CLINICAL SCIENCE

Clinical and serological manifestations associated with interferon-α levels in childhood-onset systemic lupus erythematosus

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OBJECTIVE: To determine the serum levels of interferon alpha in childhood-onset systemic lupus erythematosus patients, their first-degree relatives and healthy controls and to evaluate the associations between serum interferon alpha and disease activity, laboratory findings and treatment features.

METHODS: We screened consecutive childhood-onset systemic lupus erythematosus patients in a longitudinal cohort at the pediatric rheumatology unit of the State University of Campinas between 2009 and 2010. All patients demonstrated disease onset before the age of 16. Disease status was assessed according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). Interferon alpha levels were measured using an enzyme-linked immunoabsorbent assay.

RESULTS: We included 57 childhood-onset systemic lupus erythematosus patients (mean age 17.33±4.50), 64 first-degree relatives (mean age 39.95±5.66), and 57 healthy (mean age 19.30±4.97) controls. Serum interferon alpha levels were significantly increased in childhood-onset systemic lupus erythematosus patients compared to their first-degree relatives and healthy controls. Interferon alpha levels were significantly increased in patients with positive dsDNA antibodies, patients with cutaneous vasculitis, patients with new malar rash and patients who were not receiving medication. Interferon alpha levels correlated with C3 levels and systemic lupus erythematosus Disease Activity Index scores. In addition, we observed an inverse correlation between patient age and interferon alpha levels.

CONCLUSION: Interferon alpha may play a role in the pathogenesis of childhood-onset systemic lupus erythematosus, especially in cutaneous manifestations and dsDNA antibody formation. The observation that interferon alpha levels are increased in patients who are not taking medication should be investigated in longitudinal studies to determine whether elevated interferon alpha levels may predict systemic lupus erythematosus flares.

KEYWORDS: Interferon alpha (IFN-α); SLEDAI; Childhood-onset; Systemic lupus erythematosus.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease characterized by diverse clinical manifestations ranging from malar rash to renal involvement (1,2). Immune system disorders and abnormalities in cytokine production have been described in patients with SLE (1). The pathogenesis of SLE is multifactorial and is likely driven by a complex combination of genetic and environmental factors, which leads to an irreversible failure of immunologic self-tolerance (3).

Approximately 20% of all SLE patients experience disease onset prior to the age of 16 (4). Childhood-onset SLE demonstrates a different phenotype than adult-onset SLE. Renal (50%-67%), neurological (22-95%) and hematological (77%) involvement in addition to fever and lymphadenopathy are observed more frequently in pediatric patients than in patients with adult-onset SLE (4,5-11). In addition, childhood-onset SLE is associated with significantly more active diseases, both at disease onset and over time, compared to adult-onset SLE (9). The outcome of childhood-onset disease

No potential conflict of interest was reported.

is also generally worse than that of adult-onset disease (4,12). 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 have significantly improved survival in patients with childhood-onset SLE (13,14).

There is strong evidence indicating that cytokines play a role in the pathogenesis of SLE (15,16). The first cytokine abnormality documented in SLE was an increased serum level of interferon (IFN), subsequently characterized as IFN-α, which is produced mainly by leukocytes (17). Elevated serum IFN-α has also been observed in adult-onset SLE, and IFN-α levels correlate with both disease activity and disease severity (15,17,18). Associations have also been observed between IFN-α levels and several markers of immune activation, such as complement activation and double-stranded DNA (dsDNA) antibody titers (15). However, the role of IFN-α in childhood-onset SLE has not been investigated.

The aim of our study was to determine the serum levels of IFN-α in childhood-onset SLE patients, their first-degree relatives and healthy controls. In addition, we evaluated the association of IFN-α with disease activity, laboratory findings and treatment features.

METHODS

Subjects

Fifty-seven consecutive childhood-onset SLE patients followed at the Pediatric Rheumatology Outpatient Clinic of the State University of Campinas were invited to participate in this cross-sectional study. Patients were included in the present study if they (i) fulfilled at least four of the American College of Rheumatology (ACR) criteria (19), (ii) were younger than 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 a history of any chronic disease (including autoimmune diseases) were included as control groups. The healthy controls were matched for age, sex and demographic background. This study was approved by the ethics committee at our institution, and informed written consent was obtained from each participant and/or legal guardian.

