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Polarization of TH2 response is decreased during pregnancy in systemic lupus erythematosus

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SUMMARY

This study evaluated some cytokines involved in the Th1-Th2 shift during pregnancy in patients with systemic lupus erythematosus (SLE) and healthy women.

Twenty-seven consecutive successful pregnancies in 26 SLE patients and 28 pregnancies in 28 matched healthy subjects, as controls, were enrolled and prospectively studied. Sera obtained at first and third trimesters of pregnancy were tested for IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, INF-γ, and TNF-α with a highly sensitive, multiplexed sandwich ELISA (SearchLight Human Inflammatory Cytokine Array). Statistics were performed by SPSS package. IL-8 serum levels were higher in the first (P<0.0001) and third (P=0.003) trimesters of pregnancy in SLE patients compared with controls, INF-γ serum levels in the third trimester (P=0.009), and IL-10 serum levels in the first and third trimesters (P=0.055 and P<0.0001, respectively). IL-2 (r=0.524 P=0.010), IL-12 (r=0.549 P=0.007), IFN-γ (r=0.492 P=0.017), and IL-6 (r=0.515 P=0.020) serum levels correlated with disease activity in SLE patients in the first trimester of pregnancy. Cytokine profile was similar in patients with and without lupus nephritis both in the first and in the third trimesters of pregnancy. IL-8 serum levels were lower in patients with a previous diagnosis of antiphospholipid antibody syndrome compared with those without, both in the first and in the third trimesters of pregnancy.

In SLE patients, a lower than expected decrease in Th1 cytokine serum levels was observed in the third trimester of gestation which could contribute to a lower Th2 cytokine polarization during pregnancy.

Key words: *Systemic lupus erythematosus, lupus pregnancy, lupus cytokines.*

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INTRODUCTION

Cytokines produced by CD4+ helper T (Th) cells are traditionally subdivided into Th1 and Th2. Th1 cytokines stimulate cellular immunity and include interferon (IFN)-γ, interleukin (IL)-1, IL-2, IL-8, IL-12, and tumor necrosis factor (TNF)-α. Th2 cytokines induce humoral immunity and antibody production, and include IL-4, IL-5, IL-6 and IL-10 (1). During pregnancy, important changes occur in the maternal immune and endocrine systems (2, 3). Maternal immune response is characterized by a Th2 polarization that is driven by high levels of estrogens and progesterone (4-6).

In patients affected with systemic lupus erythematosus (SLE), an overproduction of Th2 cytokines that results in B-cell hyperactivity has been demonstrated (7). Therefore, it is expected that Th2 cytokine hyperpolarization will result in an increase in SLE complications, particularly in the third trimester of pregnancy when estrogens and progesterone are supposed to be at their highest levels (8). However, lower levels of estrogens, progesterone and Th2 cytokines were found in the third trimester of pregnancy in SLE patients compared with healthy pregnant women (9) and disease flare rate was similar in pregnant women compared with non-pregnant SLE patients in some case-control studies (9-11).

The purpose of this study was to evaluate Th1-Th2 profile in a cohort of patients affected with SLE during pregnancy.

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PATIENTS AND METHODS

Twenty-seven pregnancies in 26 SLE patients and 28 pregnancies in 28 healthy women, as controls, were prospectively evaluated. Only pregnancies ending with live births were included in the analysis. All patients fulfilled the 1997 American College of Rheumatology (ACR) classification criteria for SLE (12) and were followed-up at the Rheumatology Units of Rheumatology of the University of Padova and Brescia, Italy. All patients and controls signed written informed consent and the local ethics committees gave their approval for the study.
All patients underwent laboratory tests and rheumatology and gynecological visits every month during pregnancy and the *postpartum* period, as suggested for pregnancy monitoring in SLE (13). At baseline visit, before pregnancy, we assessed disease activity by ECLAM (European Consensus Lupus Activity Measurement) score (14). During pregnancy, we used the ECLAM score modified for pregnancy (15). Active SLE was defined by an ECLAM score of 2 or over.

