EXTENDED REPORT

Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus

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Background: Chemokines are involved in leucocyte chemotaxis. Infiltrating leucocytes play an important role of tissue injury in systemic lupus erythematosus (SLE).

Objective: To investigate the role of inflammatory chemokines and their association with interleukin 18 (IL18) in SLE pathogenesis and disease activity.

Methods: Plasma concentrations and ex vivo peripheral blood mononuclear cell production of inflammatory chemokines IP-10, RANTES, MIG, MCP-1, TARC, IL8, and GRO α , and proinflammatory cytokines IL18, IFN γ , IL2, IL4, and IL10 were assayed in 80 SLE patients with or without renal disease and 40 healthy controls by immunofluorescence flow cytometry and enzyme linked immunosorbent assay.

Results: Plasma IP10, RANTES, MIG, MCP-1, GRO α , and IL18 concentrations in all SLE patients were higher than in controls, and correlated significantly with SLEDAI score (all $p < 0.05$). In SLE patients without renal disease, IP10, RANTES, MIG, MCP-1, IL8, and IL18 correlated positively with SLEDAI score, while in those with renal derangement, IP10, IL8, IL10, and IL18 correlated with disease activity (all $p < 0.05$). Plasma IL18 concentration correlated positively with IP10, MIG, GRO α , and IL8 in all SLE patients (all $p < 0.005$). Mitogen induced increases in ex vivo production of IP10, MCP-1, TARC, IFN γ , IL4, and IL10 were higher in all SLE patients regardless of their difference in disease activity (all $p < 0.05$). Patients with renal disease had an augmented ex vivo release of RANTES.

Conclusions: The correlation of raised plasma concentration and ex vivo production of inflammatory chemokines with disease activity, and their association with IL18, supports the view that chemotaxis of Th1/Th2 lymphocytes and neutrophils is important in SLE pathogenesis.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterised by the activation of T and polyclonal B lymphocytes, the production of autoantibodies, and the formation of immune complexes causing tissue and organ damage.^{1,2} The aetiology and pathogenesis of this autoimmune disorder have not been clearly elucidated. Abnormal production of Th (helper) cell cytokines is involved in the pathogenesis of autoimmune diseases.³ It has been suggested that SLE is a Th2 polarised disease because of its production of autoantibodies specific for self antigens,⁴ and increases in plasma concentration of Th2 cytokines interleukin (IL) 6 and IL10 were found in active SLE.⁵ However, other studies have shown that concentrations of plasma cytokines for Th1 response including IL12, tumour necrosis factor α (TNF α), and interferon γ (IFN γ) are significantly higher in SLE patients.^{6–8} These phenomena imply that Th cytokine response in SLE is very complex, and the issue of polarised Th1 or Th2 cytokine expression in this disease remains undetermined. While Th1 dominant immune responses have generally been considered to be pathological in autoimmune disease through the induction of inflammatory reactions,⁹ previous reports have shown the involvement of the Th1 related cytokine IL18 in initiating both innate and acquired immune responses.^{10,11} IL18 was originally identified as a factor that enhances IFN γ production in various cell types, including macrophages, T cells, B cells, and dendritic cells.¹² It has been reported that IL18, along with IL12, is a potent inducer of the inflammatory mediators by T cells, causing severe inflammatory disorders in autoimmune diseases such as rheumatoid arthritis.^{12,13} Our previous cross sectional studies have shown an increase in plasma IL18 concentration in SLE patients;

furthermore, the plasma level correlated with SLE disease activity.^{14,15} These results indicated that IL18 may contribute to the activation of the disease.

On the other hand, there has been growing evidence suggesting that infiltration of T lymphocytes and other leucocytes into the sites of inflammation plays a critical role in organ involvement.² Leucocyte migration is mediated by the interaction of a number of chemokines and their receptors.¹⁶ These small molecules have well defined roles in directing cell movements necessary for the initiation of T cell immune response, attraction of appropriate effector cells to sites of inflammation, and regulation of differential recruitment of T helper (Th1 and Th2) lymphocytes.^{16–18} Recent studies have shown raised plasma chemokine concentrations of monocyte chemoattractant protein-1 (MCP-1), regulated upon activation normal T expressed and secreted (RANTES), and activated T cell chemokine interferon inducible protein 10 (IP10) in active SLE.^{19–21} It is also known from animal models and from studies of patients with lupus nephritis that inflammatory chemokines, especially MCP-1 and RANTES, are detectable in kidney tissues before other signs of inflammation.^{22–25} Other studies have also

