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Oxidative DNA and mitochondrial DNA change in patients with SLE

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1. ABSTRACT

We evaluated plasma IL-10, IFN-alpha, IL-23, IFN-gamma, IP-10, MCP-1, 8-OHdG, leukocyte mtDNA, serum anti-dsDNA antibodies and disease activity index (SLEDAI) in SLE patients. 93 patients (35 nephritis, 4 under dialysis, 5 under rituximab) and 50 healthy controls were recruited. Compared with healthy controls, SLE patients had higher IL-10, IFN-alpha, IL-23, IFN-γ, IP-10 and MCP-1 (p<0.05). High IFN-alpha (p=0.031) and IP-10 (p=0.026) correlated with high SLEDAI; high IFN-alpha (p<0.001), IL-23 (p=0.023) and IP-10 (p<0.001) correlated with high anti-dsDNA. High IL-10 (p=0.014), IL-23 (p<0.001), IFN-gamma (p<0.001) and MCP-1 (p=0.002) correlated with high 8-OHdG and high IL-23 (p<0.001), INF-gamma (p<0.001), IP-10 (p=0.023) and MCP-1 (p=0.002) correlated with low leukocyte mtDNA. mtDNA 4977 deletion correlated with high mtDNA (p=0.011) and low IL-10 (p=0.009). MCP-1 (p=0.043) decreased after rituximab therapy. 54 SLE patients without nephritis, 35 with nephritis but without dialysis, and 4 with nephritis under dialysis exhibited stepwise increases in IL-23 (p=0.009) and MCP-1 (p=0.015). These data suggest that oxidative DNA and mtDNA alterations and coordinate changes in cytokines/chemokines are implicated in progression of SLE and rituximab in amelioration of SLE.

2. INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune disease characterized by the production of pathogenic autoantibodies and formation of immune complex, leading to tissue inflammation and organ damage. Cytokines are soluble mediators, released mainly by immune cells, responsible

for cell-cell "cross-talk". Among them, chemokines are a group of small-sized ones with chemotactic properties. Complex networks of interactions among cytokines and chemokines are crucial for the maintenance of immune homeostasis. Growing evidence indicates that various pro- and anti-inflammatory cytokines and/or chemokines are involved in the pathogenesis of SLE. Their aberrant regulation may contribute to the disturbed immune responses, inflammation, and damage to tissues or organs in SLE patients (1-5).

Extensive studies have revealed the supreme role of interferon-α (IFN-α) in the development of SLE, i.e., the "IFN signature". Several studies showed that the IFN-α level in plasma is correlated with disease activity and anti-dsDNA levels (6, 7). Interleukin-10 (IL-10) belongs to a group of anti-inflammatory cytokines that inhibit the activity of several types of immune cells and lead to decreased production of pro-inflammatory cytokines and chemokines (8, 9). It would disrupt Th1/Th2 balance and attenuate Th17 cells, a major source of IL-17, through down-regulation of IFN-γ and IL-23 (10). On the other hand, IL-10 would promote B cell growth and differentiation, thus enhancing autoantibody production in SLE patients (11). It has been demonstrated that serum IL-10 concentration is elevated and correlated with disease activity in SLE patients (12). Th17 cells, which are characterized by the secretion of IL-17, are involved in the pathogenesis of several autoimmune and inflammatory diseases (13, 14). The expansion and survival of Th17 cells are dependent on IL-23 and the importance of IL-23/Th17 axis has been validated in SLE pathogenesis (15, 16). In addition, IL-23 can induce IFN-γ and IL-17 production by $CD4^+$ T cells, which are closely related to the development of lupus disease (17).

Nephritis is one of the severe clinical manifestations of SLE. In active lupus nephritis (LN), several chemo-attractants may be secreted from the *in-situ* and invading immune cells in the inflamed renal parenchyma and lead to deterioration of the disease (18). Recent studies showed that elevated urine chemokines, including IFN-γ-inducible protein 10 (IP-10), which is secreted from endothelial cells and monocyte chemo-attractant protein 1 (MCP-1),which is secreted from the glomerular mesangial cells, are useful markers for the prediction of the severity of LN (19-22).

