J Microbiol Immunol Infect 2010;43(1):18–25

Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

Journal homepage: http://www.e-jmii.com

Original Article

Changing Cytokine Patterns in Systemic Lupus: A Prospective Longitudinal Study

Lieh-Bang Liou*, Wan-Ju Chao

Division of Rheumatology, Allergy and Immunology, Chang Gung Memorial Hospital at Lin-kou and Chang Gung University College of Medicine, Tao-yuan, Taiwan.

BACKGROUND/PURPOSE: We have previously reported that lupus monocytes display distinctively differing patterns of C-reactive protein (CRP)-inducing cytokine interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α secretion when stimulated with either immune complexes (ICs) or lipopolysaccharide (LPS). In this study, we investigated whether the cytokine patterns of peripheral blood mononuclear cells (PBMCs) isolated from lupus patients acquired an IC or LPS pattern, either over time, or following corticosteroid or hydroxychloroquine use.

METHODS: PBMCs from lupus patients were obtained at 0, 1, 3, and 6 months post diagnosis and stimulated with ICs or LPS. Cells were obtained for polymerase chain reaction to determine the IL-6, IL-1β, and TNF-α mRNA expression, and were assigned as having acquired either an IC or an LPS pattern.

RESULTS: Upon stimulation, the mRNA expression levels of the IL-6 and IL-1β were significantly higher in IC-pattern PBMCs than in LPS-pattern PBMCs ($p=0.021$ and 0.028, respectively). Consistent with this, serum CRP levels in the IC-pattern group were significantly higher than those in the LPS-pattern groups ($p=0.027$). Total serum CRP levels were positively correlated with serum C3c and C4 concentrations, and inversely correlated with serum anti-double stranded DNA (anti-dsDNA) levels. Conversely, circulating ICs were positively correlated with serum anti-dsDNA levels and inversely correlated with serum C4 concentrations.

CONCLUSION: Within the same individual, the CRP-inducing cytokine patterns can be changed, either naturally or after medication. Pre-existing serum circulating ICs are not predisposed to either IC or LPS cytokine patterns. Finally, CRP levels were correlated with anti-dsDNA consumption.

KEYWORDS: CRP-inducing cytokines, IC or LPS cytokine patterns, mononuclear cells, systemic lupus erythematosus

*Corresponding author. Division of Rheumatology, Allergy and Immunology, Chang Gung Memorial Hospital at Lin-kou and Chang Gung University College of Medicine, Kwei-san Hsiang, Tao-yuan County, 333 Taiwan.

E-mail: b890121@adm.cgmh.org.tw

Article History:
Received: Jul 11, 2008
Revised: Aug 19, 2008
Accepted: Feb 13, 2009

Introduction

The cytokines interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α are implicated in the production of C-reactive protein (CRP) by the liver.[1] Low, but higher than normal, levels of these cytokines and CRP have been identified in the sera of patients with systemic lupus erythematosus (SLE).[2] Although the serum levels of the three CRP-inducing cytokines (IL-6, IL-1β, and TNF-α) do not correlate with either CRP levels or the SLE

Copyright © 2010 Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. All rights reserved.

03-JMII-2-08-006.indd 18 2/22/2010 6:28:30 PM

disease activity index (SLEDAI) scores in SLE patients,[2] high serum CRP levels are associated with stimulation by immune complexes (ICs), and low levels have been shown to be associated with stimulation by lipopolysaccharide (LPS).[3] As Liou's study was of a cross-sectional design, it is not known whether these cytokine secretion patterns remain constant over time, or whether they change — either naturally or by certain medications. This question underscores the importance of determining whether a lupus patient experiencing episodes of fever has an infection.[4] Furthermore, it is unclear whether serum ICs affect particular cytokine patterns. To answer these questions, this study followed lupus patients from diagnosis to 6 months post-diagnosis. However, instead of using monocytes,[3] we used peripheral blood mononuclear cells (PBMCs). This is because they are easier to isolate in high numbers, and they express IL-6, IL-1β, and TNF-α in a manner representative of the whole blood cell population.