Cytokine assays

Serum samples were collected for cytokine determinations at the first (9-11 weeks) and third (29-31 weeks) trimesters of gestation from SLE patients and healthy controls, and were stored in aliquots at -80°C until analysis.
IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IFN-γ and TNF-α serum levels were quantitatively determined by a highly sensitive enzyme-linked sandwich immunosorbent assay (ELISA) designed with multiplex technology (Search Light Human Inflammatory Cytokine Array by Pierce Biotechnology, Rockford, IL, USA) (16). This was approved as a reference method for the quantitative determination of cytokines in human biological fluids by the US National Institute of Health (Bethesda, MD, USA). The assay procedure was performed according to the manufacturer's instructions.
Ranges of measurability of the standard curve for each cytokine tested were: IL-1α=0.78-200 pg/mL; IL-1β=0.39-100 pg/mL; IL-2=0.78-200 pg/mL; IL-6=0.78-200 pg/mL; IL-8=0.78-200 pg/mL; IL-10=0.78-200 pg/mL; IL-12p70=1.2-300 pg/mL; IFN-γ=0.78-200 pg/mL; TNF-α=4.7-1200 pg/mL. Analytical sensitivity was: IL-1α=0.4 pg/mL; IL–1β=0.2 pg/mL; IL-2=0.4 pg/mL; IL-6=0.2 pg/mL; IL-8=0.4 pg/mL; IL-10=0.2 pg/mL; IL-12p70=0.4 pg/mL; IFN-γ=0.2 pg/mL; TNF-α=1.6 pg/mL.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 package. Since cytokine serum levels did not follow normal distribution, either in healthy controls or in SLE patients, a non-parametric statistical analysis was used (Wilcoxon's test for paired data and the Mann-Whitney U test). A P value <0.05 was considered significant.

RESULTS

The mean age of SLE patients at the time of diagnosis was 22±5.0 years, range 13-31 years, and at the time of conception mean age was 32.3±3.7 years, range 26-38 years. The mean disease duration at the time of conception was 9.70±5.9 years, range 1-20 years. The mean age of healthy controls at the time of conception was 30.8±5.8 years (range 21-37 years), matched with that of SLE patients.
There were no differences between the mean weight of babies born from SLE women and from healthy controls. In addition, the duration of pregnancy was similar in SLE patients and in controls.
The ECLAM score before pregnancy ranged from 0 to 6 (mean±SD 2.7±1.33, median value 3), and during pregnancy 35% of patients had an active SLE. At the time of conception, hematologic, skin and joint involvements were the most frequent manifestations of SLE patients and about one-third of these patients had renal involvement. Clinical and laboratory features of the 26 SLE patients at the time of conception are reported in Table I.
During pregnancy, 81.4% of patients received corticosteroid therapy and the mean dose taken by the patients in the first, sec-

Table I - Clinical and laboratory features at conception in 26 SLE patients.

Clinical manifestations	Patients n. (%)
Hematologic involvement	23 (85.5)
Arthralgias and/or arthritis	21 (77.7)
Skin manifestations	8 (29.6)
Glomerulonephritis	8 (29.6)
Serositis	3 (11.1)
CNS involvement	2 (7.4)
Laboratory findings	
Anti-dsDNA Ab	25 (92.5)
Anticardiolipin Ab	15 (55.5)
Anti-SSA Ab	13 (48.1)
Anti-U1RNP Ab	6 (22.2)
LAC	6 (22.2)
Double LAC + aCL Ab	5 (18.5)
Anti-Sm Ab	4 (14.8)

SLE, systemic lupus erythematosus; CNS, central nervous system; hematologic involvement, excluding hemolytic anemia. Ab, antibody; anti-dsDNA Ab, anti double-stranded-DNA antibody; LAC, lupus anticoagulant; aCL, anticardiolipin antibodies.

ond and the third trimesters of pregnancy was 5.47±4.26 mg/day (range 0-15), 5.61±4.10 mg/day (range 0-15), 5.92±4.60 mg/day (range 0-18), respectively. Patients with active SLE received a higher median dose of prednisone than patients with inactive disease: 8.06 mg/day (range 5-15) *vs* 4.6 mg/day (range 0-14.3) (P=0.03). Furthermore, 48.1% of patients received hydroxychloroquine, 29.6% azathioprine, and 3.7% were therapy free.