Previous investigations have indicated that excessive production of reactive oxygen species (ROS) may disturb the reduction-oxidation (redox) status and can modulate the expression of several chemokines, such as RANTES/CCL5, MIP-1/CCL4,MCP-1/CCL-2 and IP-10/CXCL-10, leading to inflammatory processes and thus exacerbating tissue damage in SLE patients (23). It has also been revealed that oxidative stress is related to the abnormal production of Th1 cytokines, including IL-12 and IFN-γ in SLE patients (24). Oxidative damage caused by ROS and impaired mitochondrial function have been proposed to play important roles in the pathogenesis of SLE (25-29).

In the current study, we appraised the association among fluctuated cytokines/chemokines, leukocyte mtDNA alterations (copy number and 4977 bp deletion), plasma 8-hydroxy-2'-deoxyguanosine (8-OHdG, an oxidative DNA damage marker) and disease activity with clinical parameters in SLE patients to strengthen our hypothesis that oxidative DNA damage and mitochondrial dysfunction in SLE and their adverse effects on the body may act through the imbalance of various pro-inflammatory and anti-inflammatory cytokines.

3. MATERIALS AND METHODS

3.1. Patient recruitment

According to the 1997 updated criteria of American College of Rheumatology and 2012 SLICC criteria for the classification of SLE (30, 31), 93 SLE patients (13 men; 35 with LN not under dialysis therapy and 4 with LN under dialysis therapy) and 50 HCs (8 men) were enrolled in Taipei Veterans General Hospital (Taipei VGH). This study was approved by the Institutional Review Board of Taipei VGH (2011-10-006GA). Among the 93 SLE patients, 5 (fulfilling the SLICC criteria) with severe clinical symptoms, including one with LN and interstitial lung disease (ILD, with diffusion capacity of carbon monoxide (DLCO) of 52.5.%), one with acute LN and rapidly progressive glomerulonephritis under dialysis therapy, one with LN, pleuritis and pericarditis, one with severe pulmonary arterial hypertension, and one with severe ILD (DLCO = 24%), were treated with intravenous rituximab (Mabthera™, Roche Biotech, Taiwan). The therapeutic regimen consisted of intravenous rituximab (500 mg) on days 1 and 14 with a pre-medication with 100 mg of intravenous methylprednisolone. Blood samples were collected before rituximab treatments on days 1 and 14.

3.2. Blood sample collection, leukocyte DNA extraction and plasma preservation

Approximately 10 mL of peripheral venous blood was drawn into a tube (VACUETTE®, Greiner Bio-one) containing EDTA. After centrifugation at 3000 *g* for 10 min at 4°C, supernatant plasma and leukocyte pellets were separately collected. Leukocyte DNA was extracted and preserved as described previously (28, 29). Leukocyte DNA was subject to the determination of mtDNA copy number and 4977 deletion and the plasma was used for the quantification of 8-OHdG, cytokine and chemokine.

3.3. Determination of plasma 8-OHdG level, the copy number and 4977 deletion of mtDNA in leukocytes

In part of our current cohort, including 89 of the 93 SLE patients and 45 of the 50 HCs, the plasma

8-OHdG levels and leukocyte mtDNA copy numbers have been reported (28, 29). The plasma level of 8-OHdG was measured by using the highly sensitive 8-OHdG Check ELISA (Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd. Haruoka, Fukuroi, Shizuoka, Japan) according to the manufacturer's instructions (26, 32). The leukocyte mtDNA copy number was analyzed by real-time quantitative PCR and adjusted by taking the mtDNA copy number of 143B cell line as 1.00 (28, 29). Each reaction was performed in duplicate, and the mean value was used for data presentation. The 4977bp deletion of leukocyte mtDNA was detected by modified PCR method using the primers L8150 (5'-CCGGGGGTATACTACGGTCA-3') and H13650 (5'-GGGGAAGCGAGGTTGACCTG-3'). Each 50 μl of PCR reaction mixture contained 25 μl of RBC SensiZyme® Hotstart *Taq* Premix (RBC Bioscience, New Taipei City, Taiwan), 23 μl of PCR-grade H_2O, 1 μl of each primer, and 1 μl of sample DNA (10 ng/μl). The PCR procedure included a hot start at 95˚C for 10 min, 45 cycles of 95˚C for 15 sec, 58˚C for 15 sec and 72˚C for 30 sec, and a final extension at 72˚C for 7 min. The PCR products were subject to electrophoresis on a 3% agarose gel to separate the DNA bands and were visualized by ultraviolet light illumination after ethidium bromide staining (33).

