Th Cytokine Profile in Childhood-Onset Systemic Lupus Erythematosus

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Abstract

Background: Childhood-onset systemic lupus erythematosus (cSLE) is a kind of chronic inflammatory disease characterized by a highly abnormal immune system. This study aimed to detect expression of the Th cytokines IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α in the peripheral blood of children with cSLE; clinical symptoms; and a disease index and discuss the relationship between the Th cell cytokine regulatory network and onset of systemic lupus erythematosus (SLE) in children and disease outcome.

Methods: A total of 33 children with cSLE and 30 healthy children were enrolled in this study. Children in the cSLE group were classified into the inactive cSLE group or active cSLE group according to their SLE disease activity index 2000 (SLEDAI-2K). Th cytokine profiles in peripheral blood of different groups were detected and analyzed.

Results: The levels of IL-2, IL-10 and IL-21 in the cSLE group were significantly higher than those in the healthy control group (P < 0.05, P<0.01 and P<0.01, respectively). The expression of IL-2, IL-10 and IL-21 in the active cSLE group was significantly higher than that in the healthy control group (P<0.05, P<0.01 and P<0.05, respectively), but IL-22 expression was remarkably lower in the active cSLE group than in the healthy control group (P<0.001). IL-21 in the inactive SLE group was significantly higher than that in the healthy control group (P<0.05). The levels of IL-2 and IL-10 in the active cSLE group were significantly higher than those in the inactive cSLE group (P<0.01 and P<0.05). In-depth analysis showed that the expression levels of IL-2 (r=0.382, P=0.028), IL-6 (r=0.514, P=0.002) and IL-10 (r=0.429, P=0.016) were positively correlated with disease activity. **Conclusion:** This study provides a theoretical basis for the discovery of effective methods to regulate imbalance in T lymphocyte subsets in cSLE, which may open up potential new approaches for the diagnosis of cSLE.

Background

Systemic lupus erythematosus (SLE) is an autoimmune disease caused by external environmental factors that act on the genetic susceptibility of an individual, stimulating the body's immune regulation barriers and breaking the individual's immune tolerance; this systemic autoimmune disease thus involves multiple systems and organs and shows high levels of clinical heterogeneity[1, 2]. cSLE accounts for 10%-20% of the total SLE cases. Due to its high incidence, rapid development, increased likelihood of involving vital organs, more severe clinical symptoms than adult SLE, poor prognosis, and high mortality, cSLE has become an extremely concerning disease[3].

cSLE in children, a chronic inflammatory disease involving large abnormalities in cells of the immune system (including T cells, B cells and mononuclear macrophages), is characterized by the production of autoantibodies and deposition of immune complexes[4]. One of the most common autoimmune diseases in children, cSLE is immunologically characterized by strong proliferation, increased immunoglobulin, the production of various autoantibodies and weak intracellular and extracellular immune responses[5, 6]. However, the pathogenesis of this disease has not been elucidated. A large number of cytokines,

signalling molecules and pattern recognition receptors in the immune system are involved in the pathological process of this disease[7]. Pro-inflammatory cytokines, such as those involved in local inflammatory responses that cause tissue damage, are involved in the immune disorders that are common in SLE patients[8]. Due to their key role in the pathogenesis of SLE, cytokines have become the focus of an increasing number of researchers. In recent years, Th cell subsets and cytokines have been found to play an important role in the pathogenesis of SLE[9].

cSLE is often more serious than that in adults, as the probability that each organ is involved is higher, and the disease process is more dangerous than that in adults[10, 11]. Therefore, the detection of cytokines in SLE patients is helpful to comprehensively understand the immune status of the body and correctly judge disease condition. However, studies involving the detection of Th cytokines were mostly carried out in adult SLE, and similar studies in cSLE are rare and incomplete.

This study aimed to detect the concentrations of the Th cytokines in cSLE; their clinical symptoms; and laboratory examination indexes and to discuss the relationship between the Th cytokine regulatory network and the onset of SLE in children to provide clues for the early diagnosis and timely intervention of cSLE.

Methods

General information

Patients with cSLE (n = 33; 6 males and 27 females) admitted to the Children's Hospital affiliated with Soochow University from July 2018 to October 2018 were selected as the cSLE group. All patients met the 1997 American College of Rheumatology (ACR) revised SLE classification criteria[12]. Children in the cSLE group were then scored by the SLE disease activity index 2000 (SLEDAI-2K)[13] developed at the University of Toronto, Canada. Children with an SLEDAI-2K score ≤ 4 over the past 10 days was classified in the inactive group (inactive cSLE). Children with an SLEDAI-2K score > 4 over the past 10 days was classified in the active group (active cSLE). Patients with other autoimmune diseases and inflammatory diseases were excluded. Furthermore, nephritis (diagnosed based on 24-h urinary protein > 0.5 g); haematological manifestations (including leukopenia diagnosed by two or more tests less than 4000/mm^3); and thrombocytopenia (fewer than 100×109/L thrombocytes), excluding haemolytic anaemia caused by drug factors and other reasons; were diagnosed. Additionally, malar erythema, skin mucosal lesions and arthritis, additional characteristic clinical manifestations of SLE, were diagnosed. Then, healthy volunteers who underwent outpatient physical examination (n = 30; 5 males and 25 females) were selected as the healthy control group. This study was approved by the Ethics Committee of the Children's Hospital affiliated with Soochow University.