Clinical features

The medical histories and clinical and serological characteristics of each patient were entered into a computer database at the time of SLE diagnosis. The following variables were included in this database: onset of disease, defined as the age at which the first symptoms clearly attributable to SLE occurred; age at diagnosis, defined as the age at which the patients fulfilled four or more of the 1982 revised criteria for the classification of SLE (19); and follow-up time, defined as the time from disease onset until May 2010.

All clinical manifestations and laboratory findings were recorded at disease onset on a quarterly basis throughout the follow-up period and on the day of blood withdrawal. Nephritis was defined as proteinuria exceeding 0.5 g/L with abnormal urinary sediment and/or histological findings. Nephrotic syndrome was defined as proteinuria in excess of 3 g/day. Hematological abnormalities were ascribed to lupus only in the absence of bone-marrow suppression (leukopenia <4000 cells/mm^3, thrombocytopenia $<100{,}000$ cells/mm^3, and hemolytic anemia). We also assessed 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 the ACR guidelines (20).

The treatment prescribed at the time of blood withdrawal and any adverse events related to medication use were recorded. Doses of oral and parenteral corticosteroids were analyzed and converted to the equivalent doses of prednisone.

Laboratory studies

Antinuclear antibody (ANA) levels were determined by indirect immunofluorescence using mouse liver as a substrate and were regarded as positive if the titers were higher than 1:40. The levels of dsDNA antibodies were determined by indirect immunofluorescence using *Crithidia* as a substrate and were considered positive if they were higher than 1:10. The levels of precipitating antibodies to extractable nuclear antigens (ENA), including Ro (SSA), La (SSB), and Sm, were detected using a standardized enzyme-linked immunosorbent assay (ELISA) method and were considered positive if higher than 1:80. The levels of IgG and IgM anticardiolipin antibodies (aCL) were measured by ELISA (21). Lupus anticoagulant (LA) activity was detected by coagulation assays in platelet-free plasma obtained by double centrifugation following the recommendations of the Scientific and Standardization Committee of the International Society of Thrombosis and Homeostasis subcommittee on LA (22). These measurements were performed twice at an interval of 12 weeks.

Disease Activity/Cumulative Damage Evaluation

Disease activity was measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (23). The SLEDAI consists of 24 weighted items grouped into 9 domains, or organ systems, as follows: central nervous system (assigned a weight of 8), vascular system (weight of 8), renal system (weight of 4), musculoskeletal system (weight of 4), serosal system (weight of 2), dermal system (weight of 2), immune system (weight of 2), constitutional (weight of 1), and hematologic system (weight of 1). The SLEDAI scores range between 0 and 105, and scores of ≥ 3 were considered to represent active disease (24).

Cumulative SLE-related damage in all patients was determined using the Systemic Lupus International Collaborating Clinics (SLICC)/ACR Damage Index (SDI) measured at the time of blood withdrawal. The SDI scores ranged from 0 to 47 (25).

IFN-α assay

Peripheral venous blood was collected from each subject and allowed to clot at room temperature for 30 min. Samples were then centrifuged for 15 min at 3000 rpm. Separated sera were stored in aliquots at -80℃ for subsequent use in assays. None of the samples were taken during an episode of acute or chronic infection (26).

Commercially available kits from R&D Systems (London, UK) were used to measure serum IFN-α levels by ELISA in accordance with the manufacturer's instructions.

Statistical analyses

An analysis of variance with Tukey's pairwise post hoc comparisons was used to compare IFN-α levels between groups. Spearman's correlation was used to correlate continuous variables (e.g., IFN-α levels and SLEDAI, SDI). IFN-α levels and categorical variables were compared using a 2-sample t-test. For all of the analyses, p-values <0.05 were considered statistically significant.

RESULTS

Demographics

We included 57 consecutive childhood-onset SLE patients. Fifty-four (94.7%) were female with a mean age of 17.33 years [standard deviation (SD) ±4.50 years; range 9-37]. Disease duration was 4.71 years (SD±4.57; range 0-26). We also investigated 64 first-degree relatives with a mean age of 39.95 years (SD±5.66; range 28-52). The control group consisted of 57 healthy volunteers (52 women) with a mean age of 19.30 (SD±4.97 years; range 6-30) (Table 1).

Patients and healthy controls were statistically comparable in terms of age and sex.

