

行政院國家科學委員會專題研究計畫 成果報告

全身性紅斑狼瘡患者單核性細胞的異常氧化還原狀態與其 免疫反應低下之間關係的研究(3/3) 研究成果報告(完整版)

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行政院國家科學委員會補助專題研究計畫 成果報告
 期中進度報告

全身性紅斑狼瘡患者單核性細胞的異常氧化還原狀態與其免疫反應

下之間關係的研究

(Study on the relationship of altered redox state and mononuclear cell
huporesponsiveness in patients with systemic lupus erythematosus)

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中文關鍵詞：氧化還原狀態；還原態的谷胱甘肽；谷胱甘肽過氧化酶；谷胱甘肽還原酶；CD4 陽性 T 淋巴球；多型核中性球；抗雙股 DNA 自體抗體；全身性紅斑狼瘡

中文摘要：

抗雙股 DNA 自體抗體會障害全身性紅斑狼瘡患者的單核性細胞(mononuclear cells, MNC)及多型核中性球(polymorphonuclear neutrophils, PMN)的許多活性。有報告指出狼瘡患者體內氧化壓力的增加會紊亂很多免疫細胞的功能。在本年度的計劃中,我們比較 CD4 陽性 T 淋巴球及 PMN 細胞內還原態的谷胱甘肽(reduced form glutathione,GSH)的濃度,及谷胱甘肽過氧化酶(glutathione peroxidase, GSH-Px)及谷胱甘肽還原酶(glutathione reductase, GSSR)的活性。我們發現正常人的 CD4+T cell 比 PMN 含有更高濃度的 GSH 濃度及 GSH-Px 活性。其中很有興趣的是 CD4+T cell 主要是表現 GSH-Px isoform 中的 dimer 及 tetramer。而 PMN 則是表現 monomer 及 timer。SLE-CD4+T cell 及 SLE-PMN 細胞內所含的 GSH 濃度及 GSH-Px 活性遠比正常細胞為低,但是 GSSR 的活性兩組則無顯著差異。我們也由活動性全身性紅斑狼瘡患者的血清中純化出抗雙股 DNA 自體抗體(anti-dsDNA autoantibodies)。再將 25-100IU/ml anti-dsDNA 與正常人的 CD4+T cell 培養 24-48 小時之後,發現正常人的 CD4+T cell 的 GSH-Px 活性會減少,乃因增加細胞凋亡之故。另外,SLE 細胞內 redox state 的降低與使用抗紅斑狼瘡藥物無關,因為有八位未經治療的狼瘡患者的 CD4+T cell 及 PMN 亦呈現降低的趨勢。這些結果顯示活動性全身性紅斑狼瘡患者的血清中的 anti-dsDNA autoantibodies 會抑制 CD4+T cell 的 GSH-Px 活性,而障害其免疫功能。

英文關鍵詞：redox state; reduced-form glutathione; glutathione peroxidase; glutathione reductase; CD4+T cells; polymorphonuclear neutrophils; anti-dsDNA autoantibodies; systemic lupus erythematosus

ABSTRACT

Anti-double stranded DNA antibodies (anti-dsDNA) impaired a number of functions of mononuclear cells (MNC) and polymorphonuclear neutrophils (PMN) in patients with systemic lupus erythematosus (SLE). Increased oxidative stress was found in SLE that deranged different cell functions. In this study, we compared reduced-form glutathione (GSH) levels, and GSH peroxidase (GSH-Px) and GSH reductase (GSSG-R) activities of CD4⁺T and PMN between active SLE and normal groups. We found normal CD4⁺T contained GSH level and GSH-Px activity much higher than PMN. Interestingly, CD4⁺T cells mainly expressed dimer (50kDa) and tetramer (100kDa) whereas PMN mainly expressed monomer (25kDa) and trimer (75kDa) of GSH-Px isoforms. The GSH levels in both SLE-CD4⁺T and SLE-PMN was significantly lower than normal counterparts. The GSH-Px activity in SLE-CD4⁺T was also lower than normal CD4⁺T. However, GSSG-R activity of CD4⁺T and PMN in both groups was not different. Anti-dsDNA autoantibodies (25-100IU/ml) purified from active SLE sera suppressed GSH-Px activity of normal CD4⁺T after 24h incubation via an apoptotic mechanism. The decreased redox capacity in SLE cells was not relevant to anti-SLE medications because the same tendency was also observed in 8 untreated active SLE. These results suggest that anti-dsDNA suppress GSH-Px activity of CD4⁺T that impair immune functions in patients with active SLE.