Main experimental reagents and instruments

A LEGENDplex Human Th Cytokine Mix and Match Subpanel (BioLegend, USA) and flow cytometry analyser (Beckman Coulter, USA) were used. The minimum detection concentrations for each cytokine

were as follows: IL-2, 4.44 pg/ml; IL-4, 5.02 pg/ml; IL-5, 3.84 pg/ml; IL-6, 4.07 pg/ml; IL-9, 4.22 pg/ml; IL-10, 2.73 pg/ml; IL-13, 3.88 pg/ml; IL-17A, 1.97 pg/ml; IL-17F, 4.35 pg/ml; IL-21, 3.80 pg/ml; IL-22, 6.93 pg/ml; IFN-γ, 7.47 pg/ml and TNF-α, 6.63 pg/ml.

Detection of serum Th cytokines

Blood samples were collected from all subjects; 5 mL of peripheral venous blood was collected from SLE patients and healthy controls on an empty stomach by physical examiners in the morning and left at room temperature for 2 hours to precipitate the serum. The supernatant was centrifuged at 500 r/min for 10 min, after which the upper serum was absorbed and then stored in an Eppendorf tube at -80 °C. All serum samples were rewarmed in advance, and the serum contents of IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α in each specimen were detected by cytometric bead array (CBA), namely, cytokine microsphere detection technology, after which the experimental results were recorded.

Observation of clinical symptoms and relevant laboratory indicators of the SLE patients

The clinical symptoms and data from all SLE patients were recorded; these included their mental state; the presence of joint, skin and other related clinical symptoms; and history of drug use over the past 3 months. Laboratory indicators (anti-dsDNA antibody, complement 3 (C3), and complement 4 (C4)) were all measured by the Laboratory Department of the Children's Hospital affiliated with Soochow University.

Statistical analyses

The raw data were analysed using LEGEND plex software from BioLegend (ver. 8.0) and are represented in units of pg/ml. Statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA). Normally distributed variables are expressed as the mean ± standard deviation (SD) and were compared by t-test. Nonnormally distributed variables are expressed as the median (interquartile range) and were compared by the Mann-Whitney U-test. Categorical data are described as numbers (percentages). Differences between groups were assessed by using Fisher's exact test. Correlations between different variables were analyzed by Spearman correlation analysis. A P value < 0.05 indicated statistical significance.

Results

Demographics

We enrolled 33 children with cSLE as the cSLE group; the group included 6 males and 27 females 2– 19 years of age with a median age of 13 years. The shortest course of disease was the initial diagnosis, and the longest was over 7 years. The mean age of onset was 11.21 ± 2.52 years, and the median course of disease was 6 months. Individuals in the control group were 5–18 years old, with a median age of 13 years old. The cSLE group was divided into an active group (active cSLE) and inactive group (inactive

cSLE). Sixteen children had inactive cSLE (SLEDAI-2K score ≤ 4) and were 11–17 years old. Among these children, the average age of onset was 10.56 ± 3.08 years, and the median course of disease was 30 months. Seventeen children had active SLE (SLEDAI-2K score > 4), were 9–17 years old, and had an average age of onset of 11.8 ± 1.74 years and a median course of disease of 2 months. Meanwhile, among the 30 healthy children who comprised the control group, 5 were male and 25 were female, their ages ranged from 6–17 years, and their median age was 13 years. There was no statistically significant difference (P > 0.05) in sex or age between the groups, and these values were comparable (Table 1).

Clinical manifestations

In all children with cSLE, the main clinical manifestations were malar erythema (57.58%), nephritis (75.76%), haematological disorder (18.18%), mucocutaneous disorder (6.06%) and arthritis (9.09%). The incidence rates of the above clinical symptoms in active cSLE were 82.35%, 76.47%, 23.53%, 11.76% and 17.65%, respectively. In inactive cSLE, the corresponding rates were 31.25%, 75.00%, 12.50%, 0% and 0%, respectively (Table 1).