3.4. Determination of plasma levels of cytokines and chemokines

Plasma levels of cytokines and chemokines, including IL-10, IFN-α, IL-23, IFN-γ, IP-10 and MCP-1, were determined by ProcartaPlex™ Multiplex Immunoassays (Affymetrix®, eBioscience, North America) according to the manufacturer's instructions (34).

3.5. Statistical analysis

The results are presented as the mean ± standard deviation (Mean ± S.D.). The continuous variables between two groups (SLE patients and HCs), among the three groups (LN absent SLE patients, LN present SLE patients without dialysis and LN present SLE patients with dialysis), or between paired groups (SLE patients before and after the administration of rituximab) were compared using non-parametric Mann-Whitney *U* test, Kruskal-Wallis *H* test, or Wilcoxon signed rank test when appropriate. Relationships between two continuous variables were evaluated by Spearman's correlations. Differences were considered significant if *p*-values are less than 0.05.

4. RESULTS

4.1. Differences in clinical and biological parameters between the HCs and SLE patients

As shown in Table 1, the plasma levels of 8-OHdG (p<0.001), IL-10 (p<0.001), IFN-α (p<0.001), IL-23 (p<0.001), IFN-γ (p=0.036), IP-10 (p=0.019), and MCP-1 (p<0.001) in SLE patients were significantly higher than those in the HCs.

4.2. Plasma levels of cytokines/chemokines and their relationships to the scores of SLEDAI or titers of anti-dsDNA antibody in SLE patients

For the 93 SLE patients, the mean SLEDAI score was 9.9 ± 6.6 (median, 8.5), which was generally high. As listed in Table 2 (left column), higher SLEDAI scores were related to higher levels of anti-dsDNA antibody (Spearman's correlation coefficient ρ=0.308, p=0.003), plasma IFN-α (ρ=0.224, p=0.031) and plasma IP-10 (ρ=0.230, p=0.026), but lower copy numbers of leukocyte mtDNA (ρ=-0.197, p=0.058).

The titers of anti-dsDNA antibody were available in 92 of 93 SLE patients with a mean value of 121.7mg/dL. As listed in Table 2 (right column), higher titers of anti-dsDNA antibody were correlated to higher plasma levels of IFN-α (ρ=0.414, p<0.001), IL-23 (ρ=0.236, p=0.023), and IP-10 (ρ=0.396, p<0.001).

4.3. Plasma levels of cytokines/chemokines and their relationships to plasma levels of 8-OHdG and leukocyte mtDNA copy numbers in SLE patients

As listed in Table 3 (left column), higher plasma levels of 8-OHdG were related to higher plasma levels of IL-10 (ρ=0.254, p=0.014), IL-23 (ρ=0.357, p<0.001), IFN-γ (ρ=0.435, p<0.001) and MCP-1 (ρ=0.322, p=0.002), but lower leukocyte mtDNA copy numbers (ρ= -0.324, p=0.002).

As shown in Table 3 (right column), lower leukocyte mtDNA copy numbers were related to higher plasma levels of 8-OHdG (ρ= -0.324, p=0.002), IL-23 (ρ= -0.374, p<0.001), IFN-γ (ρ=-0.395, p<0.001), IP-10 (ρ= -0.236, p=0.023), and MCP-1 (ρ= -0.312, p=0.002).

4.4. Differences in clinical and biological parameters between the SLE patients with and without 4977 bp deletion of mtDNA in leukocytes

Although SLE patients did not have a higher incidence of 4977 deletion of mtDNA in leukocytes than did HCs (26/93, 28% *vs.* 18/50, 36%, p=0.320, Table 1), SLE patients with 4977 bp deletion of mtDNA had higher mtDNA copy numbers in leukocytes (p=0.011) and lower plasma levels of IL-10 (p=0.009) than did SLE patients without 4977 bp deletion of mtDNA in leukocytes (Table 4).

4.5. Decreases in plasma levels of cytokines/chemokines in 5 SLE patients after rituximab infusion

As shown in Table 5, the plasma levels of cytokines and chemokines were decreased after administration of rituximab, but the decrease was only pronounced in MCP-1 (p=0.043).