Clinical, laboratory, and treatment features

At the time of study entry, 30 (52.6%) childhood-onset SLE patients had active disease (SLEDAI≥3) with mean SLEDAI scores of 8.37 (SD±3.80, range 3-18). Patients with inactive disease [N = 27 (47.4%)] had a mean SLEDAI score of 0.39 [(SD±0.80 range 0-2)]. Active nephritis (28.3%), new malar rash (6.6%), new alopecia (5.0%), and cutaneous vasculitis (5.0%) were the most frequently observed clinical manifestations.

At the time of blood withdrawal, 8 (13.3%) patients were not taking any medication. Thirty-nine (68.4%) patients were receiving prednisone, 32 (53.3%) were receiving hydroxychloroquine, and 22 (36.6%) patients were receiving other immunosuppressive drugs (Table 1).

Cytokine assay

The mean serum IFN-α level was 13.84±8.46 pg/mL in childhood-onset SLE patients, 10.36±6.04 pg/mL ($p = 0.012$) in first-degree relatives and 11.68±6.66 pg/mL in healthy controls ($p = 0.043$). No difference in serum IFN-α levels between first-degree relatives and healthy controls was observed ($p = 0.484$) (Figure 1a).

IFN-α levels were significantly increased in patients who were positive for dsDNA antibodies ($p = 0.011$) (Figure 1b), patients with cutaneous vasculitis ($p = 0.001$), and patients with a new malar rash ($p = 0.032$) or disease activity ($p = 0.031$). IFN-α levels were directly correlated with C3 levels (r = 0.34; $p = 0.032$) and SLEDAI scores (r = 0.43; $p = 0.012$) and indirectly correlated with age (r = -0.17; $p = 0.025$). IFN-α levels were significantly higher in patients who were not taking medication (mean = 13.01; SD±6.09) compared to patients who were receiving medication (mean = 21.59; SD±16.02; $p = 0.035$) (Figure 1c). In an analysis of individual medications, higher levels of IFN-α were observed in patients not taking prednisone (mean = 20.07; SD±14.65) compared to patients taking prednisone (mean = 12.95; SD±6.19; $p = 0.042$). No association between IFN-α levels and other clinical or laboratory variables (hematological or immunological) or SDI scores was observed. No difference in IFN-α levels was observed between patients with and without hydroxychloroquine or other immunosuppressants.

DISCUSSION

Cytokines are low-molecular weight proteins that play a key role in the immunological dysregulation observed in autoimmune diseases. The increased levels of proinflammatory cytokines are believed to be critical in the pathogenesis of SLE (27). Higher cytokine levels in SLE patients may promote the inflammatory response, apoptosis and autoantibody production, which initiate and may also maintain SLE disease activity over time (16,27).

The first cytokine abnormality documented in SLE was an increased serum level of IFN-α, which is a cytokine with both antiviral and immunoregulatory functions (17,28). The contributions of IFN-α to SLE can be explained through several distinct, but related, mechanisms. In genetically susceptible individuals, B cell precursors expressing self-reactive antibodies are not removed (15). Most likely due to a mismanagement of naturally occurring apoptotic cells, nuclear material stimulates autoreactive B-cells, leading to antibody secretion and the formation of immune complexes.

Table 1 - Demographic and clinical characteristics of the patient and control groups included in the study.

Parameter	Childhood-onset SLE patients N = 57	First-degree relatives N = 64	Controls N = 57
Sex			
Female	54 (94.7%)	59 (92.18%)	52 (91.22%)
Age (years)	17.33±4.50 (range 9-37)	39.95±5.66* (range 28-52)	19.30±4.97 (range 6-30)
Disease duration (years)	4.71 ±4.57 (range 0-26)	-----	-----
SLEDAI	4.43±4.94	-----	-----
Active disease N = 30	8.37±3.80		
Inactive disease N = 27	0.39±0.80		
SDI	0.50±0.82	-----	-----
Treatment			
No medication	8 (14%)		
Prednisone	39 (68.4%)		
Hydroxychloroquine	32 (56.1%)		
Immunosuppressive	22 (38.6%)		
IFN-α (pg/mL)	13.84±8.46*	10.36±6.04	11.68±6.66

*$p \leq 0.05$.