Laboratory parameters and treatments

The total anti-dsDNA antibody positivity rate in the children with cSLE was 45.45%, and the total incidence of low complement C3 and C4 was 54.55% and 48.48%, respectively. Among these parameters, the anti-dsDNA antibody positivity rates in the active and inactive groups were 64.71% and 25.00%, respectively. The incidence of low complement C3 was 82% and 25%, respectively, while the incidence of low complement C4 was 58% and 37%, respectively (Table 1).

In the cSLE group, 27 children (81.82%) received prednisone therapy (15 children (88.24%) in the active stage and 12 children (75%) in the inactive stage), 23 children (41%) were treated with hydroxychloroquine (10 children (58.82%) in the active stage and 13 (81.25%) in the inactive stage), 13 children (39.39%) received cyclophosphamide (10 children (58.52%) in the active stage and 2 children (12.50%) in the inactive stage), and 16 children (48.48%) were treated with mycophenolate mofetil (11 children (64.71%) in the active stage and 5 children (31.25%) in the inactive stage). Only 1 child (5.88%) with active cSLE received methotrexate therapy (Table 1).

Table 1
Characteristics of each study group

Variable	cSLE (n = 33)	Active cSLE (n = 17)	Inactive cSLE (n = 16)	Control (n = 30)	P_{ad}	P_{bd}	P_{cd}	P_{bc}
Demographics								
Female sex (%)	27 (81.82)	16 (94.12)	11 (68.75)	25 (83.33)	1.000	0.396	0.283	0.085
Age (years)	13.00 (11.00–15.00)	13.00 (12.00–14.00)	12.50 (11.00–17.00)	13.00 (11.00–14.00)	0.293	0.367	0.358	0.870
Age at disease onset (years)	11.21 ± 2.52	11.82 ± 1.74	10.56 ± 3.08	NA				
Disease duration (months)	6.00 (1.25-36.00)	2.00 (0.00–30.00)	30.00 (3.50–72.00)	NA				
SLEDAI-2K score	8.48 ± 5.85	13.53 ± 3.38	3.13 ± 1.20	NA				
Clinical manifestations								
Malar erythema (%)	19 (57.58)	14 (82.35)	5 (31.25)	NA				
Nephritis (%)	25 (75.76)	13 (76.47)	12 (75.00)	NA				
Mucocutaneous disorder (%)	2 (6.06)	2 (11.76)	0 (0.00)	NA				
Arthritis (%)	3 (9.09)	3 (17.65)	0 (0.00)	NA				
Haematological disorder (%)	6 (18.18)	4 (23.53)	2 (12.50)	NA				
Laboratory features								
Anti-dsDNA (%)	15 (45.45)	11 (64.71)	4 (25.00)	NA				
Low C3 (%)	18 (54.55)	14 (82.35)	4 (25.00)	NA				
Low C4 (%)	16 (48.48)	10 (58.82)	6 (37.50)	NA				
Treatment								

Variable	cSLE (n = 33)	Active cSLE (n = 17)	Inactive cSLE (n = 16)	Control (n = 30)	P_{ad}	P_{bd}	P_{cd}	P_{bc}
Prednisone (%)	27 (81.82)	15 (88.24)	12 (75.00)	NA				
Hydroxychloroquine (%)	23 (69.70)	10 (58.82)	13 (81.25)	NA				
Cyclophosphamide (%)	13 (39.39)	10 (58.82)	2 (12.50)	NA				
Mycophenolate mofetil (%)	16 (48.48)	11 (64.71)	5 (31.25)	NA				
Methotrexate (%)	1 (3.03)	1 (5.88)	0 (0.00)	NA				

SLEDAI-2K, systemic lupus erythematosus disease activity index 2000; *anti-dsDNA*, anti-double-stranded DNA; *C3*, complement 3; *C4*, complement 4; *NA*, not applicable; P_{ad}, cSLE group compared with the control group; P_{bd}, active cSLE group compared with control group; P_{cd}, inactive cSLE group compared with control group; P_{bc}, active cSLE group compared with inactive cSLE group; values are expressed as the mean ± SD, median (interquartile range) or number (percentage).

Th cytokine profiles of each group

The serum levels of Th cytokines (IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α) in the cSLE group and healthy control group were quantified by the CBA method (Table 2, Figs. 1 and 2). The levels of IL-2, IL-10 and IL-21 in the cSLE group were significantly higher than those in the healthy control group ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively) (Fig. 1a, 1b, 1c). Specifically, the levels of IL-2, IL-10, and IL-21 in the active cSLE group were significantly higher than those in the healthy control group ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively), and the levels of IL-22 were significantly lower in the active cSLE group than in the healthy control group ($P < 0.001$). The level of IL-21 in the inactive SLE group was significantly higher than that in the healthy control group, and the difference was statistically significant ($P < 0.05$). The levels of IL-2 and IL-10 in the active cSLE group were significantly higher than those in the inactive cSLE group, and the difference was statistically significant ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 2a, 2b, 2c, 2d).

