View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Cytokine 61 (2013) 785–791

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Th1/Th2 cytokine profile in childhood-onset systemic lupus erythematosus

Mariana Postal [a], Karina Oliveira Peliçari [a], Nailú Angélica Sinicato [a], Roberto Marini [b], Lilian Tereza Lavras Costallat [a], Simone Appenzeller [a,*]

[a] Department of Medicine, Rheumatology Unit, Faculty of Medical Science, State University of Campinas, Brazil
[b] Department of Pediatrics, Pediatric Rheumatology Unit, Faculty of Medical Science, State University of Campinas, Brazil

ARTICLE INFO

Article history:
Received 28 May 2012
Received in revised form 15 October 2012
Accepted 26 November 2012
Available online 17 January 2013

Keywords:
Interferon gamma (IFN-γ)
Tumor necrosis factor alpha (TNF-α)
Interleukin (IL) 5, 6, 10, and 12
SLEDAI
Childhood-onset systemic lupus erythematosus

ABSTRACT

Objective: To determine the serum levels of Th1 (IL-12, IFN-γ,TNF-α) and Th2 (IL-5, IL-6 and IL-10) cytokines in childhood-onset SLE, first-degree relatives and healthy controls. To elucidate their association with disease activity, laboratory and treatment features.
Methods: We included 60 consecutive childhood-onset SLE patients [median age 18 years (range 10–37)], 64 first-degree relatives [median 40 (range 28–52)] and 57 healthy [median age 19 years (range 6–30 years)] controls. Controls were age and sex-matched to SLE patients. SLE patients were assessed for clinical and laboratory SLE manifestations, disease activity (SLEDAI), damage (SDI) and current drug exposures. Mood and anxiety disorders were determined through Becks Depression (BDI) and Anxiety Inventory (BAI). Th1 (IL-12, IFN-γ,TNF-α) and Th2 (IL-5, IL-6 and IL-10) cytokines levels were measured by ELISA and compared by non-parametric tests.
Results: Serum TNF-α ($p = 0.004$), IL-6 ($p = 0.007$) and IL-10 ($p = 0.03$) levels were increased in childhood-onset SLE patients when compared to first-degree relatives and healthy controls. TNF-α levels were significantly increased in patients with active disease ($p = 0.014$) and correlated directly with SLEDAI scores ($r = 0.39$; $p = 0.002$). IL-12 ($p = 0.042$) and TNF-α ($p = 0.009$) levels were significantly increased in patients with nephritis and TNF-α in patients with depression ($p = 0.001$). No association between cytokine levels and SDI scores or medication was observed.
Conclusion: Th1 cytokines may play a role in the pathogenesis of neuropsychiatric and renal manifestations in childhood-onset SLE. The correlation with SLEDAI suggests that TNF-α may be a useful biomarker for disease activity in childhood-onset SLE, however longitudinal studies are necessary to determine if increase of this cytokine may predict flares in childhood-onset SLE.

© 2012 Elsevier Ltd. Open access under the Elsevier OA license.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic, multisystemic autoimmune disease predominantly affecting women of childbearing age [1]. Approximately 10–20% of all cases of SLE occur in the first two decades of life [1–4]. In childhood-onset patients the female-to-male ratio is 4:3 with disease onset during the first decade of life, 4:1 during the second decade when compared to 9:1 ratio in adult-onset SLE [5–7].

Childhood-onset SLE often presents more acute and severe disease features than adult-onset SLE. Renal (50–67%), neurological (22–95%) and hematological (77%) involvement, in addition to fever and lymphadenopathy are more frequently observed in children when compared to adult-onset SLE [8–13]. In relation to disease activity, childhood-onset SLE patients have a more active disease not only at disease onset, but also over time when compared to adult-onset SLE [14,15].

The impact of SLE on children is often profound, and a satisfactory outcome in this age group is not a 5 or 10-year survival, but a survival that more closely approximates the normal lifespan [16]. The awareness that SLE in childhood is a potentially fatal disease, that atypical presentations are very common, and that aggressive treatment should be introduced early in the course of the disease, has significantly improved survival in the childhood-onset SLE cohorts [14–16]. Over the last decades, morbi-mortality rates have significantly dropped in childhood-onset patients in a similar patter as in adult-onset SLE patients [14,17].

Independently of the age of onset, there is strong evidence supporting the role of cytokine in the pathogenesis of SLE [18]. Although antibody production, driven by Th2 lymphocytes and immune complex formation are key features in SLE, recent evidences have suggested that Th1 lymphocytes have an important pathogenic role in SLE [18,19]. The main cytokines associated with cellular immunity (Th1) are interleukin (IL) 12, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), while IL-5, IL-6

* Corresponding author. Address: Department of Medicine, Faculty of Medical Science, State University of Campinas, Cidade Universitária, Campinas, SP, CEP 13083-970, Brazil. Fax: +55 19 3289 1818.
E-mail address: appenzellersimone@yahoo.com (S. Appenzeller).

1043-4666 © 2012 Elsevier Ltd. Open access under the Elsevier OA license.
http://dx.doi.org/10.1016/j.cyto.2012.11.023

and IL-10 are associated with the production of antibodies and induction of humoral immunity (Th2) [19]. Not only is the cytokine profile in SLE different when compared to healthy controls, it also varies according to disease phenotypes and disease activity [18].