Abbreviations: CBA, cytometric bead array; GRO α , growth regulated oncogenes α ; IFN γ , interferon γ ; IL, interleukin; IP10, chemokine interferon inducible protein 10; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MIG, monokine induced by IFN γ ; PBMC, peripheral blood mononuclear cells; PHA, mitogen phytohaemagglutinin; RANTES, regulated upon activation normal T cell expressed and secreted; RSLE, systemic lupus erythematosus with renal disease; SLE, systemic lupus erythematosus; SLEDAI, ; systemic lupus erythematosus disease activity index, ; TARC, thymus and activation regulated chemokine; Th, T helper; TNF α , tumour necrosis factor α .

Table 1 Characteristics of patients with systemic lupus erythematosus with and without renal disease, and control subjects

Variable	RSLE	SLE	NC
Number	40	40	40
Sex (female/male)	39/1	39/1	39/1
Age (years)	36 (8)	38 (9)	40 (9)
Range	22 to 55	19 to 51	19 to 55
Duration of diagnosis (years)	12.1 (6.4)	12.8 (6.1)	NA
Range	0.3 to 29.2	1.3 to 28.2	
SLEDAI score	8 (4)	3 (2)	NA
Range	2 to 18	0 to 8	
Plasma creatinine ($\mu\text{mol/l}$)	108.7 (79.6)	76.0 (10.5)	NA
Range	57.0 to 472.0	57.0 to 106.0	
Plasma urea (mmol/l)	8.2 (6.2)	4.9 (1.4)	NA
Range	2.8 to 36.5	2.6 to 8.2	
Treatment with prednisolone			NA
Patients (n (%))	36 (90.0%)	23 (57.5%)	
Daily dose (mg)	6.1 (5.4)	3.2 (4.4)	
Treatment with hydroxychloroquine			NA
Patients (n (%))	16 (40.0%)	24 (60.0%)	
Daily dose (mg)	85.0 (112.2)	130.0 (111.4)	
Treatment with azathioprine			NA
Patients (n (%))	22 (55.0%)	3 (7.5%)	
Daily dose (mg)	35.6 (36.6)	7.5 (28.4)	

Values are mean (SD) unless stated otherwise.

NA, not applicable; NC, normal control; RSLE, systemic lupus erythematosus with renal involvement; SLE, systemic lupus erythematosus without renal involvement; SLEDAI, systemic lupus erythematosus disease activity index.

demonstrated that the CXC chemokines IL8 and growth regulated oncogenes- α (GRO α) are potent chemoattractants and activators of T cells, neutrophils, eosinophils, and basophils, thereby enhancing their proinflammatory and proangiogenic activities.^{26, 27}

A recent *in vitro* study has shown that certain chemokine and receptor responses to inflammation are strongly dependent on IL2, which links the acquisition of migratory responsiveness to T cell expansion,²⁸ suggesting that cytokines present in the microenvironment may influence the chemokine and receptor expression. Despite the above research findings, the mechanisms for accumulation of immune cells in various organs in SLE have not been fully elucidated. Furthermore, the influence of cytokines, especially IL18, on chemokine expression in these patients have not, to the best of our knowledge, been examined. The present study was undertaken to determine whether the circulating levels of chemokines preferentially chemotactic for Th1 cells (IP10 and MIG), or predominantly chemotactic for Th2 cells (MCP-1 and TARC (thymus and activation regulated chemokine)), or for both cell types (RANTES) were raised in SLE patients; and to examine their correlation with IL18. For a better understanding of the immunopathological roles of inflammatory molecules and their potential as disease markers in SLE, we further investigated the immune response of peripheral blood mononuclear cells (PBMC) by evaluating their *ex vivo* production of inflammatory chemokines, Th1 cytokines IFN γ and IL2, and Th2 cytokines IL4 and IL10 upon activation by T cell mitogen phytohemagglutinin (PHA) and B cell and macrophage mitogen lipopolysaccharide (LPS) in SLE patients and normal healthy subjects.