Table 2
Comparison of Th cytokine concentrations in the different groups

Cytokine	cSLE (n = 33)	Active cSLE (n = 17)	Inactive cSLE (n = 16)	Control (n = 30)	P_{ad}	P_{bd}	P_{cd}	P_{bc}
IL-2	53.11 (27.80-96.71)	89.99 (59.49–178.10)	28.80 (21.64–39.34)	32.51 (16.90-53.39)	0.047*	0.012*	0.395	0.003**
IL-4	31.73 (12.20-76.14)	14.17 (10.88–45.61)	51.08 (15.40-126.39)	41.28 (31.39–80.06)	0.565	0.143	0.806	0.162
IL-5	5.91 (4.36–11.89)	8.24 (4.92–13.91)	5.07 (4.54–9.34)	6.47 (4.83–9.53)	0.802	0.537	0.783	0.619
IL-6	41.80 (19.03-126.67)	41.80 (18.73–87.16)	70.02 (17.72-139.57)	36.00 (18.63–61.98)	0.245	0.438	0.302	0.490
IL-9	43.24 (23.52–64.37)	46.23 (18.00-112.15)	43.24 (26.45–62.75)	35.82 (16.38–76.85)	0.438	0.482	0.912	0.452
IL-10	11.54 (4.80-29.82)	13.30 (9.98–33.67)	5.94 (2.97–16.25)	5.71 (2.91–9.03)	0.001**	0.001**	0.162	0.039*
IL-13	18.29 (8.57–47.65)	18.20 (7.30-40.57)	18.76 (8.81–56.03)	26.81 (16.22–46.22)	0.253	0.138	0.739	0.423
IL-17A	38.90 (12.18–453.90)	94.56 (24.29–450.40)	16.25 (11.55-458.13)	45.42 (17.92–79.22)	0.291	0.522	0.483	0.937
IL-17F	17.30 (5.37–22.44)	11.34 (4.95–21.34)	20.52 (12.12–59.75)	19.19 (9.95–44.56)	0.366	0.235	0.906	0.249
IL-21	65.33 (29.17-176.17)	55.07 (27.55-183.42)	87.18 (29.17–137.60)	18.01 (10.51–39.17)	0.003**	0.047*	0.044*	0.807
IL-22	24.11 (13.35–63.48)	21.43 (10.02–26.28)	37.34 (16.43-132.33)	63.85 (30.84-133.13)	0.217	< 0.001**	0.972	0.086
IFN-γ	72.95 (16.59-227.02)	58.80 (18.17-336.42)	72.95 (8.96-205.93)	69.61 (30.04-148.88)	0.555	0.524	0.883	0.652
TNF-α	46.95 (21.35-114.53)	108 (25.29-131.23)	25.03 (13.48–77.26)	17.50 (11.68–30.65)	0.151	0.150	0.297	0.877

IL-2, Interleukin-2; *IL-4*, Interleukin-4; *IL-5*, Interleukin-5; *IL-6*, Interleukin-6; *IL-9*, Interleukin-9; *IL-10*, Interleukin-10; *IL-13*, Interleukin-13; *IL-17A*, Interleukin-17A; *IL-17F*, Interleukin-17F; *IL-21*, Interleukin-21; *IL-22*, Interleukin-22; *IFN-γ*, Interferon-γ; *TNF-a*, Tumor necrosis factor-α; P_{ad}, cSLE group compared with control group; P_{bd}, active cSLE group compared with control group; P_{cd}, inactive cSLE group compared with control group; P_{bc}, active cSLE group compared with inactive cSLE group; *P < 0.05, **P < 0.01.

Correlation analysis between Th cytokine concentrations and the occurrence of cSLE

The Spearman linear correlation test was used to analyze the correlation between the SLEDAI-2K score and levels of different cytokines. The levels of IL-2 (r = 0.382, P = 0.028), IL-6 (r = 0.514, P = 0.002) and IL-10 (r = 0.429, P = 0.016) were positively correlated with the SLEDAI-2K score (Table 3, Fig. 3a, 3b, 3c). Other Th cytokine levels and the SLEDAI-2K score were not significantly correlated. In children with cSLE, no significant association between different cytokine levels and drug therapies was found.

Table 3
Correlation between SLEDAI-2K
scores and cytokine levels in
cSLE

Cytokine	r	*P* value
IL-2	0.382	0.028*
IL-4	0.188	0.348
IL-5	0.173	0.387
IL-6	0.514	0.002**
IL-9	0.310	0.141
IL-10	0.429	0.016*
IL-13	0.104	0.565
IL-17A	0.113	0.553
IL-17F	0.260	0.157
IL-21	0.302	0.112
IL-22	-0.024	0.893
IFN-γ	0.085	0.642
TNF-α	0.217	0.277

Spearman correlation analysis; *P < 0.05, **P < 0.01.