Methods

Ethics approval

This research was approved by the Medical Ethics Committee of Chang Gung Memorial Hospital, Taoyuan County, Taiwan. Informed consent was obtained from each subject.

Patients' clinical and laboratory profiles

From October 2003 to June 2004, nine lupus patients were enrolled in this longitudinal study. Of these, six were newly diagnosed (group 1; Table 2) and three had been treated for from 3 months to >2 years (group 2; Table 3) prior to enrollment. The CRP concentration in serum (normal, <5 mg/L) was measured by nephelometry using a low detection limit of 3.19 mg/L. None of the patients had any clinical serositis, arthritis, or cardiovascular events during the 6-month follow-up. Serum C3c (normal, 10–40 mg/dL), C4 (normal, 90–180 mg/dL), and IgG anti-double stranded DNA (anti-dsDNA) antibody (normal, <35 IU/mL) concentrations were determined using nephelometry. SLEDAI-2K scores were derived according to a previously described method.[5] Blood and laboratory/clinical data were collected at specific time points, in the absence of infection.

Reagents

Human IgG and rabbit IgG were obtained from Sigma (St. Louis, MO, USA) and rabbit anti-human IgG was obtained from Jackson ImmunoResearch (West Grove, PA, USA). RPMI-1640 medium and Hank's balanced salt solution were purchased from Gibco (Grand Island, NY, USA). Fetal calf serum was obtained from Biological Industries (Kibbutz Beit Haemek, Israel) and heat-inactivated at 56°C for 1 hour before use. TRIzol reagent was acquired from Invitrogen (Carlsbad, CA, USA). Circulating ICs were examined using a circulating ICs C1q ELISA assay kit (IBL, Hamburg, Germany) to quantitatively determine the level of circulating C1q- and IgG-containing ICs in human serum.

IC formation

ICs were generated as previously described.[2] Briefly, 30 μg (or 60 μg) of human IgG in 0.5 mL phosphate-buffered saline (PBS) was incubated in 24-well flat-bottomed plates (Co-star; Corning Incorporated, Corning, NY, USA) at 37°C for 2 hours. The wells were washed three times with PBS, followed by the addition of 30 μg (or 60 μg) of rabbit anti-human IgG in 0.5 mL PBS to each well. The plate was kept at room temperature for a further 1.5 hours. The wells were again washed three times with PBS, and the adherent ICs formed used to stimulate PBMCs. Adherent rabbit IgG plus human IgG (30 μg) was used as a control for the ICs.

Cell culture and cell isolation

Each subject agreed to donate 25 mL blood at the time of diagnosis and then again 1, 3, and 6 months post-disgnosis. Heparinized blood was processed and the cells separated.[2] The PBMCs obtained were cultured with either medium alone, control ICs (rabbit IgG + human IgG at 30 μg), IC 30 (anti-human IgG + human IgG at 30 μg), IC 60 (anti-human IgG + human IgG at 60 μg), LPS 3 (3 μg/mL), or LPS 10 (10 μg/mL) for 48 hours.

Polymerase chain reaction (PCR)

The PBMCs were subsequently collected for mRNA extraction using TRIzol reagent (Invitrogen). mRNA (30 ng/μl) was subjected to reverse transcriptase-PCR amplification using the One step reverse transcription-PCR kit (Qiagen, Valencia, CA, USA). Primer sequences are listed in Table 1.[6,7] PCR conditions were as follows: reverse transcription

03-JMII-2-08-006.indd 19 2/22/2010 6:28:31 PM

Table 1. Primer sequence used in this study[6,7]