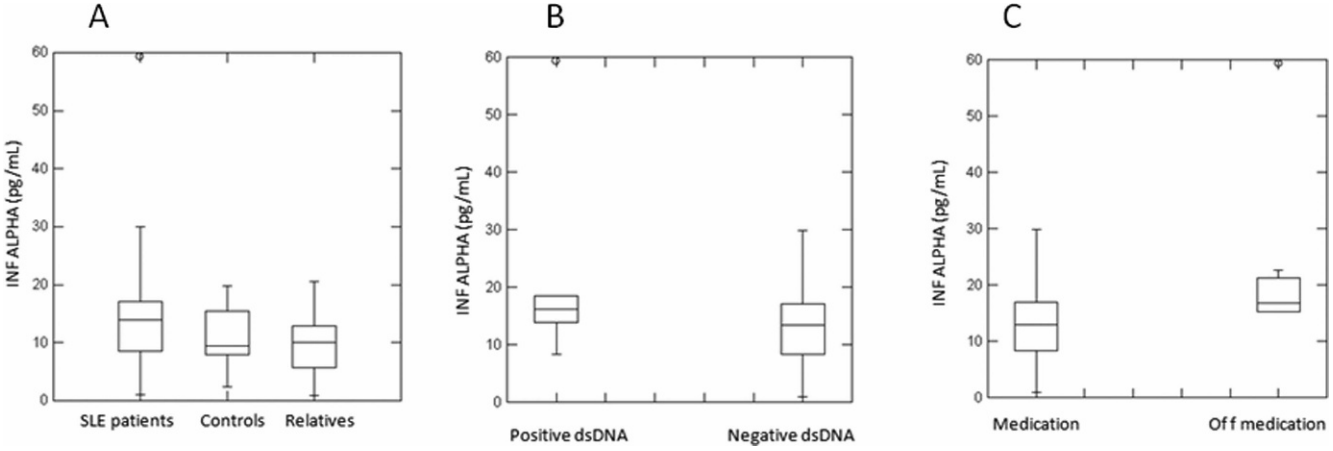

Figure 1 - IFN-α association in SLE. 1a- Analysis of variance with Tukey's pairwise post-hoc comparisons among SLE patients, their first-degree relatives and controls; 1b- 2-sample t-test comparing SLE patients positive and negative for dsDNA antibodies; 1c- 2-sample t-test comparing SLE patients taking and not taking medication. Data are presented as box plots; the box represents the 25th to 75th percentiles, the line within the box represents the 50th percentile, and the lines outside the box represent the minimum and maximum values.

These immune complexes and apoptotic bodies stimulate plasmacytoid dendritic cells to produce IFN-α, which in turn enhances antigen presentation by dendritic cells to T-cells while promoting memory T-cell expansion and survival (15,29).

Increased IFN-α serum levels are often observed in SLE patients (3,17,18,30-34). Here, we observed increased serum IFN-α in childhood-onset SLE patients compared to their first-degree relatives or healthy controls. Our data support the results of previous studies that demonstrated elevated IFN-α levels in the sera of adult-onset SLE patients (3,17,18,30-35).

The clinical significance of IFN-α pathway activation in SLE is multifaceted. IFN-α has been implicated in the pathogenesis of SLE, and therefore, therapies targeted against IFN-α are currently in clinical trials (36,37). Furthermore, IFN-α activation may determine specific subset of SLE patients with potential diagnostic, prognostic, and therapeutic implications. Importantly, a change in IFN-α activity levels may reflect changes in disease activity and thus inform the clinical management of the disease (38). In our study, IFN-α was significantly higher in patients with active disease (SLEDAI ≥3) than in patients with inactive disease. Furthermore, we observed a direct correlation between SLEDAI scores and IFN-α levels, suggesting that IFN-α could be a biomarker for disease activity in childhood SLE. Similar results have been observed in adult-onset SLE (3,17,18,30-35).

Previous studies suggest that IFN-α plays an important role in the immunopathogenesis of SLE (3,17,18,30-35). Serum IFN-α is correlated with multiple clinical and serological features of SLE (29,35). We observed that IFN-α levels were increased in patients with cutaneous manifestations of the disease. Our data also showed increased IFN-α levels in childhood-onset SLE patients positive for dsDNA antibodies as well as a direct correlation between IFN-α and C3 levels. However, no association with renal disease was observed. The increased expression of IFN-inducible genes (IFIGs) in peripheral blood mononuclear cells (PBMCs) has been associated with the presence of lupus nephritis and proteinuria, cutaneous manifestations, and the presence of anti-Ro, anti-Smith (anti-Sm), anti-RNP, and anti-dsDNA antibodies (32,39). Anti-dsDNA antibodies have been associated with lupus nephritis (40), and studies have linked anti-Ro antibody to lupus-related skin findings (41). It remains unclear whether the association between IFN-α and cutaneous and renal disease manifestations found in previous studies is primary or secondary due to an association between autoantibodies and IFN-α (39). We did not observe any associations between IFN-α levels and the levels of other antibodies, such as anti-Ro, anti-Sm, or anti-RNP.