Serum IL-12, IFN-γ, TNF-α, and IL-5, IL-6 and IL-10 and the relation between these Th1 and Th2 cytokines have been studied in adult-onset SLE [19–22]. However, the role of these cytokines in childhood-onset SLE has never been investigated. The aim of our study was to determine the serum levels of Th1 (IL-12, IFN-γ, TNF-α) and Th2 (IL-5, IL-6 and IL-10) cytokines in childhood-onset SLE patients, first-degree relatives and healthy controls. In addition we evaluated their association with disease activity, laboratory and treatment features.

2. Patients and methods

2.1. Subjects

Sixty consecutive childhood-onset SLE patients, recruited from the Pediatric Rheumatology Outpatient Clinic of State University of Campinas were included in this study. Patients were included in the present study if they: (i) fulfilled at least four criteria of American College of Rheumatology (ACR) [23]; (ii) were below 16 years of age at disease onset; and (iii) had a follow-up duration of at least 6 months.

Sixty-four first-degree relatives and 57 healthy controls without history of any chronic disease (including autoimmune diseases) were included as a control group. The healthy volunteers were matched by age and gender to the patients.

This study was approved by the ethics committee at our institution, and informed written consent was obtained from each participant and/or legal guardian.

2.2. Clinical features

All patients had their medical histories, clinical and serological characteristics evaluated at study entry according to the ACR [23]. Features included in this protocol were age at onset of disease (defined as the age at which the first symptoms clearly attributable to SLE occurred), age at diagnosis (defined as the age when patients fulfilled four or more of the 1982 revised criteria for the classification of SLE [23], and follow-up time (defined as the time from disease onset until May 2010).

All clinical manifestations and laboratory findings were recorded at the day of blood withdrawal. Nephritis was diagnosed on the basis of proteinuria exceeding 0.5 g/L with abnormal urinary sediment and/or histological findings. Nephrotic syndrome was defined as proteinuria in excess of 3.0 g/day. Hematological alterations were ascribed to lupus only in the absence of bone-marrow suppression (leukopenia <4000 cells/mm^3; thrombocytopenia <100,000 cells/mm^3; hemolytic anemia). We also analyzed the presence of malar rash, discoid lesions, subacute cutaneous lesions, cutaneous vasculitis, photosensitivity, oral ulcers, arthritis and serositis. Neurological and psychiatric involvement was defined according to ACR [24].

Treatment prescribed at time of blood withdrawal, as well as its adverse events related to drug use, was recorded. Doses of oral and parenteral corticosteroids were analyzed and converted to the equivalent doses of prednisone.

2.3. Laboratory studies

Antinuclear antibodies (ANAs) were determined by indirect immunoflurescence using HEp-2 cells as substrate, and regarded as positive if higher than 1:40. Anti-double stranded DNA (dsDNA) antibodies were determined by indirect immunoflurescence using *Crithidia* as substrate and considered positive if higher than 1:10. Precipitating antibodies to extractable nuclear antigens (ENAs), including Ro (SSA), La (SSB), and Sm were detected by a standardized ELISA method, and considered positive if higher than 1:40. Rheumatoid factor (RF) was detected by nefelometry, and regarded as positive if higher than 10. Anticardiolipin antibodies (aCL) of the IgG and IgM isotypes were measured by an ELISA method [25]. The lupus anticoagulant (LA) activity was detected by coagulation assays in platelet-free plasma obtained by double centrifugation, following the recommendation of the subcommittee on LA of the Scientific and Standardization Committee of the International Society of Thrombosis and Homeostasis [26]. These measurements were carried out twice, at an interval of 12 weeks.

2.4. Disease activity and damage

Disease activity was measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [27]. SLEDAI scores range between 0 and 105. Scores of $\geqslant 3$ were considered active disease [28]. Active nephritis was diagnosed on the basis of renal items of the SLEDAI (proteinuria exceeding 0.5 g/L, abnormal urinary sediment, low complement levels).

Cumulative SLE-related damage in all patients was determined using the Systemic Lupus International Collaborating Clinics (SLICCs)/ACR Damage Index (SDI) [29] at time of blood withdrawal. SDI score range from 0 to 47. Damage was considered if scores $\geqslant 1$ [29].

2.5. Mood and anxiety evaluation

All subjects completed the Beck Depression (BDI) [30] and Beck Anxiety Inventory (BAI) [31] at study entry. For patients under 16 years old, Children's Depression Inventory (CDI) was applied. These scales consist of 21 items, each describing a common symptom of depression/anxiety. The respondent is asked to rate how much he or she has been bothered by each symptom over the past month on a 4-point scale ranging from 0 to 3. The items are summed to obtain a total score that can range from 0 to 63. The cutoffs used for the BDI are: 0–13: no/minimal depression; 14–19: mild depression; 20–28: moderate depression; and 29–63: severe depression and for the BAI: 0–7: no/minimal level of anxiety; 8–15: mild anxiety; 16–25: moderate anxiety; 26–63: severe anxiety. The cutoff used for CDI is 17.

2.6. Cytokines assays

A blood sample was collected from all participants, centrifuged at 3000 rpm for 15 min after being allowed to clot for 30 min at room temperature. Sera were separated as soon as possible from the clot of red cells after centrifugation to avoid TNF-α production by blood cells that falsely could increase its values [32]. Separated sera were kept in aliquots at −80 °C until the time of assay. None of the samples was taken during an episode of a severe bacterial infection requiring hospitalization because TNF-α could be increased due to a secondary cause [33]. The samples were performed in duplicate to guarantee the reproducibility of the kits.