Cytokine serum levels

Median cytokine levels in SLE patients and healthy controls in the first and third trimesters of pregnancy are reported in Table II.

Compared with controls, SLE patients had higher IL-10 serum levels in the first (P=0.055) and in the third (P<0.0001) trimesters of pregnancy, IL-8 serum levels in the first (P<0.0001) and third (P=0.003) trimesters (Fig. 1), and INF-γ serum levels in the third trimester (P=0.009) (Fig. 2).

The following cytokine serum levels correlated with ECLAM score in SLE patients in the first trimester of pregnancy: IL-2 r=0.524, P=0.010; IL-12 r=0.549, P=0.007; IFN-γ r=0.492, P=0.017; IL-6 r=0.515, P=0.020. Cytokine profile was similar in patients with and without lupus nephritis both in the first and in the third trimesters of pregnancy.

IL-8 serum levels were lower in patients with a previous diagnosis of antiphospholipid antibody syndrome compared with those without, both in the first (4.40 (range 1.00-10.750) *vs* 8.70 (range 4.05-39.850) P=0.045) and in the third trimester of pregnancy (1.90 (range 0.25-4.050) *vs* 6.40 (1.65-29.200), P=0.008).

There were no differences in cytokine serum levels among patients receiving low-dose and high-dose corticosteroid therapy in either the first or the third trimesters of gestation.

Furthermore, there were no differences in cytokine serum levels among patients treated with or without antimalarials or azathioprine.

Table II - Th1 and Th2 cytokine serum levels during pregnancy in 28 healthy women compared with 26 SLE patients. The median values (25^{th}-75^{th} percentile) are expressed in pg/mL.

	Healthy women			SLE patients		
	1st trimester	3rd trimester	P	1st trimester	3rd trimester	P
TNF-α	11.0 (5.8-26.9)	7.6 (4.5-15.0)	0.003	13.5 (6.5-38.9)	18.2 (6.8-36.5)	n.s.
IFN-γ	3.4 (1.7-6.1)	1.5 (1.0-3.3)	0.001	4.1 (1.5-11.1)	3.8 (1.5-11.0)	n.s.
IL-1α	1.5 (0.4-2.9)	0.9 (0-2.2)	0.003	1.0 (1.0-6.9)	1.2 (0.4-4.6)	0.044
IL-1β	0.8 (0.5-1.4)	0.5 (0.3-0.9)	0.026	1.4 (0.6-4.3)	1.1 (0.4-3.7)	n.s.
IL-2	4.0 (1.6-7.3)	3.4 (0.5-6.4)	0.012	3.1 (1.5-12.5)	4.7 (1.3-9.7)	n.s.
IL-8	5.3 (3.4-7.7)	4.5 (2.2-6.5)	n.s.	14.2 (7.0-43.3)	12.2 (4.3-45.7)	n.s.
IL-12	4.5 (2.0-9.1)	3.0 (1.5-7.2)	0.016	6.0 (2.0-20.8)	4.1 (2-25.5)	n.s.
IL-10	1.3 (0.8-3.1)	1.0 (0.4-1.7)	n.s.	2.9 (1.5-6.1)	4.4 (1.9-6.3)	n.s.
IL-6	6.0 (2.2-11.8)	4.8 (2.9-9.5)	n.s.	6.3 (3.5-24.5)	9.4 (3.0-25.3)	n.s.

