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

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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.

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

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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.