METHODS

SLE patients, control subjects, and blood samples

Eighty Chinese SLE patients (78 female, two male) managed at the rheumatology outpatient clinic of the Prince of Wales Hospital, Hong Kong, were recruited with informed consent. Ethical approval was obtained from clinical research ethics committee of The Chinese University of Hong Kong–New Territories East Cluster Hospitals, and informed consent was obtained from all participants. Diagnosis of SLE was

established according to the 1982 revised American Rheumatism Association (ARA) criteria,²⁹ and disease activity evaluated by the SLE disease activity index (SLEDAI) score.³⁰ Active lupus disease was defined as a SLEDAI score of ≥ 6 .³⁰ The SLE patients were divided into two groups: 40 with renal disease (RSLE group) and 40 without renal disease (SLE group), the rationale being that SLE patients with advanced or severe disease will gradually develop renal impairment, thus representing the active disease group. RSLE patients were defined by persistent of proteinuria (>0.5 g/24 h) or the presence of cellular casts, persistent haematuria, or renal biopsy showing mesangial, focal proliferative, diffuse proliferative, or membranous glomerulonephritis. Forty sex and age matched healthy Chinese subjects were recruited as controls (NC group). EDTA blood (20 ml) was collected from each patient and control subject.

Whole blood assay

The method of Viallard *et al*³¹ was adopted with modification.³² After a maximum storage period of one hour at room temperature, blood was diluted 1:1 with RPMI 1640 culture medium (Gibco Laboratories, New York, USA), and 1 ml aliquots were dispensed in each well of a 24-well plate (Nalge Nunc International, Illinois, USA). The blood culture was then incubated without or with PHA (Sigma Co, MO, USA) at 5 $\mu\text{g/ml}$ and LPS at 25 $\mu\text{g/ml}$ (Sigma) for 24 hours at 37°C in a 5% CO₂ atmosphere. After incubation, the cell-free culture supernatant was harvested and stored at -70°C until analysis.

Chemokine and cytokine assays

Chemokines (IP10, RANTES, MIG, MCP-1 and IL8) and Th1/Th2 cytokines (IFN γ , IL2, IL4 and IL10) in plasma and blood culture supernatant were simultaneously quantified by immunofluorescence flow cytometry (FASCalibur flow cytometer, Becton Dickinson, California, USA) using Human Chemokine and Th1/Th2 Cytokine Cytometric Bead Array (CBA) reagent kits (BD Biosciences Pharmingen, San Diego, California, USA). Enzyme linked immunosorbent assay (ELISA) was used to measure TARC, GRO α (R&D System, Minneapolis, Minnesota, USA) and IL18 (BioSource

Table 2 Plasma chemokine and cytokine concentrations of patients with systemic lupus erythematosus and normal control subjects

Chemokine/ cytokine	Subgroup	Median (IQR) (pg/ml)
IP10	SLE	2220 (1590 to 3510)***
	RSLE	840 (1310 to 2940)***
	SLE+RSLE	2030 (1370 to 3290)***
	NC	737 (597 to 864)
RANTES	SLE	1740 (718 to 4360)**
	RSLE	1500 (530 to 4000)**
	SLE+RSLE	1640 (655 to 4360)**
	NC	580 (300 to 1660)
MIG	SLE	625 (369 to 1350)***
	RSLE	508 (332 to 777)***
	SLE+RSLE	16 (342 to 1070)***
	NC	203 (159 to 250)
MCP-1	SLE	82.0 (49.8 to 123)***
	RSLE	87.1 (64.3 to 141)***
	SLE+RSLE	86.2 (54.9 to 132)***
	NC	33.5 (22.3 to 47.1)
GRO α	SLE	334 (241 to 844) ***
	RSLE	362 (263 to 1015)***
	SLE+RSLE	335 (248 to 1015)***
	NC	219 (158 to 466)
IL4	SLE	0.01 (0.00 to 0.14)
	RSLE	0.09 (0.03 to 0.13)*
	SLE+RSLE	0.05 (0.00 to 0.13)
	NC	1.41 (0.72 to 2.26)
IL10	SLE	0.01 (0.00 to 0.10)
	RSLE	1.68 (0.94 to 2.96)*
	SLE+RSLE	1.58 (0.79 to 2.81)
	NC	0.98 (0.38 to 2.34)
IL18	SLE	242 (174 to 334)***
	RSLE	263 (184 to 362)***
	SLE+RSLE	248 (180 to 335)***
	NC	158 (137 to 219)

*p<0.05; **p<0.01; ***p<0.001 v NC.