SLE, systemic lupus erythematosus; TNF-α, tumor necrosis factor alpha, IFN-γ, interferon gamma, IL, interleukin; ns, not significant.

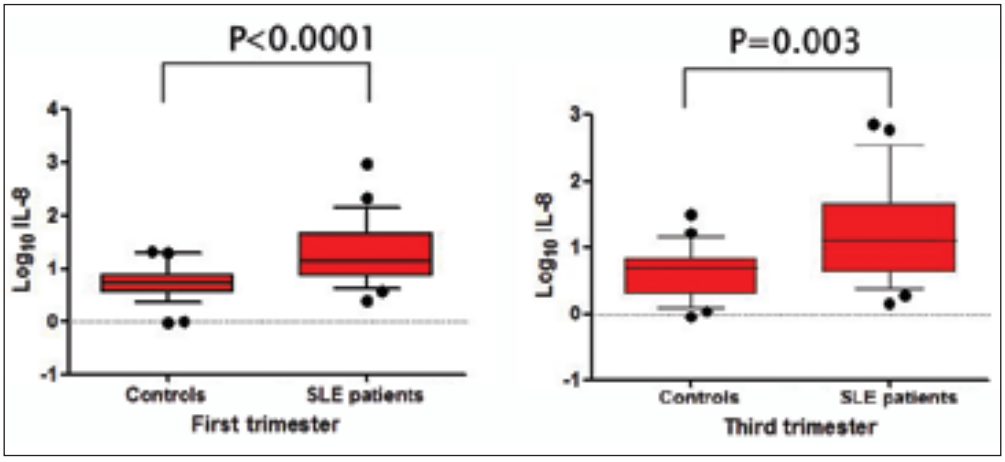

Figure 1 - IL-8 serum levels in controls and SLE patients in the first and third trimesters of pregnancy.

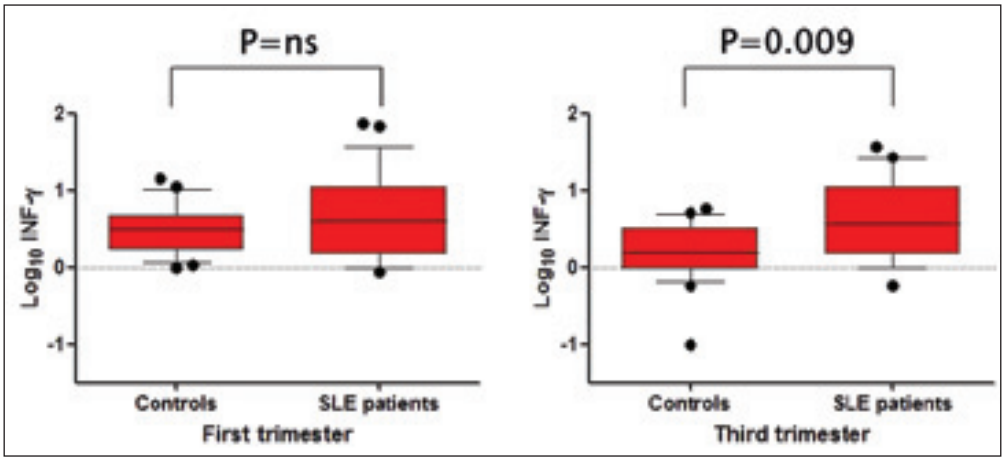

Figure 2 - IFNγ serum levels in controls and SLE patients in the first and third trimesters of pregnancy.

