X, Cai L, Wang J, Zheng SG. The imbalance between regulatory and IL-17-secreting CD4⁺ T cells in lupus patients. *Clin Rheumatol.* 2010;29:1251-8.
- Bălănescu P, Bălănescu E, Tănăsescu C, Nicolau A, Tănăsescu R, Grancea C, et al. T helper 17 cell population in lupus erythematosus. *Rom J Intern Med.* 2010;48:255-9.
- Prado C, de Paz B, Gómez J, López P, Rodríguez-Carrio J, Suárez A. Glucocorticoids enhance Th17/Th1 imbalance and signal transducer and activator of transcription 3 expression in systemic lupus erythematosus patients. *Rheumatology (Oxf).* 2011;50:1794-801.
- Wong CK, Ho CY, Li EK, Lam CW. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. *Lupus.* 2000;9:589-93.
- Wong CK, Lit LC, Tam LS, Li EK, Wong PT, Lam CW. Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in auto-immunity. *Clin Immunol.* 2008;127:385-93.
- Shah K, Lee WW, Lee SH, Kim SH, Kang SW, Craft J, et al. Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. *Arthritis Res Ther.* 2010;12:R53.
- Dong G, Ye R, Shi W, Liu S, Wang T, Yang X, et al. IL-17 induces autoantibody overproduction and peripheral blood mononuclear cell overexpression of IL-6 in lupus nephritis patients. *Chin Med J (Engl).* 2003;116:543-8.
- Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8⁺ T cells. *Blood.* 2006;107:4781-9.
- De Rosa SC, Lu FX, Yu J, Peretto SP, Falloon J, Moser S, et al. Vaccination in humans generates broad T cell cytokine responses. *J Immunol.* 2004;173:5372-80.
- Chattopadhyay PK, Price DA, Harper TF, Betts MR, Yu J, Gostick E, et al. Quantum dot semiconductor nanocrystals for

- immunophenotyping by polychromatic flow cytometry. *Nat Med.* 2006;12:972-7.
22. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997;40:1725.
 23. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum.* 1992;35:630-40.
 24. Cook RJ, Gladman DD, Pericak D, Urowitz MB. Prediction of short term mortality in systemic lupus erythematosus with time dependent measures of disease activity. *J Rheumatol.* 2000;27:1892-5.
 25. Kallas EG, Gibbons DC, Soucier H, Fitzgerald T, Treanor JJ, Evans TG. Detection of intracellular antigen-specific cytokines in human T cell populations. *J Infect Dis.* 1999;179:1124-31.
 26. Mesquita D, de Melo Cruvinel W, Araujo J, Pucci F, Salmazi K, Kallas E, et al. Systemic lupus erythematosus exhibits a dynamic and continuum spectrum of effector/regulatory T cells. *Scand J Rheumatol.* 2011;40:41-50.
 27. Wouters CH, Diegenant C, Ceuppens JL, Degreef H, Stevens EA. The circulating lymphocyte profiles in patients with discoid lupus erythematosus and systemic lupus erythematosus suggest a pathogenetic relationship. *Br J Dermatol.* 2004;150:693-700.
 28. Yu JJ, Gaffen SL. Interleukin-17: a novel inflammatory cytokine that bridges innate and adaptive immunity. *Front Biosci.* 2008;13:170-7.
 29. Yang J, Yang X, Zou H, Chu Y, Li M. Recovery of the immune balance between Th17 and regulatory T cells as a treatment for systemic lupus erythematosus. *Rheumatology (Oxf).* 2011;50:1366-72.
 30. Mok MY, Wu HJ, Lo Y, Lau CS. The relation of interleukin 17 (IL-17) and IL-23 to Th1/Th2 cytokines and disease activity in systemic lupus erythematosus. *J Rheumatol.* 2010;37: 2046-52.
 31. Henriques A, Inês L, Couto M, Pedreiro S, Santos C, Magalhães M, et al. Frequency and functional activity of Th17, Tc17 and other T-cell subsets in Systemic Lupus Erythematosus. *Cell Immunol.* 2010;264:97-103.