Table 1. Differences in clinical and biological parameters between the healthy controls (HCs) and SLE patients

Clinical and biological parameters (Case number=HCs, SLE)	Mean±S.D.		*p*-value
	HCs (n=50)	SLE (n=93)	Mann-Whitney *U* test
SLEDAI (n= -, 93)	-	9.9. ± 6.6	
Anti-dsDNA antibodies (mg/dL) (n= -, 92)	-	121.7±130.8	
Plasma 8-OHdG (ng/mL) (n= 50, 93)	0.155±0.037	0.247±0.126	< 0.001
Leukocyte mitochondrial DNA (mtDNA)			
Relative mtDNA copy number (n= 50, 93)	0.199±0.089	0.205±0.113	0.744
4977 bp deletion			0.320
Yes	18 (36.0%)	26 (28.0%)	
No	32 (64.0%)	67 (72.0%)	
Plasma cytokines (pg/mL)			
IL-10 (n= 41, 93)	0.903±0.541	3.698±6.136	< 0.001
IFN-α (n= 50, 93)	1.218±0.414	5.378±7.436	< 0.001
IL-23 (n= 50, 93)	103.2±32.9	138.8±55.8	< 0.001
IFN-γ (n= 46, 90)	1.300±0.714	1.689±1.423	0.036
Plasma chemokines (pg/mL)			
IP-10 (n= 50, 93)	25.4±12.3	37.4±33.5	0.019
MCP-1 (n= 50, 93)	33.2±14.8	47.7±28.4	< 0.001

Table 2. Plasma levels of cytokines/chemokines and their relationships to the scores of SLEDAI and titers of anti-dsDNA antibody in SLE patients

	Correlation to SLEDAI (n=93)		Correlation to anti-dsDNA antibodies (n=92)	
Clinical and biological parameters (case number)	Spearman's coefficient (ρ)	*p*-value	Spearman's coefficient (ρ)	*p*-value
Anti-dsDNA antibodies (mg/dL) (n= 92)	0.308	0.003	-	-
SLEDAI	-	-	0.308	0.003
Oxidative DNA damage marker (plasma 8-OHdG (ng/mL) (n= 93))	0.012	0.907	-0.107	0.308
Leukocyte mtDNA copy number (n= 93)	-0.197	0.058	0.015	0.889
Plasma cytokines (pg/mL)				
IL-10 (n= 93)	0.185	0.076	0.049	0.644
IFN-α (n= 93)	0.224	0.031	0.414	< 0.001
IL-23 (n= 93)	0.127	0.226	0.236	0.023
IFN-γ (n= 90)	0.186	0.079	-0.061	0.567
Plasma chemokines (pg/mL)				
IP-10 (n= 93)	0.230	0.026	0.396	<0.001
MCP-1 (n= 93)	0.185	0.075	0.186	0.075

SLE, Systemic lupus erythematosus; SLEDAI, SLE disease activity index

Table 3. Plasma levels of cytokines/chemokines and their relationships to plasma levels of 8-OHdG and mtDNA copy numbers in leukocytes of SLE patients

	Correlation to plasma level of 8-OHdG (n=93)		Correlation to relative leukocyte mtDNA copy number (n=93)	
Clinical and biological parameters	Spearman's coefficient (ρ)	*p*-value	Spearman's coefficient (ρ)	*p*-value
SLEDAI (n= 93)	0.012	0.907	-0.197	0.058
Anti-dsDNA antibodies (mg/dL) (n= 92)	-0.107	0.308	0.015	0.889
Leukocyte mtDNA copy number (n= 93)	-0.324	0.002	-	-
Plasma 8-OHdG (ng/mL)(n= 93)	-	-	-0.324	0.002
Plasma cytokines (pg/mL)				
IL-10 (n= 93)	0.254	0.014	-0.065	0.536
IFN-α (n= 93)	-0.102	0.329	-0.022	0.836
IL-23(n= 93)	0.357	<0.001	-0.374	<0.001
IFN-γ (n= 90)	0.435	<0.001	-0.395	<0.001
Plasma chemokines (pg/mL)				
IP-10 (n= 93)	0.059	0.575	-0.236	0.023
MCP-1 (n= 93)	0.322	0.002	-0.312	0.002

Table 4. Differences in clinical and biological parameters between the SLE patients with and without 4977 bp deletion of mtDNA in leukocytes