DISCUSSION

During pregnancy, immune and endocrine systems undergo profound changes involving both hormones and cytokines: cortisol, progesterone, estradiol and testosterone physiologically increase during gestation and drive Th-2 cytokine polarization at the feto-maternal interface, as well as in systemic circulation. In fact, high levels of estrogens and progesterone inhibit Th1 type cytokine production from T cells (2) and stimulate the secretion of Th2 cytokines, including IL-4, IL-5, IL-6 and IL-10 (17-19), which lead to antibody production (20). Among Th2 cytokines, IL-6 stimulates the differentiation of Th0 cells toward a Th2 phenotype and inhibits the development of a Th1-mediated response (21); IL-10 stimulates B-cell proliferation, differentiation and antibody secretion (22). In addition, IL-10 inhibits antigen presenting cell and T-cell functions (22). High serum levels of IL-6 and IL-10 (23, 24) were observed in SLE patients and they correlated with disease activity or anti-double-stranded DNA antibodies (25-28).

The role of Th1 cytokines in the pathogenesis of SLE remains unclear. Probably there are differences between systemic and local effects of these cytokines. For example, some patients with rheumatoid arthritis

who were treated with anti-TNF-α agents developed anti-double-stranded DNA antibodies and in some cases SLE or lupus-like disease (29). By contrast, the level of TNFα messenger RNA was high in kidney-biopsy specimens from patients with lupus nephritis (30) and TNFα expression was increased in skin biopsy specimens from patients with cutaneous SLE (31). Aringer *et al.* reported that the anti-TNF-α monoclonal antibody, infliximab, administered to 6 patients with lupus led to resolution of joint swelling in 3 patients with arthritis and the reduction of urinary protein loss by 60% in 4 patients with glomerulonephritis (32). Since Th2 cytokine production seems to play a key role in SLE, and an overproduction of IL-6 and IL-10 is reported, we should expect a Th2 polarization of immune response in SLE patients compared with healthy controls, especially during pregnancy.

The most relevant features of our study were a lower than expected increase in Th2 cytokine during pregnancy in SLE patients and an increase in some Th1 cytokines, such as IL-8 and INF-γ, in SLE pregnant women compared with controls (Figs. 1 and 2). In our study, serum levels of Th2 cytokines were similar in SLE patients compared with controls and only IL-10 was higher in SLE patients than in healthy women. These data are comparable to those recently published by Torricelli et al. (33) who found higher serum levels of IL-10 in pregnant SLE patients compared with healthy pregnant women, and IL-10 serum levels progressively increased during pregnancy. Similarly, Muñoz-Valle et al. (34) showed higher levels of IL-10 in pregnant SLE patients than in healthy pregnant women, particularly during the last trimester of pregnancy. This lower than expected Th2 cytokine polarization may be explained by the lower levels of estrogen and progesterone that we found in a previous study in pregnant SLE patients compared with pregnant healthy women during the second and third trimesters of pregnancy (9). Furthermore, the lower Th2 polarization observed during pregnancy could explain why SLE flare rate was found to be similar between pregnant and non-pregnant patients in some controlled studies (10, 35, 36).

The increased levels of IL-8 and IFN-γ in SLE patients compared with healthy pregnant women are unexpected. IL-8 is a 8 kDa protein produced by many types of cells, not only monocytes, lymphocytes, granulocytes, fibroblasts and endothelial cells, but also trophoblast and placental macrophages (37) and IL-8 plays a key role in stimulation of trophoblast cell migration and invasion during pregnancy (38). By contrast, IFN-γ and TNF-α that are produced in the deciduas are thought to act synergistically to inhibit trophoblast invasion (39). Furthermore, we found IL-8 serum levels lower in SLE patients with a previous diagnosis of antiphospholipid antibodies syndrome compared with those without, both in the first and in the third trimesters of pregnancy. Notably, it has been recently shown that anti-β2GPI antibodies could trigger an inflammatory response in trophoblast, (40) with an increased expression of Th1 cytokines, including IL-8. However, the reason why IL-8 serum levels were higher in SLE patients compared with controls, but were reduced in patients with antiphospholipid antibody syndrome remains to be clarified.

In conclusion, our study shows that in pregnant SLE patients the polarization towards Th2 cytokines is lower than expected and some Th1 cytokine serum levels are higher in SLE patients compared with healthy women.

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