Discussion

cSLE, a common chronic systemic autoimmune disease in children, involves multiple systems and organs throughout the body; its clinical manifestations are complex, the course of the disease is protracted, and the disease can recur[14]. The pathogenesis of cSLE is not fully clear, but this disease mainly involves dysregulation of the immune system, including excessive T and B cell activation, providing large amounts of a variety of autoantibodies, and immune complex deposition, which causes multiple organ damage[4, 15]. Disruption of the immune state may be the key mechanism in a range of autoimmune diseases, including SLE. T cell-mediated immunoreaction plays an important role in specific immunity in the human body. One of the characteristics of SLE is abnormality in the differentiation and regulation of T cells. Previous studies found that the pathogenesis of SLE is related to an imbalance in the proportion of regulatory T (Tregs) cells and helper T (Th) cells and an increase in the proportion of Th cells[16]. The Th subgroup itself is also altered during the development of SLE. In recent years, Th cells have been found to play an important role in autoimmune diseases by secreting a variety of cytokines and mediating interactions between cells. Overexpression of IL-6, IL-10, IL-17, and TNF-α, etc., plays an important role in the pathogenesis of SLE, polymyositis (PM), dermatomyositis (DM) and rheumatoid arthritis (RA) and is significantly related to disease activity[17–19]. SLE is a prototypical autoimmune disease. Cytokines play an important role in the pathogenesis of SLE and determine the degree of disease activity.

In this study, serum IL-2 levels were increased in children with cSLE and positively correlated with disease activity. The level of IL-2 in active cSLE was significantly higher than that in inactive cSLE. IL-2 is a soluble molecule that promotes the clonal proliferation of T cells. Mice and people deficient in IL-2 were shown to develop severe autoimmune diseases, possibly due to the uncontrolled proliferation of autoreactive T cells and B cells and the proliferation of immature, non-functional Tregs caused by their defects. Recent studies have shown that small doses of IL-2 can selectively promote the growth of Treg cells[20]. In addition, low-dose IL-2 treatment in adult SLE patients increased Tregs in the blood and reduced the number of Th17 cells, thus exerting an immunosuppressive effect. At the same time, the SLEDAI-2K score after 12 weeks of treatment was found to be significantly reduced compared with that before treatment, and hormone levels were reduced, significantly alleviating disease[21]. It was also found in mouse studies that IL-2 can regulate the Th17 cell/Treg balance, enhance the function of Tregs, and inhibit Th17 cell differentiation[22]. Interestingly, based on laboratory test results in the literature and in our study, serum IL-2 deficiency was not observed in either adults with SLE or children with cSLE, especially active SLE. The role of IL-2 in the pathogenesis of SLE and whether low-dose treatment with IL-2 is as equally effective in children with cSLE compared with adults with SLE remain to be clarified in further studies.

IL-6, which can be secreted by a variety of immune cells, mainly functions to stimulate the proliferation and differentiation of B cells and their development into mature B cells capable of secreting antibodies. IL-6 also stimulates the IL-2 receptor, which, as it induces the growth of T cells and differentiation of cytotoxic T cells, plays an important role in immune activation. The expression of IL-6 and IL-6r in various

lupus mouse models was upregulated. However, the rates of IgG and complement C3 deposition and the permeability of macrophages in IL-6-deficient mice were decreased compared with those in normal mice. Increased expression of IL-6 in SLE patients is associated with anti-dsDNA, an indicator of SLE. Our study also shows that the expression level of serum IL-6 in children with cSLE was positively correlated with the degree of disease activity, indicating a potential certain correlation between IL-6 and the pathogenesis of SLE. In studies of adults with SLE, IL-6 was found to be associated with anaemia in patients with lupus nephritis. Furthermore, the higher the IL-6 concentration is, the more severe the anaemia is[23]. Additionally, IL-6 can stimulate activation of the STAT3 signalling pathway, reduce the cell circulation speed in the blood and prevent the apoptosis of immune cells[24]. In addition, IL-6 can inhibit the proliferation of Tregs and promote the development of autoimmunity[25]. All these results suggest the possibility of IL-6 as a predictor of SLE.