Gene	Sequence
Human IL-6	5′-AAC TCC TTC TCC ACA AGC G-3′ 5′-TGG ACT GCA GGA ACT CCT T-3′
Human IL-1β	5′-GAC CTG GAC CTC TGC CCT CTG-3′ 5′-AGG TAT TTT GTC ATT ACT TTC-3′
Human TNF-α	5′-ATG AGC ACT GAA AGC ATG ATC-3′ 5′-TCA CAG GGC AAT GAT CCC AAA GTA GAC CTG CCC-3′
Human GAPDH	5′-TTT GCA GGG GGG AGC CAA AAG G-3′ 5′-GGA GTG GGT GTC GCT GTT GAA GTC-3′

for 30 minutes at 50°C, and denaturation at 95°C for 15 minutes, followed by 35 PCR cycles at 95°C for 59 seconds, 54°C for 59 seconds, and 72°C for 59 seconds in a Mastercycler gradient 5331 (Eppendorf, Hamberg, Germany). Each PCR product (10 μL) were then electrophoresed on 2% agarose gels and band densities were determined using a Fluor-STM MultiImager densitometer (BioRad, Hercules, CA, USA). The specificities of IL-6, IL-1β, and TNF-α PCR products were confirmed by sequence analysis.

Data were normalized as the ratio of cytokine/GAPDH (mean ± standard deviation) for comparison. The IC pattern was determined at each time point (Tables 2 and 3) when the ratio of cytokine/GAPDH induced by the ICs divided by cytokine/GAPDH induced by LPS was ≤ 1.00 for two or more of the three cytokines. The LPS pattern was determined for each time point (Tables 2 and 3) when the ratio of cytokine/GAPDH induced by the ICs divided by cytokine/GAPDH induced by LPS was > 1.00 for two or more of the three cytokines. These parameters were used because the expression levels of IL-6, IL-1β, and TNF-α in the PBMCs after challenge with ICs or LPS were not as distinctive as those for monocytes.[3]

Statistical analysis

Statistical analyses were undertaken using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Correlations were assessed using Spearman's correlation coefficient. Between-group comparative analysis was performed using a χ^2 test, Fisher's exact test, Odds ratios, one-way analysis of variance (F-test), or the Mann-Whitney U test. A p value of < 0.05 was considered statistically significant.

Results

One time point was counted as a unit for comparison or correlation. Thus 33 and 31 time points were available for correlating CRP versus SLEDAI and cytokine pattern versus CRP, respectively (Tables 2 and 3).

Effects of medication and disease on cytokine patterns in individual patients

The percentage of patients in group 1 showing a change in cytokine pattern between Month 0–1 was greater than that seen in group 2 over the same time period (75.0% *vs.* 33.3%; Odds ratio = 6; 95% confidence interval, 0.22–162.54; Tables 2 and 3). There are no significant differences in the percentage of patients showing cytokine pattern changes between group 1 and 2 in time periods covering Month 1–3 (or Month 3–6). Therefore, we suggest that natural course of the disease and/or medications taken by the patients may alter the cytokine patterns of lupus mononuclear cells.

Comparison of clinical and laboratory variables between the cytokine pattern groups

We next investigated whether the two different cytokine patterns were associated with differences in clinical and laboratory variables. Clinical symptoms did not differ between the IC- and LPS-pattern groups. For example, the frequency of arthritis did not differ significantly between the LPS-pattern and IC-pattern groups (4/20 *vs.* 0/13 sections; $p = 0.10$). A new rash was seen in 4/20 LPS-pattern patients and similarly in 2/13 IC-pattern patients ($p = 1.00$). Other clinical symptoms had a considerably lower frequency of occurrence than arthritis or a new rash, and there were no differences in the frequency of other clinical symptoms between the two pattern groups.