GRO α , growth regulated oncogenes α ; IL, interleukin; IP10, chemokine interferon inducible protein 10; MCP-1, monocyte chemoattractant protein-1; MIG, monokine induced by interferon γ ; NC, normal control; RANTES, regulated upon activation normal T cell expressed and secreted; RSLE, systemic lupus erythematosus with renal involvement; SLE, systemic lupus erythematosus without renal involvement.

Corporation, Bethesda, Maryland, USA). To normalise for the individual difference in leucocyte number of each whole blood sample, the amounts of chemokines and cytokines released in the ex vivo supernatant were expressed as pg/10⁶ leucocytes. The absolute number of leucocytes (CD45+) of each whole blood sample was ascertained by Multitest IMK kit with TruCOUNT tubes (Becton Dickinson) using the lyse/no-wash method on a four colour FASCalibur flow cytometer (Becton Dickinson).

Statistical analysis

Numerical data were expressed as median (interquartile range, IQR) if they were not in Gaussian distribution. Difference in plasma concentration among groups was compared with Kruskal-Wallis analysis of variance (ANOVA), followed by Dunn's multiple comparisons post test. Spearman's rank correlation test was used to assess the correlations of plasma chemokine and cytokine concentrations with SLEDAI score. Comparison of basal and ex vivo culture supernatant concentrations was made with the Wilcoxon paired test. Statistical analysis was done using the statistical package for the Social Sciences (SPSS) statistical software for Windows, version 9.0 (SPSS Inc,

Table 3 Correlations between plasma chemokine and cytokine concentrations with SLEDAI score in the various groups

Chemokine/ cytokine	Group	Spearman <i>r</i>	p Value
IP10	SLE	0.363	0.021*
	RSLE	0.356	0.024*
	SLE+RSLE	0.136	0.230
RANTES	SLE	0.354	0.025*
	RSLE	0.145	0.378
	SLE+RSLE	0.103	0.368
MIG	SLE	0.311	0.048*
	RSLE	0.150	0.337
	SLE+RSLE	0.102	0.369
MCP-1	SLE	0.356	0.024*
	RSLE	0.066	0.684
	SLE+RSLE	0.241	0.032*
IL8	SLE	0.333	0.036*
	RSLE	0.333	0.035*
	SLE+RSLE	0.309	0.005*
IL10	SLE	0.160	0.326
	RSLE	0.311	0.048*
	SLE+RSLE	0.252	0.024*
IL18	SLE	0.377	0.017*
	RSLE	0.326	0.040*
	SLE+RSLE	0.258	0.021*

Results are expressed as correlation coefficient *r*; *p<0.05.

IL, interleukin; IP10, chemokine interferon inducible protein 10; MCP-1, monocyte chemoattractant protein-1; MIG, monokine induced by interferon γ ; RANTES, regulated upon activation normal T cell expressed and secreted; RSLE, systemic lupus erythematosus with renal involvement; SLE, systemic lupus erythematosus without renal involvement.

Chicago, Illinois, USA). Probability (p) values of <0.05 was considered significant.

RESULTS

SLE patients and control subjects

Forty SLE patients with renal disease (RSLE group), 40 patients without renal disease (SLE group), and 40 sex and age matched control subjects were studied. Their age, sex, duration of diagnosis, SLEDAI score, plasma urea and creatinine concentrations, and drug treatment are summarised in table 1. Using a SLEDAI cut off score of ≥ 6 , the proportions of the SLE group and RSLE group with active disease were 7.5% and 65%, respectively. Active kidney disease was shown in 67.5% of the RSLE patient group according to the presence of urinary casts, haematuria, proteinuria, or pyuria. The mean duration of glomerulonephritis in the RSLE patients was 9.6 years.

Plasma concentrations of chemokines and Th1/Th2 cytokines

Plasma IP10, RANTES, MIG, MCP-1, GRO α , and IL18 concentrations were significantly higher in all SLE patients as well as in RSLE and SLE groups than in control subjects (table 2). IP10, MIG, MCP-1, GRO α , and IL18 showed the greatest differences between patients and controls (p<0.001), followed by RANTES (p<0.01). There was also a significant increase in circulating IL10 and IL4 concentration in RSLE patients, but not in the other subgroups (all p<0.05). In contrast, there were no significant differences in plasma TARC, IL8, or IL2 concentrations between patient and control groups. The plasma IFN γ concentrations of patients and control subjects were close to the lowest detection limit of the assay, so they were not taken into consideration.