Relatives of individuals with SLE are at a higher risk of developing not only SLE but also other autoimmune diseases (3,31). A heritable predisposition to increased IFN-α pathway activation in SLE patient families could explain some of the burden of both SLE and non-SLE autoimmunity in the population. Single nucleotide polymorphisms (SNPs) in the IFN-α pathway genes IFN regulatory factor 5 (IRF5) and non-receptor tyrosine-protein kinase (TYK2) (42-45) are associated with SLE, suggesting that genetic variability in endogenous IFN-a signaling may be involved in the etiology of this disease, although the impact of these polymorphisms on IFN-α activity *in vivo* is not known (3,43). We did not observe any differences in serum IFN-α levels between first-degree relatives of SLE patients and controls. However, the limited sample size may have affected these results.

We found an inverse correlation between patient age and IFN-α levels. Similar findings have been reported in adult SLE patients as well as in healthy controls, independent of menopause status (31). It is not clear whether the higher serum IFN-α activity observed in young SLE patients is a cause or a result of disease activity, but this correlation may explain some of the differences in the clinical and serologic manifestations of childhood-onset and adult-onset SLE patients.

In addition, we observed higher IFN-α levels in patients who were not receiving medication. None of these patients had any evidence of disease activity at the time of evaluation. Previous studies have shown a dramatic decrease in the expression of IFN-inducible genes (IFIGs) in patients who received pulse glucocorticoid (GC) therapy

(46,47). Data from other studies suggest that intravenous pulse GC treatment may decrease the number of IFN-producing cells, transiently reducing the stimulus for IFIG expression (47).

Although previous studies have analyzed IFN-α levels in childhood-onset SLE, none of these studies have analyzed the clinical and laboratory features associated with increased IFN-α levels (31,45). Serum IFN-α activity was found to be higher in younger individuals in SLE family cohorts, and this tendency was accentuated in affected individuals (31). In addition, one other study revealed that childhood-onset SLE patients are characterized by a potent IFN-α response (45). Genomic approaches have shown that >95% of childhood-onset SLE patients display an ''IFN signature'', as measured by PBMC gene expression profiling (45,48). The PBMC transcriptional signature in childhood-onset SLE corresponds to neutrophil-specific genes, and the differential expression of these genes correlates with disease activity (45). Notably, SLE neutrophils undergo accelerated spontaneous apoptosis *in vitro*, and SLE sera induce apoptosis in healthy neutrophils, both of which are correlated with disease activity (49). In addition, the IFIG expression signature can be used to establish subgroups of patients with severe SLE characterized by renal disease, complement activation, and autoantibody production to RNA-associated autoantigens (33,50).

Genetic association studies of SLE patients have identified several genes, especially components of the pathways both upstream and downstream of IFN (mainly type I), including signal transducer and activator of transcription 4 (STAT4) and IRF5 (38,51-53). STAT4 interacts with type I IFN receptors and is directly involved in IFN signaling. IRF5 is a transcription factor that induces IFN transcription in response to Toll-like receptor (TLR) signaling. In fact, the IRF5 risk haplotype in SLE patients is associated with high serum IFN-α activity (34). These findings are consistent with the fundamental observations from a previous study that identified gene expression profiling of SLE PBMCs (38). These experiments demonstrate a significant upregulation of IFN-regulated gene transcripts in adult and childhood-onset SLE PBMCs (32,34). This characteristic is referred to as the ''IFN signature'' and is assessed as a biomarker for disease activity (38).

In summary, our findings suggest that IFN-α may play a role in the pathogenesis of childhood-onset SLE. The higher levels observed in younger children may explain the different clinical and serologic manifestations when compared to older patients. The pattern of increased IFN-α levels in patients not taking medication should be investigated further in longitudinal studies to determine whether elevated IFN-α levels may predict SLE flares.

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AUTHOR CONTRIBUTIONS

Postal M designed the study and was also responsible for data collection, data analysis and manuscript writing. Sinicato NA was responsible for the collection and analysis of data. Peliçari KO was responsible for the data collection. Marini R designed the study and was also responsible for the data collection and manuscript writing. Costallat LTL was responsible for the manuscript writing. Appenzeller S designed the study and was also responsible for the collection and analysis of data and manuscript writing.

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