When all the time points were divided into groups based on SLEDAI scores > 7 ($n = 4$) and SLEDAI scores ≤ 7 ($n = 29$), thus defining active or non-active lupus, as previously described,[8] no differences were found in their corresponding mRNA expression patterns (Tables 2 and 3; $p = 3.84$). Additionally, serum CRP levels were found to be uncorrelated with SLEDAI scores (Tables 2 and 3; $p = 0.833$

03-JMII-2-08-006.indd 20 2/22/2010 6:28:31 PM

Table 2. Clinical and laboratory data for group 1: newly diagnosed lupus patients

		Mo 0	Mo 1	Mo 3	Mo 6
1	SLEDAI	8	8	4	4
	CRP (mg/L)	<3.19	16.3	<3.19	<3.19
	Anti-dsDNA (IU/mL)	430	343	80	60
	Medicine	None	Methylprednisolone (40 mg IV bid started)	Methylprednisolone (12 mg qd for 3 wk)	Methylprednisolone (6 mg qd for 2 mo)
	Cytokine pattern[a]	LPS	ND	LPS	IC
2	SLEDAI	4	4	2	0
	CRP (mg/L)	ND	6.00	<3.19	<3.19
	Anti-dsDNA (IU/mL)	—	—	—	—
	Medicine	None	HCQ (200 mg qd for 1 mo)	HCQ continued; Prednisolone (10 mg qd for 2 mo)	Same, continued
	Cytokine pattern[a]	LPS	LPS	LPS	IC
3	SLEDAI	4	2	2	0
	CRP (mg/L)	<3.19	<3.19	<3.19	<3.19
	Anti-dsDNA (IU/mL)	178	116	151	164
	Medicine	None	HCQ (200 mg qd for 1 mo)	Same, continued	Same, continued
	Cytokine pattern[a]	IC	LPS	IC	LPS
4	SLEDAI	13	5	5	5
	CRP (mg/L)	<3.19	<3.19	<3.19	<3.19
	Anti-dsDNA (IU/mL)	90	57	46	66
	Medicine	None	HCQ (200 mg qd for 1 mo)	HCQ (300 mg qd for 2 mo)	HCQ (400 mg qd for 3 mo)
	Cytokine pattern[a]	LPS	IC	LPS	IC
5	SLEDAI	4	4	ND	16
	CRP (mg/L)	11.10	<3.19	ND	39.20
	Anti-dsDNA (IU/mL)	—	—	—	—
	Medicine	None	HCQ (200 mg qd for 1 mo)	None	Prednisolone (15 mg qd for 3 wk)
	Cytokine pattern[a]	IC	LPS	ND	IC
6	SLEDAI	7	4	0	0
	CRP (mg/L)	16.50	6.84	6.73	88.80
	Anti-dsDNA (IU/mL)	45	97	151	193
	Medicine	None	HCQ (200 mg qd and Prednisolone 5 mg bid for 1 mo)	Same, continued	Same, continued
	Cytokine pattern[a]	LPS	ND	LPS	IC

[a]Immune complex or lipopolysaccharide pattern was designated for each section when the ratio of IC-induced cytokine/GAPDH over LPS-induced cytokine/GAPDH was > 1.00 or ≤ 1.00 for two or more of the three cytokines. SLEDAI = systemic lupus erythematosus disease activity index; CRP = C-reactive protein; Mo = month; IV = intravenous; bid = twice daily; qd = daily; LPS = lipopolysaccharide; IC = immune complex; ND = not determined; — = negative.

03-JMII-2-08-006.indd 21 2/22/2010 6:28:31 PM

Table 3. Clinical and laboratory data for group 2: already treated lupus patients