Commercially available kits from R&D Systems (London, UK) were used for the measurement of serum IFN-γ, TNF-α, IL-5, 6, 10 and 12 levels by enzyme-linked immunosorbent assay (ELISA), carried out in accordance with the manufacturer's instructions. The minimum detectable dose (MDD) was 8.0 pg/mL for IFN-γ, 0.29 pg/mL for IL-5, 0.039 pg/mL for IL-6 and 3.9 pg/mL for IL-10. For IL-12, MDD was 0.5 pg/mL. Assay sensitivity was 0.106 pg/ml for TNF-α.

2.7. Statistical analysis

All statistical analyses were performed using SPSS Statistics software version 17.0 and results were given as median and range. Kruskal–Wallis Test was used to compare cytokines levels between groups. Spearman's correlation was used to correlate continuous variables (e.g. cytokines levels and SLEDAI, SDI, BDI and BAI scores). Cytokine levels and categorical variables were compared by Mann–Whitney *U* test. For all analyses, a p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographics

We included 60 consecutive childhood-onset SLE patients. Fifty-seven (95%) were female with a median age of 18 years (range 10–37). The median of the disease duration was 4 years (range 0–26 years). Sixty-four first-degree relatives [59 women; mean age of 40 years (range 28–52)] agreed to participate in the study. The control group consisted of 57 healthy controls (52 women) with a median age of 19 years (range 6–30 years). Patients and healthy controls were statistically comparable in terms of age and sex (Table 1).

3.2. Clinical, laboratory, and treatment features

All patients had disease onset before the age of 16 and clinical and laboratory manifestations at disease onset are shown in Table 2. At time of study entry, 30 (50%) childhood-onset SLE patients had active disease (SLEDAI $\geqslant$3) with median SLEDAI scores of 8 (range 4–18). The 30 (50%) inactive patients had a median SLEDAI score of 0 (range 0–2). Active nephritis (33.3%), new malar rash (6.6%), new alopecia (5.0%) and cutaneous vasculitis (5.0%) were the clinical manifestations more frequently observed (Table 2).

At time blood withdrawal, 8 (13.3%) patients were not taking any immunosuppressant medication. Forty-two (70%) patients were receiving prednisone, 32 (53.3%) hydroxychloroquine and 22 (36.6%) patients were receiving other immunosuppressive drugs (Table 2).

Depression was identified in 10 (16.7%) patients and in no healthy control or first-degree relatives. Mild depression was identified in 5 (8.3%) patients and 5 (8.3%) patients had moderate/severe depression. Anxiety was observed in 21 (35%) childhood-onset SLE patients. Twelve (20%) patients had mild and 9 (15%) had moderate/severe anxiety.

Table 2
Clinical, laboratory and treatment features at day of blood withdrawal.

Manifestations	Patients, N = 60
Clinical features	
Alopecia	3 (5%)
Malar rash	4 (6.6%)
Nephritis	20 (33.3%)
Neurologic manifestations	21 (35%)
Serositis	2 (3.3%)
Vasculitis	3 (5%)
Laboratory features	
Anticardiolipine or LA	13 (21.6%)
Anti-SM	9 (15%)
Anti-SSA/Ro	8 (13.3%)
dsDNA	25 (41.6%)
Leukopenia	2 (6.7%)
Thrombocytopenia	2 (6.7%)
Treatment	
No medication	8 (13.3%)
Prednisone	42 (70%)
Hydroxychloroquine	32 (53.3%)
Immunosuppressive drugs	29 (48.3%)
Azathioprine	15 (25%)
Ciclophosphamide	2 (3.3%)
Cyclosporine	5 (8.3%)
Methotrexate	1 (1.6%)
Mycophenolate mofetil	6 (10%)

dsDNA: double-stranded DNA, LA: lupus anticoagulant

3.3. Cytokines assays

Serum levels of Th1 cytokines and Th2 cytokines, in SLE patients are shown in Fig. 1. Sera TNF-α (p = 0.004), IL-6 (p = 0.007) and IL-10 (p = 0.03) levels were significantly increased in childhood-onset SLE when compared to first-degree relatives and healthy controls (Table 3). No significant difference in serum TNF-α, IL-6 and IL-10 levels was observed between first-degree relatives and healthy controls. No significant difference in serum levels of IFN-γ, IL-5 and IL-12 was observed among childhood-onset SLE, first-degree relatives and healthy controls. TNF-α levels (p = 0.014) were significantly increased in patients with active disease (SLEDAI $\geqslant$3) when compared to patients with inactive disease. In addition, TNF-α levels correlated directly with SLEDAI scores (r = 0.39; p = 0.002). Although IL-6 was increased in patients with active disease when compared to patients with inactive disease, no statistically significance was noted.

IL-12 (p = 0.042) and TNF-α (p = 0.009) levels were significantly increased in patients with active nephritis when compared to patients without nephritis. IL-6 levels were significantly increased

Table 1
Demographic and clinical characteristics of patients and controls included in the study.

Parameter	Childhood-onset SLE patients	First-degree relatives	Healthy controls
	N = 60	N = 64	N = 57
Sex			
Female	57 (95%)	59 (92.18%)	52 (91.22%)
Age (years)	18 (range 10–37)	40 (range 28–52)[a]	19 (range 6–30)
Disease duration (years)	4 (range 0–26)	–	–
SLEDAI	2 (range 0–18)	–	–
Active disease N = 30	8 (range 4–18)		
Inactive disease N = 30	0 (range 0–2)		
SDI	0 (range 0–3)	–	–

[a] $P \leqslant 0.05$.