	4977 bp deletion of mtDNA in leukocytes		*p*-value
Clinical and biological parameters (Case number=HCs, SLE)	**No**	**Yes**	**Mann-Whitney *U* test**
SLEDAI (n= 67, 26)	9.7±6.4	10.4±7.1	0.763
Anti-dsDNA antibodies (mg/dL) (n= 66, 26)	108.8±121.4	154.6±149.6	0.135
Plasma 8-OHdG (ng/mL) (n= 67, 26)	0.252±0.139	0.233±0.085	0.851
Leukocyte mtDNA			
Relative mtDNA copy number (n= 67, 26)	0.193±0.119	0.234±0.090	0.011
Plasma cytokines (pg/mL)			
IL-10 (n= 67, 26)	4.247±6.877	2.282±3.287	0.009
IFN-α (n= 67, 26)	5.743±7.375	4.438±7.654	0.451
IL-23 (n= 67, 26)	133.9±56.8	151.4±52.2	0.183
IFN-γ (n= 64, 26)	1.770±1.647	1.490±0.566	0.996
Plasma chemokines (pg/mL)			
IP-10 (n= 67, 26)	36.5±33.4	39.7±34.2	0.504
MCP-1 (n= 67, 26)	48.2±30.9	46.1±21.1	0.857

Table 5. Decreases in plasma levels of cytokines/chemokines in 5 SLE patients after Rituximab infusion

	Before treatment (Mean±S.D.)	After treatment (Mean±S.D.)	*p*-value (Wilcoxon signed rank test)
Plasma cytokines (pg/mL)			
IL-10	2.370±1.974	1.286±0.504	0.080
IFN-α	3.726±1.788	2.852±1.179	0.080
IL-23	210.9±31.1	157.8±57.3	0.225
IFN-γ	4.778±4.452	3.510±2.954	0.144
Plasma chemokines (pg/mL)			
IP-10	51.9±24.6	34.2±10.3	0.138
MCP-1	78.2±28.1	47.4±7.6	0.043

4.6. Changes in plasma levels of cytokines/chemokines in SLE patients with or without LN and/or under dialysis

Compared to the 54 SLE patients without LN, the 39 patients with LN had higher plasma levels of IL-23 (p=0.039) and MCP-1 (p=0.015) (Table 6). Interestingly, we noted that, from SLE patients without LN (n=54) to SLE patients with LN without dialysis (n=35) and SLE patients with LN under dialysis (n=5), there was a steady increase in plasma levels of IL-23 (p=0.009) and MCP-1 (p=0.015) (Table 6, Figure 1).

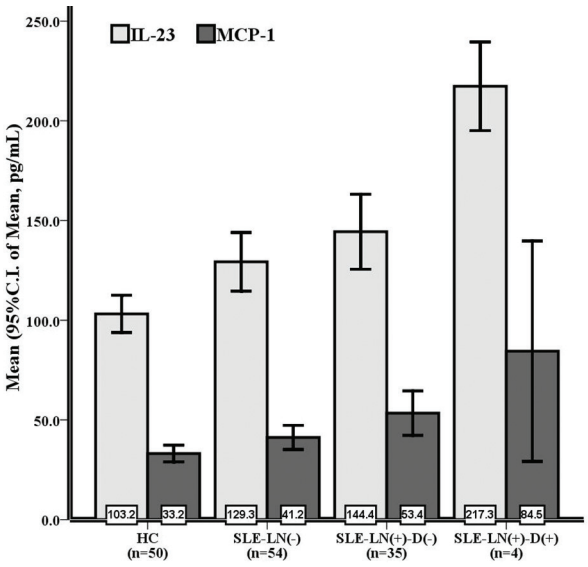

Figure 1. Compared to the 50 HCs, 54 LN (-) patients, 35 LN (+) patients without dialysis and 4 LN (+) patients under dialysis, showed a stepwise increase in plasma levels of IL-23 and MCP-1 (both $p<0.0.01$, Kruskal-Wallis *H* test). The heights of gray and black bars denote the mean plasma levels of IL-23 and MCP-1, respectively, which are also shown in boxes in the lower margin of individual bars. HCs, healthy controls; SLE, systemic lupus erythematosus; LN, lupus nephritis; D, dialysis.

5. DISCUSSION

In this study, we demonstrated that SLE patients had higher levels of plasma pro-inflammatory or anti-inflammatory cytokines (IL-10, IFN-α, IL-23, IFN-γ) and chemokines (IP-10 and MCP-1) than did the HCs (Table 1). Higher plasma levels of cytokines and chemokines were related to higher scores of SLEDAI (IFN-α, IP-10) or higher titers of anti-dsDNA antibody (IFN-α, IL-23 and IP-10) in SLE patients (Table 2). These findings are consistent with the previous reports (2-6). Therefore, we suggest that fluctuation of these cytokines and/or chemokines may be involved in the pathogenesis and progression of SLE.