Table 4 Ex vivo production of chemokines and cytokines from mitogen activated peripheral blood mononuclear cells in the various groups

Chemokine/ Th cytokine	Group	Basal median (IQR) (pg/10 ⁶ leucocytes)	Post stimulation median (IQR) (pg/ 10 ⁶ leucocytes)
IP10	SLE	543 (419 to 715)	4090 (2623 to 5560)*
	RSLE	823 (521 to 1490)	5520 (3690 to 6930)***
	SLE+RSLE	621 (442 to 1040)	4420 (2900 to 6520)**
	NC	166 (138 to 241)	2750 (650 to 4780)
RANTES	SLE	1030 (752 to 2140)	1890 (1170 to 4390)
	RSLE	3460 (1730 to 4960)	5690 (1730 to 8360)*
	SLE+RSLE	2070 (795 to 4730)	3300 (1530 to 6690)*
	NC	1020 (632 to 2110)	2403 (1120 to 3640)
MCP-1	SLE	34.8 (19.1 to 68.6)	75.0 (62.1 to 141)***
	RSLE	50.7 (34.8 to 117)	108 (67.4 to 248)***
	SLE+RSLE	42.1 (24.9 to 84.3)	89.1 (65.7 to 227)***
	NC	7.90 (4.70 to 12.6)	40.1 (28.8 to 47)
TARC	SLE	8.60 (5.97 to 14.40)	12.9 (6.80 to 20.4)*
	RSLE	10.5 (9.90 to 16.50)	37.1 (12.4 to 82.6)**
	SLE+RSLE	12.1 (6.50 to 15.70)	15.5 (9.80 to 39.5)*
	NC	5.30 (2.50 to 11.30)	10.2 (5.20 to 15.0)
IFN γ	SLE	3.30 (0.10 to 6.20)	4.50 (0.80 to 6.80)
	RSLE	4.50 (0.10 to 12.1)	6.50 (0.10 to 17.1)*
	SLE+RSLE	3.50 (0.10 to 6.90)	5.20 (0.10 to 10.3)*
	NC	1.10 (0.10 to 3.05)	3.30 (0.10 to 4.80)
IL4	SLE	0.10 (0.10 to 0.50)	0.12 (0.10 to 1.30)*
	RSLE	0.10 (0.10 to 0.20)	0.20 (0.10 to 3.30)**
	SLE+RSLE	0.10 (0.10 to 0.31)	0.10 (0.10 to 2.55)*
	NC	0.02 (0.10 to 0.20)	0.05 (0.02 to 0.10)
IL10	SLE	1.50 (0.20 to 2.00)	54.3 (28.5 to 75.9)**
	RSLE	1.30 (0.15 to 4.20)	35.9 (28.7 to 58.6)**
	SLE+RSLE	1.40 (0.35 to 2.95)	43.8 (28.7 to 67.2)**
	NC	0.12 (0.10 to 0.20)	21.8 (11.0 to 33.6)

The culture supernatant was derived from whole blood cultured with medium in the presence of phytohaemagglutinin (5 μ g/ml) and lipopolysaccharide (25 μ g/ml) for 24 hours.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ v NC.

IL, interleukin; IP10, chemokine interferon inducible protein 10; IQR, interquartile range; MCP-1, monocyte chemoattractant protein-1; NC, normal control; RANTES, regulated upon activation normal T cell expressed and secreted; RSLE, systemic lupus erythematosus with renal involvement; SLE, systemic lupus erythematosus without renal involvement.

Correlations between disease activity and plasma concentrations of chemokines and Th1/Th2 cytokines

As shown in table 3, there were significant and positive correlations of plasma MCP-1, IL8, IL10, and IL18 concentrations with SLEDAI score in all SLE patients (all $p < 0.05$). Upon subgroup analysis, IP10, RANTES, MIG, MCP-1, IL8, and IL18 correlated significantly with SLEDAI score of the SLE group, while IP10, IL8, IL10, and IL18 concentrations correlated significantly with SLEDAI of RSLE patients (all $p < 0.05$). Weak correlations of disease activity with TARC and IL4 concentrations were also noted in the SLE group although they missed statistical significance ($p = 0.099$ and 0.058 , respectively).