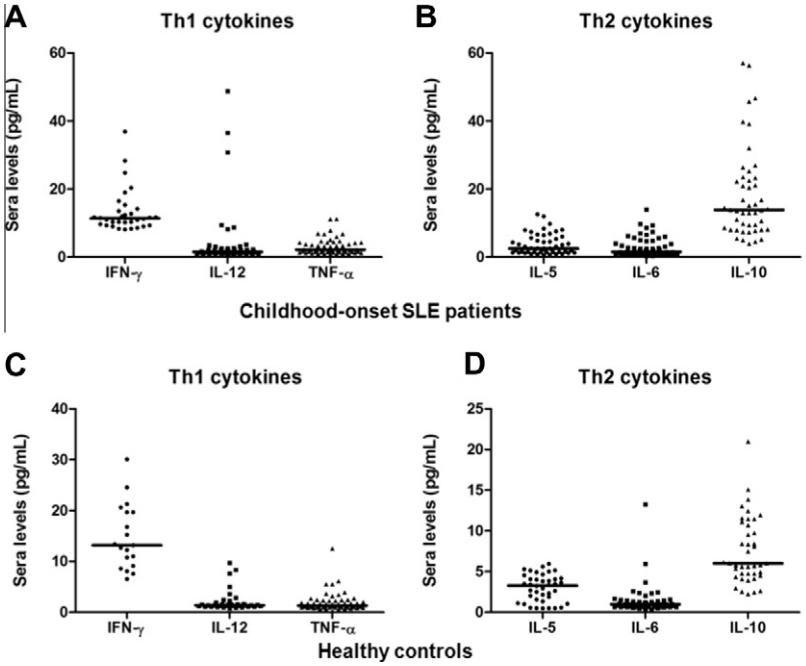

Fig. 1. Sera Th1 and Th2 cytokine levels of childhood-onset SLE patients and healthy controls.

Table 3
Sera cytokines levels of the individuals included in the study.

Sera levels (pg/mL)	Childhood-onset SLE patients, *N* = 60	First-degree relatives, *N* = 64	Healthy controls, *N* = 57
Th1 cytokines			
IL-12 median (range)	1.54 (0.58–48.79)	1.38 (0.5–5.77)	1.38 (0.74–9.76)
IFN-γ	11.36 (8.1–36.88)	11.7 (6.76–26.43)	13.39 (8.08–30.08)
TNF-α	2.18 (0.8–11.17)[a]	1.63 (0.21–9.65)	1.30 (0.25–12.53)
Th2 cytokines			
IL-5	2.5 (0.61–12.59)	2.76 (0.33–8.78)	3.29 (0.49–8.86)
IL-6	1.5 (0.22–13.98)[a]	1.45 (0.56–13.84)	0.98 (0.39–13.29)
IL-10	14 (3.93–57)[a]	10.7 (3.91–24.94)	6.01 (2.24–20.99)

[a] $P \leqslant 0.05$.

in patients with dysmorphic hematuria ($p = 0.003$) and IL-10 in patients with positive dsDNA ($p = 0.01$). An indirect correlation between the TNF/IL-10 ratio and dsDNA was observed ($r = -0.45$; $p = 0.001$).

TNF-α levels were significantly increased in patients with depression moderate/severe ($p = 0.01$) when compared to patients without depression. IL-10 levels had a negative correlation with the severity of depression ($r = -0.45$; $p = 0.013$).

No association between other SLEDAI variables or SDI scores and IL-12, IFN-γ, TNF-α, IL-5, IL-6 and IL-10 levels was observed. In addition, no difference in these cytokine levels in patients with and without medication was observed.

4. Discussion

Cytokines are low-weight proteins that play a key role in immunological dysregulation observed in autoimmune diseases. The development of SLE can be viewed as Th1 and Th2 imbalance. The increased levels of proinflammatory cytokines are believed to play a key role in the pathogenesis of SLE [34–47]. Higher cytokine levels in SLE patients may promote inflammatory response, apoptosis and autoantibody production that not only initiate, but may also maintain SLE disease activity over time [34,41,42,44,46].

In our study, we observed increased TNF-α, IL-6 and IL-10 levels in childhood-onset SLE when compared to healthy controls and first-degree relatives, as previously observed in adult-onset SLE patients [18–22,48–54].

Although several studies have analyzed TNF-α levels in adult-onset SLE patients, the clinical significance is less clear [19,20,48,50–54]. In addition to increased TNF-α levels in patients with active disease, we observed a positive correlation between SLEDAI scores, suggesting that TNF-α could be a biomarker for disease activity in SLE. Several studies [19,50–52,54] analyzing adult-onset SLE have shown higher TNF-α levels in SLE patients with active disease. However, this association was never studied in a childhood-onset cohorts.

We also observed significantly higher levels of TNF-α in patients with nephritis when compared to patients without nephritis. SLE nephritis is a prototype of immune-complex induced kidney damage [55]. In SLE nephritis, the pattern of glomerular injury is primarily related to dsDNA and anti-C1q antibodies and the formation of immune complexes. These immune complexes are deposited on the tissue surface, inducing inflammatory response by activating adhesion molecules on endothelium. This response leads to the recruitment of pro inflammatory leukocytes. Renal injury results from activated and damaged glomerular cells, infiltrating macrophages, and cytokines [56].

Recently, it has been shown that dysregulated apoptosis is also an important factor for developing proliferative lupus nephritis [54,57]. Higher TNF-α levels were also observed in one previous

study that compared active SLE nephritis with inactive nephritis [20], and in other non-SLE nephropathies, including membranous nephropathies and nephritic syndromes [58,59]. These findings support the hypothesis that TNF-α may play a pathogenic role in the induction or maintenance of glomerular barrier dysfunction in renal diseases [59].