		Mo 0	Mo 1	Mo 3	Mo 6
7	SLEDAI	4	ND	2	2
	CRP (mg/L)	<3.19	ND	<3.19	27.00
	Anti-dsDNA (IU/mL)	71	66	80	98
	Medicine	Diagnosed and treated with HCQ (200 mg qd for 3 mo) & Prednisolone (10 mg qd for 2 mo)	HCQ (200 mg qd continued) & Prednisoline (7.5 mg qd for 1 mo)	HCQ (200 mg qd continued) & Prednisolone (5 mg qd for 2 mo)	HCQ (200 mg qd continued) & Prednisolone (2.5 mg qd for 1 mo)
	Cytokine pattern[a]	LPS	LPS	IC	LPS
8	SLEDAI	2	2	0	0
	CRP (mg/L)	5.99	5.93	6.12	5.86
	Anti-dsDNA (IU/mL)	–	–	–	–
	Medicine	Diagnosed and treated for 2.5 yr; HCQ (200 mg qd) & Prednisolone (2.5 mg qd for 3 mo)	Same, continued	Same, continued	Same, continued
	Cytokine pattern[a]	IC	LPS	LPS	IC
9	SLEDAI	2	2	4	4
	CRP (mg/L)	<3.19	<3.19	3.87	<3.19
	Anti-dsDNA (IU/mL)	52	116	–	–
	Medicine	Diagnosed and treated for >3 yr; HCQ (200 mg qd)	Same, continued	Same, continued	Same, continued
	Cytokine pattern[a]	LPS	LPS	IC	LPS

[a]Immune complex or lipopolysaccharide pattern was designated for each section when the ratio of IC-induced cytokine/GAPDH over LPS-induced cytokine/GAPDH was >1.00 or ≤1.00 for two of the three cytokines. SLEDAI = systemic lupus erythematosus disease activity index; CRP = C-reactive protein; Mo = month; HCQ = hydroxychloroquine; qd = daily; LPS = lipopolysaccharide; IC = immune complex; – = negative.

for those with SLEDAI >7, and $p=0.129$ for those with SLEDAI ≤ 7). When all time points were taken into account, serum CRP levels were found to be uncorrelated with SLEDAI score ($p=0.882$; $n=33$). Serum CRP levels within the individual LPS or IC groups were also found to be uncorrelated with SLEDAI score ($p=0.300$; $n=18$ and $p=0.727$; $n=13$, respectively).

As expected, serum CRP levels in the IC-pattern groups were higher than those in the LPS-pattern groups (Table 4). However, no difference existed between the groups with respect to serum anti-dsDNA levels (data not shown). Notably, when all IC- and LPS-pattern groups were mixed, the serum CRP levels were positively correlated with serum C3c and C4 concentrations (Figure 1). Conversely, the CRP or C3c levels were negatively correlated with serum anti-dsDNA levels (Figure 1). In particular, the CRP concentrations within the subgroup with an anti-dsDNA concentration >70 IU/mL was significantly lower than that in the subgroup with an anti-dsDNA concentration <35 IU/mL (Figure 2). Similarly, when all sections were divided into two subgroups [i.e. anti-dsDNA < 35 IU/mL ($n=12$) and >35 IU/mL ($n=19$)], the former had higher serum CRP levels than the latter ($p=0.007$).

Comparison of cytokine genes between cytokine-pattern groups

We then went on to examine whether the mRNA expression levels of the CRP-inducing cytokines in PBMCs

03-JMII-2-08-006.indd 22 2/22/2010 6:28:31 PM

Table 4. Comparison of serum C-reactive protein levels between immune complex-pattern groups and lipopolysaccharide-pattern groups

	Immune complex induced (n=13)	Lipopolysaccharide induced (n=18)	p
Percentages <3.19 mg/L (%)	53.8	66.7	0.226
C-reactive protein levels (mg/L)[a]	11.91±25.47 (0–88.8)	3.79±7.26 (0–27.0)	0.027[b]

[a]Data presented as mean±standard deviation (range), C-reactive protein levels <3.19 mg/L was calculated as 0 mg/L; [b]F ratio=5.395.