Ex vivo production of chemokines and Th1/Th2 cytokines

The above findings prompted us to investigate the immunocompetence of the cells in the ex vivo production of inflammatory chemokines and cytokines from PBMC in the patient cohort. PHA and LPS significantly induced the release of chemokines IP10, RANTES, MIG, MCP-1, and IL8, and cytokines IFN γ , IL10, and TARC from PBMC in all SLE patients and normal control subjects compared with the spontaneous production of chemokines/cytokines under basal condition (all $p < 0.001$). In the presence of exogenous stimuli, the increases in ex vivo production of IP10, MCP-1, TARC, IFN γ , IL4, and IL10 after incubation with PHA and LPS were significantly greater in all SLE patients than in the

control groups (all $p < 0.05$, table 4). An increase in the ex vivo release of RANTES was also observed in patients with renal diseases (RSLE). However, the activated production of MIG and IL8 did not differ significantly between SLE patients and healthy subjects.

Correlations between plasma IL18 concentration and chemokines

Figure 1 shows that plasma IL18 concentration showed a significant positive correlation with Th1 chemokines IP10 and MIG, and neutrophil activation chemokines GRO α and IL8 in all SLE patients ($r = 0.328$, $p = 0.003$; $r = 0.377$, $p < 0.001$; $r = 0.403$, $p < 0.001$; and $r = 0.337$, $p = 0.002$, respectively).

DISCUSSION

SLE is an idiopathic disease characterised by variable autoimmune inflammatory tissue destruction. Defective T cell regulation has been postulated to play a crucial role in its pathogenesis and in the disease manifestations.³³ However, the predominance of Th1 or Th2 cytokines in SLE has not been well defined, and the mechanisms that lead to the aberrant autoinflammatory syndrome are not clearly understood. Recent studies have shown that chemokines and their receptors are intimately involved in regulating organ specific leucocyte trafficking and inflammation, suggesting their important roles in the pathophysiology of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and

Figure 1 Correlations of plasma concentration of IL18 with chemokines (A) interferon inducible protein 10 (IP10), (B) monokine induced by interferon γ (MIG), (C) interleukin 8 (IL8), and (D) growth regulated oncogenes α (GRO α).

SLE.^{34–36} Given that the complex network of cytokines and imbalance of Th1 and Th2 immune responses may contribute to the pathogenesis and activation of SLE, studies on chemokines and T cell expression of chemokine receptors have shed lights on the underlying mechanisms causing the immune dysregulation, such as inappropriate T cell activation or trafficking in this disease.^{21–37} However, there are few reports addressing the balance of these Th1 or Th2 chemoattractants, nor the possible influence of proinflammatory cytokines such as IL18 on chemokine expressions.

Our results showed that the plasma concentrations of inflammatory chemokines that are preferentially chemotactic for Th1 and Th2 cells (IP10, MIG, MCP-1, and RANTES) were significantly higher in all SLE patients than in healthy individuals. Parallel to the derangements of these lymphocyte chemoattractants, neutrophil chemokines IL8 and GRO α , proinflammatory cytokine IL18, and Th2 cytokines IL4 and IL10 were also found to be increased in all SLE patients and in the RSLE group (table 2). Moreover, the raised plasma concentrations of MCP-1, IL8, IL18, and IL10 correlated positively with SLEDAI score in all SLE patients (table 3). It is known from animal models^{23–24, 38} and from studies in patients with lupus nephritis^{25–39} that inflammatory chemokines, especially RANTES and MCP-1, are readily detectable in the kidney tissues and urine. However, when our patient cohort was stratified into renal involvement subgroup analysis, we found that SLE patients with impaired renal function showed decreased plasma concentrations of IP10, RANTES, MIG, and TARC compared with patients without renal disease. A recent paper proposed that urine chemokines could serve as biomarkers for renal SLE flare,⁴⁰ suggesting that the reduced plasma concentration of these circulating chemokines in our patients with renal involvement may result from a protein leak in the urine.

IP10, RANTES, and MIG are chemoattractants for Th1 lymphocytes. Their synthesis and expression from neutrophils, macrophages, and other immune cells is induced by IFN γ and this response is suppressed by IL10 and IL4.^{41–42} Th1 cells and IFN γ are shown to be important for cell mediated inflammation in developing autoimmune disease such as SLE.⁶ In our separate longitudinal study, we observed a general decrease in plasma median concentrations of IP10, RANTES, MIG, and IL8, but not MCP-1, in SLE patients who received prednisolone and leflunomide treatment for 12 months. Taking these results into consideration, our study further supports the view that the studied Th1 and neutrophil chemokines could play an important role in the development of the inflammatory response.