Rituximab, a monoclonal antibody that depletes B cells, is effective to suppress many recalcitrant lupus manifestations (35). We demonstrated that MCP-1 was decreased after the administration of rituximab (Table 5). Blood samples were collected before the treatment protocol and two weeks after the first treatment and before the second treatment. Thus, the possible effect of pre-medication of methylprednisolone could be excluded. MCP-1 could recruit monocytes, memory T cells, and dendritic cells to the sites of inflammation (36, 37). Based on our preliminary results, we conclude that the inhibition of B cells by rituximab may result in the suppression of MCP-1 production and thus the amelioration of pro-inflammatory reaction.

IP-10 was also high in our patients with high disease activity, indicated by high score of SLEDAI or high titer of anti-dsDNA antibody (Table 2). This is conceivable because IP-10 (also known as CXCL-10) is a chemoattractant for monocytes/macrophages, T cells, natural killer cells and dendritic cells (38, 39). It can promote T cell adhesion to endothelial cells, thus create an environment that favors the autoimmune reaction. Interestingly, IP-10 was also high in patients with low leukocyte mtDNA copy numbers, and the degree of low leukocyte mtDNA copy number was correlated with a higher plasma 8-OHdG (Table 3). This may imply that increased oxidative stress as well as dysfunctional mitochondria predisposes to the increased pro-inflammatory chemokines such as IP-10 that in turn causes autoimmune reaction. Due to limited case number and analysis of only selected cytokines and chemokines in the present study, these scenarios cannot be completely established and require validation by further longitudinal studies. However, these cytokines or chemokines may be potential novel biomarkers to monitor the fluctuation of disease activity as well as treatment response of SLE patients.

Many pro-inflammatory/anti-inflammatory cytokines and chemokines have been known to contribute to the impaired immune reactions in SLE as well as the initiation and progression of LN (40, 41). However, conflicting data exist in the literature regarding the stimulatory or inhibitory roles of these molecules in the autoimmune processes. It is thus unclear as to whether the alterations of cytokines/chemokines contribute to the heterogeneous characteristics of the clinical conditions of SLE. Although not all the analyses in this study yielded significant results for LN, we observed a stepwise increase in plasma levels of IL-23 and MCP-1 when the diseases worsened (from HCs through SLE patients without LN, those with LN but without dialysis and finally to those with LN and under dialysis, as shown in Figure 1 and Table 6. IL-23 is a potent pro-inflammatory cytokine excreted mainly by macrophages and has been implicated in the pathogenesis of LN (42-44). In the present study, we found that IL-23 was high in SLE patients with active LN (Table 6), high titers of dsDNA antibodies (Table 2), high plasma levels of 8-OHdG and low copy numbers of leukocyte mtDNA (Table 3). These findings suggest an interrelationship among IL-23, disease activity and oxidative stress in SLE. Since IL-23 is a preceding pro-inflammatory cytokine that promote the excretion of IL-17 responsible for various inflammatory and autoimmune reaction (45, 46), the finding in the present study indicates that IL-23 can be used as a sensitive biomarker to monitor lupus activity. On the other hand, MCP-1, an inflammation trigger (36, 37), was found high in urine of patients with LN and could be regarded as a potential biomarker for predicting the severity of LN as well as the likelihood of subsequent dialysis (20). Although we measured the plasma MCP-1 instead of urine MCP-1, this particular finding deserves further validation in the future. In addition, as it is an important stimulator of dendritic

Table 6. Changes in plasma levels of cytokines/chemokines in SLE patients with or without LN and dialysis

Subject	LN(-) (n=54)	LN(+)(n=39)			*p*-value	
Cytokines/ chemokines (Mean±S.D.)	LN(-) (n=54)	LN(+)(n=39)	Without dialysis (n=35)	With dialysis (n=4)	Mann-Whitney *U* test*	Kruskal-Wallis *H* test**
Continuous model						
Plasma cytokines (pg/mL)						
IL-10 (n= 54, 39, 35, 4)	2.863±2.676	4.853±8.877	5.127±9.317	2.458±2.407	0.165	0.357
IFN-α (n= 54, 39, 35, 4)	5.375±8.012	5.382±6.659	5.450±6.885	4.788±4.876	0.821	0.892
IL-23 (n= 54, 39, 35, 4)	129.3±53.8	151.9±56.6	144.4±54.7	217.3±14.0	0.039	0.009
IFN-γ (n= 53, 37, 33, 4)	1.711±1.716	1.658±0.868	1.623±0.902	1.945±0.484	0.221	0.196
Plasma chemokines (pg/mL)						
IP-10 (n= 54, 39, 35, 4)	36.6±34.4	38.5±32.4	39.0±33.2	34.0±28.2	0.785	0.920
MCP-1 (n= 54, 39, 35, 4)	41.2±22.2	56.6±33.6	53.4±32.4	84.5±34.7	0.015	0.015