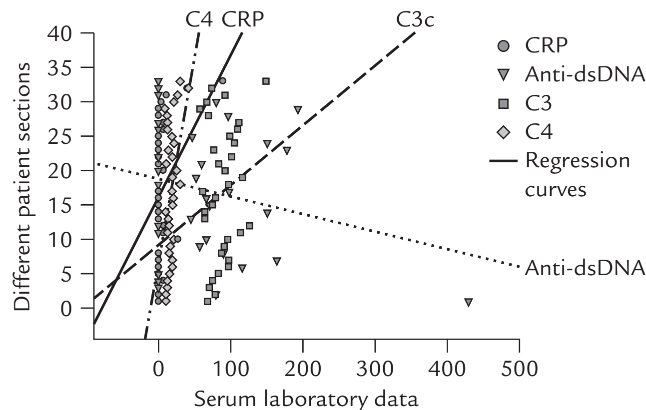

Figure 1. Correlation curves showing laboratory variables. When all immune complex and lipopolysaccharide pattern groups were mixed (n=31), serum C-reactive protein levels were positively correlated with serum C3c at rho=0.416 and with C4 at rho=0.390 (p=0.002, and p=0.001, respectively). Additionally, serum CRP and C3c levels were negatively correlated with anti-double stranded DNA (anti-dsDNA) at rho=–0.599 and rho=–0.452 (p<0.001 and p=0.008, respectively). The x-axis represents real laboratory data without units. Different symbols on the same horizontal level represent data from one section. Therefore, when one set of data from a particular section had a high anti-dsDNA level (right side of the figure), its C3c, C4, and CRP levels were low (left side of the figure) and *vice versa*.

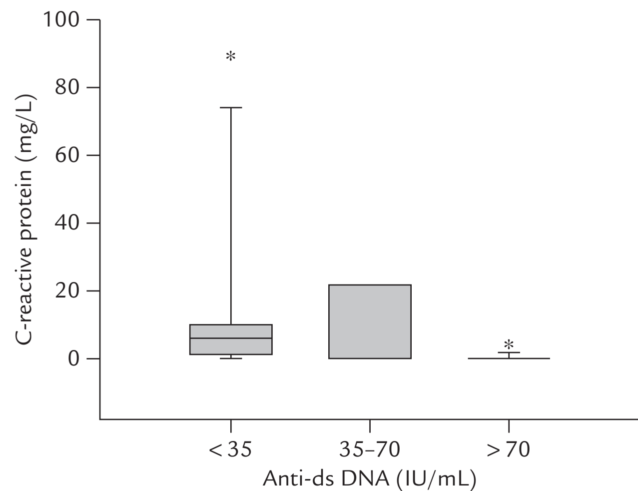

Figure 2. Comparison of serum C-reactive protein levels between subgroups classified by different anti-double stranded DNA antibody levels. All 31 sections were divided into three subgroups, with cut-off points as shown on the x-axis (70 IU/mL was arbitrarily selected as twice the value of 35 IU/mL, which is the lower normal limit). Data presented as mean±standard deviation. Box-whiskers indicate 25th–75th and 10th–90th percentiles. *p=0.038 and a 95% confidence interval of 0.89–27.85.

differed between the two cytokine-pattern groups. Interestingly, following IC stimulation, the IL-6/GAPDH and IL-1β/GAPDH mRNA expression ratio was higher in the IC-pattern groups than in the LPS-pattern groups (Table 5). These experimental results indicate that PBMCs showing an IC-pattern of cytokine expression in lupus patients have constitutively express higher levels of the CRP-inducing cytokine than LPS-pattern cells, mirroring the expression of cytokine proteins in lupus monocytes.[3] However, no differences were observed in the CRP-inducing cytokine mRNA expression in either pattern group upon LPS stimulation (data not shown). Thus, with respect to combined serum CRP levels and cytokine gene patterns, individuals with an IC pattern are predisposed to higher serum CRP levels than those with LPS patterns.