In this study, the serum IL-10 level in children with cSLE was significantly higher than that in the normal group, and the IL-10 level in the active stage was significantly higher than that in the inactive stage. IL-10 is an anti-inflammatory cytokine, and Th2 cells and various types of regulatory T cells are generally thought to be the source of its production in T cells. IL-10 stimulates B cell proliferation and IgG synthesis. Previous studies have confirmed that the IL-10 gene is a susceptibility gene for SLE. In adult patients with SLE, serum levels of IL-10 were positively correlated with the SLEDAI-2K score and anti-dsDNA antibody levels, consistent with the results of this study. IL-10 can inhibit cytokine production, downregulate monocyte antigen presentation and co-stimulation, and inhibit T cell proliferation, thereby increasing immunosuppression and achieving anti-inflammatory effects[26]. Although IL-10 plays a typical role in the immune process, recent studies have shown that IL-10 also plays a role in cytokine activation and induction. In the investigation of SLE patients, IL-10 was found to be associated with the pathogenesis of SLE, and when IL-10 mRNA levels in peripheral blood mononuclear cells from the patients and healthy controls were detected by competitive primer PCR, a difference was found between the two. In a study by Liu et al., the serum IL-10 level was significantly higher in patients than in the healthy control group, and IL-10 was positively correlated with the SLEDAI-2K score[27], consistent with the conclusions of our study.

The authors found that serum IL-21 levels in children with cSLE were significantly higher than those in the normal group, and IL-21 levels in the children with inactive cSLE were remarkably higher than those in the healthy control group. IL-21 can activate B cells to secrete IgG1 and IgG3 and induce all B cell subsets to differentiate into Ig-secreting cells, thus producing large amounts of IgM, IgG and IgA[28]. IL-21 produced by Tfh cells plays a major role in the initial immune response, secondary immune response, and long-term maintenance of humoural immunity of B cells to T cell-dependent antigens. Terrier et al found that serum IL-21 levels in SLE patients were significantly increased compared with those in a healthy control group, which is consistent with the results of this study. The study also indicated that IL-21 is closely related to changes in peripheral blood T cells and B cell subtypes, and IL-21 has certain therapeutic prospects as a target in SLE patients[29]. Thus, the significant increase in Tfh cell-related cytokine IL-21 levels in the serum of children with cSLE suggests that Tfh cells also play a crucial role in the pathogenesis of cSLE in children. The immune system stimulates B cells by producing cytokines such as IL-21, causing abnormal

humoural immune responses and participating in the pathogenesis of cSLE. In view of this, IL-21 may become a target molecule for the treatment of SLE in children, and the study of Tfh cell-related cytokines will also open up new approaches for the treatment of SLE in children.

IL-22 is produced by a variety of cell types, including Th17 cells, natural killer (NK) cells, and Th22 cells. Th22 cells are the main cell type that secretes IL-22. Research by Lin et al. showed that in patients with newly diagnosed SLE, the concentration of IL-22 was reduced compared with that of patients with relapsed SLE and healthy controls[30]. In our study, the serum IL-22 concentration in cSLE was lower than that of the normal group, and the IL-22 level in children with active disease was significantly different from that of children in the normal group, consistent with the results of a previous study on SLE in adults. This finding suggests that IL-22 plays an opposite role in the pathogenesis of this disease. However, in a study by Zhao et al., the serum IL-22 concentration in SLE patients was significantly higher than that in the normal group. After glucocorticoids were administered to the patients, the number of cells associated with IL-22 secretion was reduced. IL-22 is believed to play a role in the development of this disease. Under normal circumstances, Th22 cells, Th17 cells, Treg cells and other cell subsets interact and regulate each other to maintain the body in a state of immune equilibrium. In chronic inflammatory diseases, loss of the functions of key transcription factors that regulate Th22 cell differentiation exacerbates the occurrence of chronic inflammatory diseases[31]. The decreased number of Th22 cells in SLE patients may be because AhR, a key transcription factor that regulates Th22 cell differentiation, directly or indirectly regulates the production and secretion of inflammatory cytokines such as IL-22, leading to immune imbalance in different immune cell subsets[32, 33]. This also suggests that changing or promoting the pathways upstream or downstream of Th22 cell differentiation may reduce the immune inflammatory response in SLE, and cytokine levels in Th22 cells may be used as a potential target and auxiliary marker for the diagnosis and treatment of SLE.

Our study has several limitations. First, there was a minimum detection concentration in our detection kit, so we have to eliminate the data below the detection lower limit, which may bring some interference to the research results. Second, patients were treated with drugs before the test, although no correlation between different drugs and cytokine levels were found in our study, it may still influence the results to some extent. Third, it is a small sample study, further randomized and controlled clinical trials are needed to determine the efficacy of this cytokines in predicting activity in cSLE. However, the strength of the study was that for the first time, we comprehensively analyzed the level of serum Th cytokines in cSLE and its correlation with disease activity.