SLE, systemic lupus erythematosus; LN, lupus nephritis, *Compared between LN (-) and LN (+) SLE patients, **Compared among LN (-), LN (+) without dialysis and LN (+) under dialysis in SLE patients

cells (including plasmacytoid dendritic cell, *p*DC) (36, 37), MCP-1 may indirectly augment autoimmune reaction either in *p*DC or B cells consecutively by triggering innate immune activation in these cells.

Recent studies suggest that oxidative damage plays a role in the pathogenesis of SLE (27, 47). The 8-OHdG is a common oxidative DNA damage marker, which was found elevated in blood cells of SLE or LN patients (26, 28). Concerning the intracellular oxidative DNA damage, mtDNA is more susceptible to oxidative damage than did nuclear DNA (48). Oxidative damage to mtDNA may cause mitochondrial dysfunction, and the defective mitochondria would produce more ROS and culminate in a vicious cycle. Thus, we focused on the alterations of mtDNA copy number and cellular oxidative DNA damage in leukocytes of SLE patients. Under low or moderate oxidative stress, an increase of mtDNA copy number might compensate for the damaged mtDNA to rescue mitochondrial function. Once the damages are accumulated beyond the threshold of tolerance, a decrease of mtDNA copy number or mitochondrial dysfunction might ensue (49). In this and our previous studies, we have demonstrated that the decrease in leukocyte mtDNA copy number was correlated with the increases in SLEDAI (ρ=-0.1.97, p=0.058) and the elevation in plasma level of 8-OHdG (ρ=-0.324, p=0.002, Table 3) (28, 29). Furthermore, we showed that higher plasma levels of some cytokines/chemokines were related to higher plasma 8-OHdG (IL-10, IL-23, IFN-γ, and MCP-1) or lower leukocyte mtDNA copy number (IL-23, INF-γ, IP-10 and MCP-1) (Table 3). On the contrary, leukocytes mtDNA of SLE patients harboring 4977 bp deletion have higher leukocyte mtDNA copy numbers (p=0.011) and lower plasma levels of IL-10 (p=0.009) than those without 4977 bp deletion of mtDNA. These dynamic alterations of mtDNA copy number in leukocytes suggest that mitochondrial dysfunction may play a role in the ROS production and oxidative damages in SLE. It was demonstrated that mitochondrial ROS may directly drive the production of pro-inflammatory cytokines (50). On the other hand, pro-inflammatory cytokines also influence mitochondrial ROS production. For example, IFN-γ is capable of up-regulating the expression of many nuclear genes encoding subunits that constitute the mitochondrial electron transport chain and inducing mitochondrial ROS by activation of estrogen-related receptor α and coactivator peroxisomeproliferator-activated receptor γ coactivator-1 β (PGC1β) (51). Previous studies also revealed that persistent mitochondrial hyperpolarization in SLE increased necrosis of T cells, which might initiate a pro-inflammatory state, activation of dendritic cells and IFN-α production in SLE (52). It is possible that mitochondrial dysfunction contributes to excess ROS production, which disturbs redox homeostasis and modulates the cytokines/chemokines secretion, resulting in tissue/organ inflammation and initiation of the immune cascades as well as autoimmune damage in SLE (24). However, there might be complex interactions among these factors to form a vicious cycle and subsequently break the immune tolerance in SLE patients.

In conclusion, elevated plasma levels of IL-10, IFN-α, IL-23, IFN-γ, IP-10 and MCP-1 may participate in the pathogenesis and progression of SLE and some of these cytokines and chemokines are associated with the disease activity, anti-dsDNA levels, degree of oxidative DNA damage and mtDNA alterations in the leukocytes of SLE patients. Furthermore, rituximab, targeting selectively to B cells, might modulate the expression levels of these cytokines and chemokines and improve the symptoms in SLE patients.

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