Comparison of circulating ICs between groups and their correlations with laboratory parameters

We then looked at whether the cytokine patterns of PBMCs stimulated with IC or LPS were affected by the presence of pre-existing serum ICs. In cases where sera were obtained on the same day that gene experiments were carried out, the serum concentration of circulating ICs was 64.86±12.15 μg/mL in the LPS-pattern groups (n=18; range, 37.59–88.79) and 59.93±15.50 μg/mL in the IC-pattern groups (n=12; range, 20.74–80.66). These values were not significantly different (p=0.446). Even when all time points were divided into those with SLEDAI>7 (66.56±31.25 μg/mL, n=3) and SLEDAI>7 (63.05±10.30 μg/mL, n=28), no significant differences were found (p=0.593). Notably, CIC concentration was positively correlated with serum anti-dsDNA antibody levels, and negatively correlated with

03-JMII-2-08-006.indd 23 2/22/2010 6:28:31 PM

Table 5. Different cytokine mRNA expression after Immune complex stimulation[a]

Ratio	Immune complex ($n=13$)	Lipopolysaccharide ($n=18$)	p
IL-6/GAPDH	203.55 (193.60–207.38)	191.63 (179.34–200.13)	0.028
IL-1β/GAPDH	196.10 (191.35–204.83)	183.60 (162.31–192.46)	0.003
TNF-α/GAPDH	171.45 (153.95–178.15)	164.88 (155.19–175.75)	0.921

[a]Data presented as median (25th–75th percentiles) of the combined Immune complex stimulation (i.e. at 30 μg and 60 μg).

serum C4 concentrations (CIC *vs*. CRP, $p=0.065$; CIC *vs*. anti-dsDNA, $p=0.028$; CIC *vs*. C3c, $p=0.314$; CIC *vs*. C4, $p<0.001$).

Discussion

In this study, we demonstrate that hydroxychloroquine, corticosteroids, and natural disease course can all alter the serum cytokine profiles in lupus patients (Tables 2 and 3). Moreover, the classification of the different cytokine patterns in this study was also reflected by the CRP differences in these patients (Table 4). This latter result for PBMCs confirms similar results obtained for using monocyte subsets isolated from the peripheral blood of lupus patients.[3] Although serum CRP levels were not significantly correlated with SLEDAI scores in the different pattern groups (Tables 2 and 3), serum CRP levels were positively correlated with serum C3c and C4 concentrations, and negatively correlated with anti-dsDNA antibody levels (Figures 1 and 2). These results imply that in the absence of infection CRP is synthesized in the liver of SLE patients (together with C3 and C4) only after tissue damage has occurred. In other words, low levels of C3, C4, and serum anti-dsDNA antibody levels appear following tissue damage, with a subsequent quick rebound in C3 and C4 production from the liver.[2] In addition, only when anti-dsDNA antibody concentrations declined (possibly due to deposition in the tissues or cells) did serum CRP levels increase (together with increased complement levels), consistent with its role as an acute phase reactant (Figure 1). The clinical importance of this finding is highlighted by a recent study that suggests that reductions in anti-dsDNA levels are associated with concurrent 'flares' in patients with SLE.[9] The time-gap (i.e. kinetic change over hours) between low serum anti-dsDNA levels and elevated serum CRP, C3, and C4 levels, must be considered as a focus of future research. Moreover, no serum complement data were shown that may have correlated with lupus disease "flares", although flares were shown to correlate with reductions in anti-dsDNA levels.[9] Therefore, a considerably larger study is needed to confirm this observation. Moreover, whether combined CRP and anti-dsDNA levels accurately reflect lupus flares, or whether serum CRP levels can be employed as an indicator for pathogenic anti-dsDNA antibodies needs to be clarified by future research.

High levels of IL-6 and IL-1β mRNA expression in the IC-pattern groups, in combination with higher mean CRP levels than in LPS-pattern groups (Tables 4 and 5), indicate that, in the absence of infection, IC-pattern white blood cells have an intrinsic potential to generate a greater volume of CRP-inducing cytokines, as has been described previously for lupus monocytes.[3] Since IC or LPS patterns are interchangeable under different conditions (Tables 2 and 3), determining whether patients have an infection by analyzing CRP levels alone is difficult when no obvious source of infection exists. This is particularly true when serum CRP levels are < 60 mg/L.[10] However, most patients in this study did not exhibit severe lupus disease activity (Tables 2 and 3; Figure 1) so a large patient population is required to confirm this hypothesis.