Conclusion

Our research results showed significant immunomodulatory disorder and the abnormal expression of various Th cytokines closely related to the clinical manifestations, results of laboratory examination and disease activity in children with cSLE. The cytokines IL-2, IL-6, IL-10, IL-21 and IL-22 are candidate biomarkers for predicting childhood cSLE activity and can be used as potential therapeutic targets for immunotherapy.

Abbreviations

SLE: Systemic lupus erythematosus; cSLE: Childhood-onset systemic lupus erythematosus; SLEDAI-2K; Systemic lupus erythematosus disease activity index 2000; dsDNA: Double-stranded DNA; GC: Germinal centre; ACR: American College of Rheumatology; CBA: Cytometric bead array; C3: Complement 3; C4: Complement 4; IL: Interleukin; IFN: Interferon; TNF: Tumor necrosis factor; Tfh; Follicular helper T; Treg: Regulatory T; Th: Helper T; NK: Natural killer; PM: Polymyositis; DM: Dermatomyositis; RA: Rheumatoid arthritis.

Declarations

Acknowledgements

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XZL and XHH contributed to research design, YYX contributed to experimental guidance, SL contributed to sample collection, WQ and XHH contributed to data collection, analysis and manuscript writing, JNA and GL contributed to assisting in data acquisition, analysis and interpretation, XLZ, GHQ, MFJ and CXF contributed to critically revised the manuscript. All authors read and approved of the final manuscript.

Ethics approval and consent to participate

We confirmed that all methods were carried out in accordance with relevant guidelines and regulations, and we confirmed that informed consent was obtained from all subjects or, if subjects were under 18, from the parents and/or legal guardians. The study was approved by the Ethics Committee of the Children's Hospital of Soochow University (approval No. SUDA20201110A03). All participants of this study were informed and signed a consent form to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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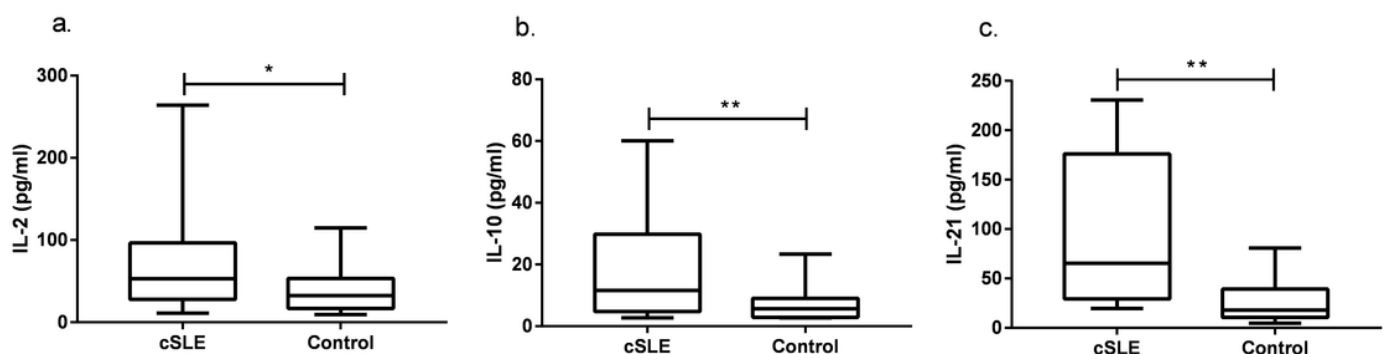
Figures