The involvement of TNF-α in lupus nephritis is further supported by the improvement of lupus nephritis under TNF-α blocking therapy [60–65]. In particular, nephritis may remain in long-term remission after just a few infusions of infliximab [64]. Although an increase in the autoantibody response to chromatin was observed in patients treated with TNF-α blocking therapy, these were transient and without pathologic consequences [66]. It is important to consider some limitations of the study analyzing TNF-α blocking therapy, such as the small number of SLE patients included (seven patients) and the short follow up period (4–10 weeks) [63].

It is important to stress that no controlled trials about TNF-α blocking therapy have been published and that the number of patients treated is still very limited [61,65]. Therefore, no firm conclusions can be drawn. Nevertheless, the available studies are of interest in that they not only provide a rationale for controlled trials, but also add to our understanding of the role of TNF-α in the pathophysiology of SLE [67].

We also detected higher levels of IL-12 in patients with nephritis. Previous studies have demonstrated that increased IL-12 production was associated closely with renal disease in parallel with Th1 polarization and increased IFN-γ solubilization in vitro [68,69]. In addition, the increase in urinary IL-12 apparently reflected both its serum and its glomerular accumulation [68,69].

Recently, it has been shown that nutrition may contribute to renal disorders in SLE. Dietary factors that suppress Th2 cytokines such as IL-4 at early phase, and inhibit inflammatory cytokines IL-1β, IL- 6, TNF-α and IFN-γ, and enhance TGF-β or IL-10 production at late phase, are beneficial for delay onset of proteinuria and for longer life span of murine lupus [70]. However, we did not evaluate the diet of the individuals included in our study.

IL-10 boosts B cell proliferation and immunoglobulin class switching resulting in enhanced antibody secretion with the capacity to enter extravascular compartments and promote inflammation in SLE [42,44]. In SLE patients, it has been shown that elevated production of IL-10 is capable of promoting generation of dsDNA antibodies [71], as observed in our study. Although SSA and SSB were also reported to be associated with high TNF/low IL-10 genotype [72], we did not find such association in our cohort. In addition, IL-6 was demonstrated to be highly expressed in kidneys and to be significantly increased in the serum SLE patients with nephritis [20]. We did not find an association between IL-6 and nephritis; however we observed that patients with dysmorphic hematuria had increased serum levels of IL-6.

Although depression was identified in only 17% of our cohort, we observed increased levels of TNF-α in patients with moderate/severe depression when compared to patients with no/mild depression.

In the past 20 years since the initial reports of neural-immune interactions in depression, several studies have shown a clear association between activation of the immune system, levels of proinflammatory cytokines, and psychiatric symptoms [73–75]. TNF-α exerts its biological effect mainly by binding to tumor necrosis factor receptor 1 (TNFR1) and receptor 2 (TNFR2), causing activation of complex signaling cascades that mediate different intracellular effects [76]. In the brain, TNFR1 seems to show a constitutive pattern of expression whereas TNFR2 is mainly expressed under stimulatory conditions [76]. The highest concentrations of TNF-α receptors in the brain are found in several regions involved in mood regulation and cognitive functioning like the hypothalamus, hippocampus, and areas of the cerebral cortex [77]. Although an associative link between neuroinflammation and mood disorders is widely accepted, further studies are necessary to establish the cause-effect relationship [78]. Several studies connect cytokines with the pathogenesis of depression in Alzheimer's disease [79], in atypical depression [80], in major depressive disorder [73–75,81,82], and in multiple sclerosis [72], however this association has not been reported in SLE so far.

We did not observed correlation between depression and other cytokines studied, however we found a negative correlation between IL-10 levels and the severity of depression. There is no data in the literature exploring this correlation between depression and IL-10 levels so far.

Hydroxychloroquine has been shown to reduce the probability of flares, the accrual of damage, to possibly protect patients with SLE from the occurrence of vascular and thrombotic events and even to increase survival [83]. Literature data suggests that antimalarials interfere with TNF-α release from human and murine cells, although their exact mode of action is not fully understood [34]. In one previous study [34] chloroquine was shown to lower TNF-α levels, however in our study we did not observe differences in TNF-α levels between hydroxychloroquine users and non-users.

The pathogenesis of SLE is a combination of multifactorial, genetic and environmental influences, which lead to an irreversible break in immunologic self-tolerance [83]. SLE family members are at higher risk of developing not only SLE, but also other autoimmune diseases [84–86]. However, IL-12, IFN-γ, TNF-α, IL-5, IL-6 and IL-10 levels have never been studied in first-degree relatives. In our study we did not observe any difference in these cytokine levels of first-degree relatives when compared to healthy controls.

To the best of our knowledge, this is the first study to evaluate Th1 (IL-12, IFN-γ, TNF-α) and Th2 (IL-5, IL-6 and IL-10) cytokines in childhood-onset SLE. Th1 cytokines may play a role in the pathogenesis of neuropsychiatric and renal manifestations in childhood-onset SLE. The correlation with SLEDAI suggests that TNF-α may be a useful biomarker for disease activity in childhood-onset SLE, however longitudinal studies are necessary to determine if increase of this cytokine may predict flares in childhood-onset SLE.

Acknowledgments

Fundação Amparo À Pesquisa Estado São Paulo-Brasil (FAPESP 2008/02917-0 and 2009/06049-6 and 2009/11076-2), Conselho Nacional Pesquisa Desenvolvimento-Brasil CNPq (300447/2009-4).