Parallel to the positive correlation of plasma IL8 concentration with SLE disease activity (table 3), a significant positive correlation was also shown between the plasma concentrations of IL8 and GRO α ($r = 0.392$, $p = 0.0003$). IL8 and GRO α are potent chemoattractants and activators of neutrophils. They stimulate neutrophil degranulation and release of reactive oxygen radicals, thereby inducing an acute inflammatory reaction.^{27–43–44} Seemingly, our results simply reflected the proinflammatory activity of these molecules in staging SLE exacerbations.

It is clear that several factors determine the fate of activated T cells—including antigen form, dose, type of antigen presenting cells, co-stimulatory molecules, chromatin structure, and most importantly, cytokines—present in the local environment of the cells at the time of stimulation. IL18 has proinflammatory properties and is known to induce severe inflammatory disorders. In our recent studies, we have investigated the role of IL18 in stimulating Th1 immune responses in SLE.^{14–15} Consistent to the results of our previous reports, we also showed that the increased production of the inflammatory mediator IL18 correlated with disease activity in all SLE patients (table 3). In addition, we found that the plasma IL18 concentration showed strong positive correlations with other inflammatory chemokines (IP10, MIG, IL8, and GRO α) (fig 1). As aberrant synthesis of inflammatory mediators is a fundamental mechanism for leucocyte recruitment to the inflamed tissue. The successful delivery of the appropriate population of leucocytes to sites of acute inflammation will depend on the repertoire of inducible chemokines synthesised locally, and the temporal expression of chemokine receptors on the leucocytes. Meanwhile, the

chemokine expressions are, in part, influenced by pro-inflammatory cytokines present in the local environment of the cells at the time of stimulation. Indeed, in a separate *in vitro* study, we have been able to show preliminarily significant increase in *ex vivo* production of the inflammatory chemokines IL8, IP10, RANTES, and MIG in SLE patients when their PBMC were cultured in the presence of IL18, indicating that IL18 is a potent co-stimulus for the induction of chemokine release from activated PBMC. Furthermore, when acting in conjunction with the inflammatory activities of IL18—such as the induction of Th1 cytokine IFN γ , and activation of Th cells, natural killer cells (NK), and cytotoxic T lymphocytes¹²—inflammatory chemokines may even enhance the Th1 mediated inflammatory process, the activation of NK and T cells, and the migration of macrophages for initiating and perpetuating the Th1 immune response in SLE.

Another significant finding from our study is the enhanced *ex vivo* production of the inflammatory chemokines as observed from SLE patients, particularly in those with renal diseases (table 4). Previous studies on the role of cytokines in association with the disease have often relied on the investigation of plasma concentrations or cytokine production by immunocompetent cells harvested directly from the peripheral blood, or using multistep procedures to isolate the blood cells.^{20–21, 45} The purification procedure may lead to uncontrolled cell activation and elimination of essential cellular and molecular components or mediators present in the whole blood *in vivo*. In this regard, the use of the direct whole blood assay method should preserve the molecular environment for cell activation better, and thus our results could reflect more accurately the immunocompetence or secretory capacity, and hence the immunological response, of the cells in our patient cohort.

In summary, we have shown that derangements of chemoattractants for Th1/Th2 lymphocytes and neutrophils are involved in the autoinflammatory processes of SLE, as exemplified by their positive correlation with disease activity. Taken together with our findings on the association of the proinflammatory cytokine IL18 with inflammatory chemokines, the results should provide new clues for their potential roles in the exacerbation of SLE disease, and shed light on the development of SLE disease markers. An increase in plasma inflammatory chemokines could play a critical role in the inflammatory responses in SLE through leucocyte recruitment and trafficking to organs and tissues suffering autoimmune injury. However, more intensive studies are required to clarify the possible roles of these distinct chemokine superfamilies and their receptor interactions that are involved in the inflammatory mechanisms—including cell activation, intercellular adhesion, tissue destruction, and other processes underlying the immunopathogenesis of SLE. Such understanding may lead to the development of anti-chemokine treatment for the treatment of SLE.

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