Figure 1

Differences in serum Th cytokine levels between the cSLE and control groups. a. IL-2; b. IL-10; c. IL-21. Mann-Whitney U-test; * P <0.05; ** P <0.01.
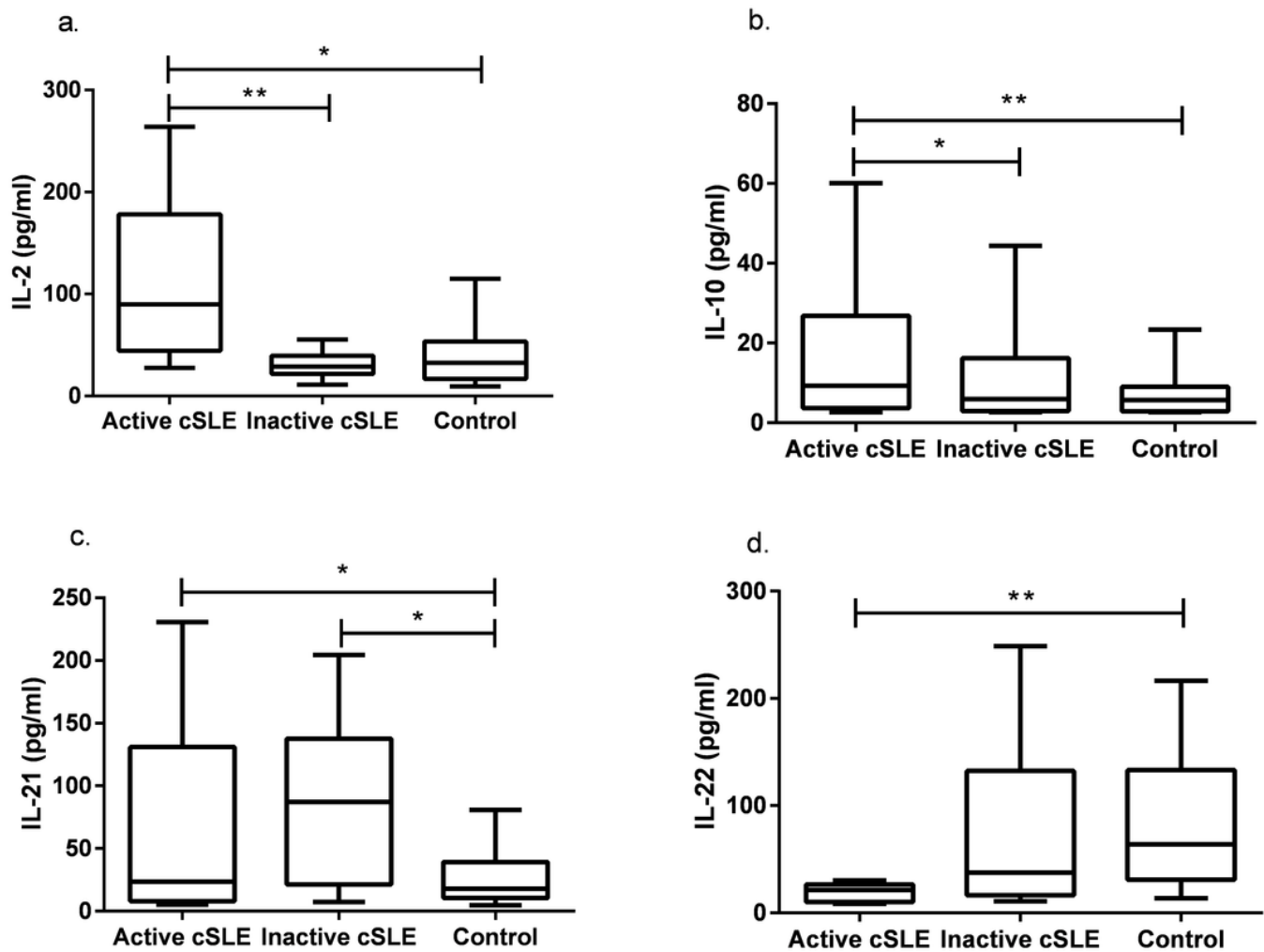

Figure 2

Differences in serum Th cytokines between the active cSLE, inactive cSLE and control groups. a. IL-2; b. IL-10; c. IL-21; d. IL-22. Mann-Whitney U-test; * P <0.05; **P <0.01.
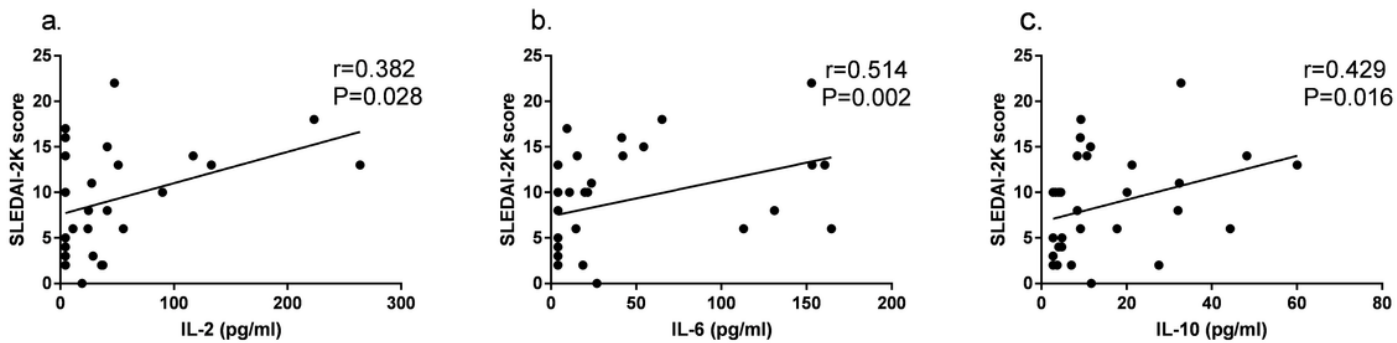
Figure 3

Correlations between serum concentrations of Th cytokines and SLEDAI-2K scores in cSLE. a. IL-2; b. IL-10; c. IL-10.