References

[1] Papadimitraki ED, Isenberg DA. Childhood- and adult-onset lupus: an update of similarities and differences. Expert Rev Clin Immunol 2009;5:391–403.
[2] Klein-Gitelman M, Reiff A, Silverman ED. Systemic lupus erythematosus in childhood. Rheum Dis Clin N Am 2002;28:561–77.
[3] Stichweh D, Arce E, Pascual V. Update on pediatric systemic lupus erythematosus. Curr Opin Rheumatol 2004;16:577–87.
[4] Tucker LB, Menon S, Schaller JG, Isenberg DA. Adult and childhood-onset systemic lupus erythematosus: a comparison of onset, clinical features, serology and outcome. Br J Rheum 1995;34:866–72.
[5] Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. Lupus 2006;15:308–18.
[6] Malleson PN, Fung MY, Rosenberg AM. The incidence of pediatric rheumatic diseases: results from the Canadian Pediatric Rheumatology Association Disease Registry. J Rheumatol 1996;23:1981–7.
[7] McCarty DJ, Manzi S, Medsger Jr TA, Ramsey-Goldman R, LaPorte RE, Kwoh CK. Incidence of systemic lupus erythematosus Race and gender differences. Arthritis Rheum 1995;38:1260–70.
[8] Mina R, Brunner HI. Pediatric lupus-are there differences in presentation, genetics, response to therapy, and damage accrual compared with adult lupus? Rheum Dis Clin North Am. 2010;36:53–80.
[9] Font J, Cervera R, Espinosa G, Pallarés L, Ramos-Casals M, Jiménez S, et al. Systemic lupus erythematosus (SLE) in childhood: analysis of clinical and