Since pre-existing serum IC concentrations did not differ between the IC- and LPS-pattern groups, or among the different SLEDAI groups, CIC does not affect the predisposition of lupus PBMCs to either IC or LPS patterns. Conversely, from a clinicopathological perspective, serum CIC concentrations may be an epiphenomenon for the anti-dsDNA antibody and complement interactions (see the end of the Results section).

A limitation of this study is the small number of patients enrolled. This drawback can be remedied, in part, by undertaking a longitudinal study over a greater time period, with a greater number of data points. However, in

03-JMII-2-08-006.indd 24 2/22/2010 6:28:31 PM

order to reliably confirm the findings of the present study, a further investigation with a considerably larger patient population will be necessary.

In summary, this is the first longitudinal study demonstrating that CRP-inducing cytokine patterns in lupus PBMCs change over time, either naturally or as a result of medication. The results indicate that IC-pattern white blood cells have the inherent capability to secrete higher levels of CRP-inducing cytokines than LPS-pattern cells. Notably, serum CRP levels were positively correlated with serum C3c or C4 concentrations, and inversely correlated with serum anti-dsDNA antibody levels during the 6-month follow-up period (Tables 2 and 3; Figure 1). This latter phenomenon is the first reported temporal relationship among CRP, C3c, C4, and anti-dsDNA antibodies in lupus patients. However, studies examining a larger lupus population are needed to confirm these findings.

Acknowledgments

This work was supported by the National Science Council of the Republic of China, Taiwan, under Contract No. NSC91-2314-B182A-027.

References

1. Baumann H, Gauldie J. Regulation of hepatic acute phase plasma protein genes by hepatocyte stimulating factors and other mediators of inflammation. *Mol Biol Med* 1990;7:147–59.
2. Liou LB. Serum and *in vitro* production of IL-1 receptor antagonist correlate with C-reactive protein levels in newly diagnosed, untreated lupus patients. *Clin Exp Rheumatol* 2001;19: 515–23.
3. Liou LB. Different monocyte reaction patterns in newly diagnosed, untreated rheumatoid arthritis and lupus patients probably confer disparate C-reactive protein levels. *Clin Exp Rheumatol* 2003;21:437–44.
4. Honig S, Gorevic P, Weissman G. C-reactive protein in systemic lupus erythematosus. *Arthritis Rheum* 1997;20:1065–70.
5. Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29: 288–91.
6. Sorg R, Enczmann J, Sorg U, Heermeier K, Schneider EM, Wernet P. Rapid and sensitive mRNA phenotyping for interleukins (IL-1 to IL-6) and colony stimulating factors (G-CSF, M-CSF, and GM-CSF) by reverse transcription and subsequent polymerase chain reaction. *Exp Hematol* 1991;19:882–7.
7. James SP. Detection of cytokine mRNA expression by PCR. In: Coligan JE, Kruisbeek AM, Margulies DH, Strober W, editors. *Current protocols in Immunology*, John Wiley & Sons, 1994; section 10:23.1–10.
8. Chen HS, Tsai WP, Leu HS, Ho HH, Liou LB. Invasive fungal infection in systemic lupus erythematosus: An analysis of 15 cases and a literature review. *Rheumatology* 2007;46:539–44.
9. Ho A, Magder LS, Barr SG, Petri M. Decreases in anti-double-stranded DNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. *Arthritis Rheum* 2001;44:2342–9.
10. Ter Borg EJ, Horst G, Limbrug PC, Van Rijswijk MH, Kallenberg CGM. C-reactive protein levels during disease exacerbations and infections in systemic lupus erythematosus: A prospective longitudinal study. *J Rheumatol* 1990;17:1642–8.

03-JMII-2-08-006.indd 25 2/22/2010 6:28:31 PM