immunological findings in 34 patients and comparison with SLE characteristics in adults. Ann Rheum Dis 1998;57:456–9.
[10] Hoffman IE, Lauwerys BR, De Keyser F, Huizinga TW, Isenberg D, Cebecauer L, et al. Juvenile-onset systemic lupus erythematosus: different clinical and serological pattern than adult-onset systemic lupus erythematosus. Ann Rheum Dis 2009;68:412–5.
[11] Sibbitt Jr WL, Brandt JR, Johnson CR, Maldonado ME, Patel SR, Ford CC, et al. The incidence and prevalence of neuropsychiatric syndromes in pediatric onset systemic lupus erythematosus. J Rheumatol 2002;29:1536–42.
[12] Carreno L, Lopez-Longo FJ, Monteagudo I, Rodríguez-Mahou M, Bascones M, González CM, et al. Immunological and clinical differences between juvenile and adult onset of systemic lupus erythematosus. Lupus 1999;8: 287–92.
[13] Brunner HI, Gladman DD, Ibanez D, Urowitz MD, Silverman ED. Difference in disease features between childhood-onset and adult-onset systemic lupus erythematosus. Arthritis Rheum 2008;58:556–62.
[14] Tucker LB, Menon S, Schaller JG, Isenberg DA. Adult- and childhood-onset systemic lupus erythematosus: a comparison of onset, clinical features, serology, and outcome. Br J Rheumatol 1995;34:866–72.
[15] Hersh AO, von Scheven E, Yazdany J, Panopalis P, Trupin L, Julian L, et al. Differences in long-term disease activity and treatment of adult patients with childhood- and adult-onset systemic lupus erythematosus. Arthritis Rheum 2009;61:13–20.
[16] Appenzeller S, Marini R, Costallat LT. Damage did not independently influence mortality in childhood systemic lupus erythematosus. Rheumatol Int. 2005;25:619–24.
[17] von Scheven E, Bakkaloglu A. What's new in paediatric SLE? Best Pract Res Clin Rheumatol 2009;23:699–708.
[18] Yap DY, Lai KN. Cytokines and their roles in the pathogenesis of systemic lupus erythematosus: from basics to recent advances. J Biomed Biotechnol 2010;2010:365083.
[19] Gómez D, Correa PA, Gómez LM, Cadena J, Molina JF, Anaya JM. Th1/Th2 cytokines in patients with systemic lupus erythematosus: is tumor necrosis factor alpha protective? Semin Arthritis Rheum 2004;33:404–13.
[20] Sabry A, Elbasyouni SR, Sheashaa HA, Alhusseini AA, Mahmoud K, George SK, et al. Correlation between levels of TNF-alpha and IL-6 and hematological involvement in SLE Egyptian patients with lupus nephritis. Int Urol Nephrol 2006;38:731–7.
[21] Mellor-Pita S, Citores MJ, Castejon R, Yebra-Bango M, Tutor-Ureta P, Rosado S, et al. Monocytes and T lymphocytes contribute to a predominance of interleukin 6 and interleukin 10 in systemic lupus erythematosus. Cytometry B Clin Cytom 2009;76:261–70.
[22] Park YB, Lee SK, Kim DS, Lee J, Lee CH, Song CH. Elevated interleukin-10 levels correlated with disease activity in systemic lupus erythematosus. Clin Exp Rheumatol 1998;16:283–8.
[23] Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
[24] ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum 1999;42:599–608.
[25] Harris EN, Gharavi AE, Patel SP, Hughes GR. Evaluation of the anti-cardiolipin antibody test: report of an international workshop held 4 April 1986. Clin Exp Immunol 1987;68:215–22.
[26] Brandt JT, Triplett DA, Alving B, Scharrer I. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. Criteria for the diagnosis of lupus anticoagulants: an update. Thromb Haemost 1995;74:1185–90.
[27] 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.
[28] Yee CS, Farewell VT, Isenberg DA, Griffiths B, Teh LS, Bruce IN, et al. The use of Systemic Lupus Erythematosus Disease Activity Index-2000 to define active disease and minimal clinically meaningful change based on data from a large cohort of systemic lupus erythematosus patients. Rheumatology (Oxford) 2011;50:982–8.
[29] Gladman DD, Urowitz MB, Goldsmith CH, Fortin P, Ginzler E, Gordon C, et al. The reliability of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index in patients with systemic lupus erythematosus. Arthritis Rheum 1997;40:809–13.
[30] Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561–71.
[31] Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol 1988;56:893–7.
[32] Leroux-Roels G, Offner F, Philippe J, Vermeulen A. Influence of blood collecting systems on concentrations of tumor necrosis factor in serum and plasma. Clin Chem 1988;134:2373–4.
[33] Galley HF, Webster NR. The immuno-inflammatory cascade. Br J Anaesth 1996;77:11–6.
[34] Wozniacka A, Lesiak A, Narbutt J, McCauliffe DP, Sysa-Jedrzejowska A. Chloroquine treatment influences proinflammatory cytokine levels in systemic lupus erythematosus patients. Lupus 2006;15:268–75.
[35] Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 2003;3:133–46.
[36] Trinchieri G. Interleukin-12: a cytokine at the interface of inflammation and immunity. Adv Immunol 1998;70:83–243.
[37] Farrar MA, Schreiber RD. The molecular cell biology of interferon-gamma and its receptor. Annu Rev Immunol 1993;11:571–611.
[38] Joseph A, Brasington R, Kahl L, Ranganathan P, Cheng TP, Atkinson J. Immunologic rheumatic disorders. J Allergy Clin Immunol 2010;125:S204–15.
[39] Kollias G, Douni E, Kassiotis G, Kontoyiannis D. The function of tumor necrosis factor and receptors in models of multi-organ inflammation, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. Ann Rheum Dis 1999;58:132–9.
[40] Bradley JR. TNF-mediated inflammatory disease. J Pathol 2008;214:149–60.
[41] Wen X, Zhang D, Kikuchi Y, Jiang Y, Nakamura K, Xiu Y, et al. Transgene-mediated hyper-expression of IL-5 inhibits autoimmune disease but increases the risk of B cell chronic lymphocytic leukemia in a model of murine lupus. Eur J Immunol 2004;34:2740–9.
[42] Sun KH, Yu CL, Tang SJ, Sun GH. Monoclonal anti-double-stranded DNA autoantibody stimulates the expression and release of IL-1beta, IL-6, IL-8, IL-10 and TNF-alpha from normal human mononuclear cells involving in the lupus pathogenesis. Immunology 2000;99:352–60.
[43] Battaglia M, Gianfrani C, Gregori S, Roncarolo MG. IL-10-producing T regulatory type 1 cells and oral tolerance. Ann NY Acad Sci 2004;1029:142–53.
[44] Gerli R, Nocentini G, Alunno A, Bocci EB, Bianchini R, Bistoni O, et al. Identification of regulatory T cells in systemic lupus erythematosus. Autoimmun Rev 2009;8:426–30.
[45] Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK. Type 1 T regulatory cells. Immunol Rev 2001;182:68–79.
[46] Pot C, Apetoh L, Kuchroo VK. Type 1 regulatory T cells (Tr1) in autoimmunity. Semin Immunol 2011;23:202–8.
[47] Groux H, Bigler M, de Vries JE, Roncarolo MG. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. J Exp Med 1996;184:19–29.
[48] Al-Mutairi S, Al-Awadhi A, Raghupathy R, Al-Khawari H, Sada P, Al-Herz A, et al. Lupus patients with pulmonary involvement have a pro-inflammatory cytokines profile. Rheumatol Int 2007;27:621–30.
[49] Sabry AA, Elbasyouni SR, Kalil AM, Abdel-Rahim M, Mohsen T, Sleem A. Markers of inflammation and atherosclerosis in Egyptian patients with systemic lupus erythematosus. Nephrology (Carlton) 2006;11:329–35.
[50] Sabry A, Sheashaa H, El-Husseini A, Mahmoud K, Eldahshan KF, George SK, et al. Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: its correlation with disease activity. Cytokine. 2006;35:148–53.
[51] Gabay C, Cakir N, Moral F, Roux-Lombard P, Meyer O, Dayer JM, et al. Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. J Rheumatol 1997;24:303–8.
[52] Mahmoud RA, El-Gendi HI, Ahmed HH. Serum neopterin, tumor necrosis factor-alpha and soluble tumor necrosis factor receptor II (p75) levels and disease activity in Egyptian female patients with systemic lupus erythematosus. Clin Biochem 2005;38:134–41.
[53] Jones BM, Liu T, Wong RW. Reduced in vitro production of interferon-gamma, interleukin-4 and interleukin-12 and increased production of interleukin-6, interleukin-10 and tumour necrosis factor-alpha in systemic lupus erythematosus. Weak correlations of cytokine production with disease activity. Autoimmunity 1999;31:117–24.
[54] Studnicka-Bencke A, Steiner G, Petera P, Smolen JS. Tumor necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. Br J Rheumatol 1996;35:1067–74.
[55] Masutani K, Akahoshi M, Tsuruya K, Tokumoto M, Ninomiya T, Kohsaka T, et al. Predominance of Th1 immune response in diffuse proliferative lupus nephritis. Arthritis Rheum 2001;44:2097–106.
[56] Gigante A, Gasperini ML, Afeltra A, Barbano B, Margiotta D, Cianci R, et al. Cytokines expression in SLE nephritis. Eur Rev Med Pharmacol Sci 2011;15:15–24.
[57] Gloor JM. Lupus nephritis in children. Lupus 1998;7:639–43.
[58] Ihm CG, Park JK, Hong SP, Lee TW, Cho BS, Kim MJ. Circulating factors in sera or peripheral blood mononuclear cells in patients with membranous nephropathy or diabetic nephropathy. J Korean Med Sci 1997;12:539–44.
[59] Lionaki S, Siamopoulos K, Theodorou I, Papadimitraki E, Bertsias G, Boumpas D, et al. Inhibition of tumour necrosis factor alpha in idiopathic membranous nephropathy: a pilot study. Nephrol Dial Transplant 2009;24:2144–50.
[60] Dean GS, Tyrrell-Price J, Crawley E, Isenberg DA. Cytokines and systemic lupus erythematosus. Ann Rheum Dis 2000;59:243–51.
[61] Aringer M, Smolen JS. Efficacy and safety of TNF-blocker therapy in systemic lupus erythematosus. Expert Opin Drug Saf 2008;7:411–9.
[62] Aringer M, Smolen JS. Tumor necrosis factor and other proinflammatory cytokines in systemic lupus erythematosus: a rationale for therapeutic intervention. Lupus 2004;13:344–7.
[63] Aringer M, Steiner G, Graninger WB, Höfler E, Steiner CW, Smolen JS. Effects of short-term infliximab therapy on autoantibodies in systemic lupus erythematosus. Arthritis Rheum 2007;56:274–9.
[64] Zhu LJ, Yang X, Yu XQ. Anti-TNF-alpha therapies in systemic lupus erythematosus. Biomed Biotechnol 2010;2010:465898.
[65] Murdaca G, Colombo BM, Puppo F. Emerging biological drugs: a new therapeutic approach for systemic lupus erythematosus. An update upon efficacy and adverse events. Autoimmune Rev 2011;11:56–60.
[66] Mageed RA, Isenberg DA. Tumors necrosis factor alpha in systemic lupus erythematosus and anti-DNA autoantibody production. Lupus 2002;11:850–5.
[67] Aringer M, Smolen JS. Therapeutic blockade on TNF in patients with SLE-promising or crazy? Autoimmune Rev 2012;11:321–5.

[68] Tucci M, Lombardi L, Richards HB, Dammacco F, Silvestris F. Overexpression of interleukin-12 and T helper 1 predominance in lupus nephritis. Clin Exp Immunol 2008;154:247–54.
[69] Nagai T, Yanagida T, Hirohata S. Anti-ribosomal P protein antibody induces Th1 responses by enhancing the production of IL-12 in activated monocytes. Mod Rheumatol 2011;21:57–62.
[70] Hsieh CC, Lin BF. Dietary factors regulate cytokines in murine models of systemic lupus erythematosus. Autoimmun Rev. 2011;11:22–7.
[71] López P, Gómez J, Prado C, Gutiérrez C, Suárez A. Influence of functional interleukin 10/tumor necrosis factor-alpha polymorphisms on interferon-alpha, IL-10, and regulatory T cell population in patients with systemic lupus erythematosus receiving antimalarial treatment. J Rheumatol 2008;35: 1559–66.
[72] Suárez A, López P, Mozo L, Gutiérrez C. Differential effect of IL10 and TNF-α genotypes on determining susceptibility to discoid and systemic lupus erythematosus. Ann Rheum Dis 2005;64:1605–10.
[73] Mikova O, Yakimova R, Bosmans E, Kenis G, Maes M. Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. Eur Neuropsychopharmacol 2001;11:203–8.
[74] Tuglu C, Kara SH, Caliyurt O, Vardar E, Abay E. Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder. Psychopharmacology (Berl) 2003;170:429–33.
[75] Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry 2010;67:446–57.
[76] Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol 2001;11:372–7.
[77] Khairova RA, Machado-Vieira R, Du J, Manji HK. A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. Int J Neuropsychopharmacol 2009;12:561–78.
[78] Kaster MP, Gadotti VM, Calixto JB, Santos AR, Rodrigues AL. Depressive-like behavior induced by tumor necrosis factor-α in mice. Neuropharmacology 2012;62:419–26.
[79] Clark IA, Alleva LM, Vissel B. The roles of TNF in brain dysfunction and disease. Pharmacol Ther 2010;128:519–48.
[80] Yoon HK, Kim YK, Lee HJ. Role of cytokines in atypical depression. Nord J Psychiatry 2012;66:183–8.
[81] Himmerich H, Fulda S, Linseisen J, Seiler H, Wolfram G, Himmerich S, et al. Depression, comorbidities and the TNF-alpha system. Eur Psychiatry 2008;23:421–9.
[82] Kim YK, Na KS, Shin KH, Jung HY, Choi SH, Kim JB. Cytokine imbalance in the pathophysiology of major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:1044–53.
[83] Alarcón GS, McGwin G, Bertoli AM, Fessler BJ, Calvo-Alén J, Bastian HM, et al. LUMINA study group. Effect of hydroxychloroquine on the survival of patients with systemic lupus erythematosus: data from LUMINA, a multiethnic US cohort (LUMINA L). Ann Rheum Dis 2007;66:1168–72.
[84] Niewold TB, Hua J, Lehman TJ, Harley JB, Crow MK. High serum IFN-alpha activity is a heritable risk factor for systemic lupus erythematosus. Genes Immunol 2007;8:492–502.
[85] Niewold TB, Adler JE, Glenn SB, Lehman TJ, Harley JB, Crow MK. Age- and sex-related patterns of serum interferon-alpha activity in lupus families. Arthritis Rheum 2008;58:2113–9.
[86] Scofield RH, Bruner GR, Harley JB, Namjou B. Autoimmune thyroid disease is associated with a diagnosis of secondary Sjogren's syndrome in familial systemic lupus. Ann Rheum Dis 2007;66:410–3.