Key words: anti-dsDNA autoantibodies; reduced-form glutathione; glutathione peroxidase; CD4⁺T lymphocyte; systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is an archetype of systemic autoimmune disorder characterized by the presence of different autoantibodies in the serum. Among these autoantibodies, the titer of anti-double stranded DNA antibodies (anti-dsDNA) is correlated with disease activity, particularly the lupus nephritis.¹ Functionally, anti-dsDNA can suppress mitogen-activated T lymphocyte proliferation but enhance immunoglobulin synthesis by B cells resembling immune dysfunctions in SLE patients *in vivo*.² Recently, we demonstrated that telomere shortening was accelerated in both MNC and PMN of SLE patients.³ It is conceivable that telomere shortening during human aging was accelerated by oxidative stress.⁴⁻⁶ Glutathione-dependent anti-oxidants play a key role in the prevention of telomere shortening in different cells.⁷ Many authors found that enhanced oxidative stress in patients with SLE increased serum protein oxidation and disease activity of these patients.⁸⁻¹¹ In addition, changes in intracellular reduction-oxidation (redox) state affect lymphocyte proliferation and NK-mediated cytotoxicity.¹² Glutaredoxin-glutathione system composes of NADPH, reduced-form glutathione (GSH), the flavoprotein glutathione peroxidase (GSH-Px), and glutathione reductase (GSSG-Rx) that are the key players in redox regulation of the cells.¹³ Because glutathione is present in all animal cells with high concentration, it acts as the most important endogenous modulating molecule for redox, cell proliferation, DNA synthesis, immune response, and arachidonic acid metabolism.^{14,15} Based on these facts, we hypothesize that defective intracellular redox status in immune-related cells of SLE contributes to florid immune dysfunctions of SLE. In the present study, we compared the redox capacity of CD4⁺T and PMN between normal and active SLE. The

role of anti-dsDNA purified from active SLE sera on redox state of normal CD4⁺T cell was also investigated.

PATIENTS AND METHODS

Patients and controls

Patients fulfilling 1982 ACR Revised Classification Criteria of SLE were enrolled in the present study. Age- and sex-matched normal individuals were the control. All of the SLE patients were disease active [SLEDAI score >6 plus anti-dsDNA titer >50 IU/ml (normal <12 IU/ml)] judging from SLEDAI.¹⁶ The daily medications (mean±s.d.) of these 23 patients were 17.6±10.7mg of prednisolone, 200-400mg of hydroxychloroquine, with/without 71.6±22.7mg of azathioprine. Besides, 8 active SLE patients without receiving anti-SLE medications were recruited for comparing redox capacity before and after effective treatment with immunosuppressants including glucocorticoids, hydroxychloroquine and/or azathioprine for 2 weeks. The study was approved by the Institutional Review Board of National Taiwan University Hospital (Taipei, Taiwan) and informed consent was obtained from each participant.

Isolation of CD4⁺T cells and PMN from peripheral blood

The purification of PMN and CD4⁺T cells was followed by the method described in our previous report.¹⁷ via positively selected by specific antibody (anti-CD₁₆ for PMN and anti-CD₄ for CD4⁺T)-conjugated magnetic beads and AutoMACs (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of PMN was confirmed >95% by flow cytometry after staining with FITC-conjugated monoclonal anti-CD₁₆ antibody (Sigma-Aldrich). The purity of CD4⁺T population was >98%, confirmed by flow cytometry after staining with FITC-conjugated monoclonal anti-CD₄ antibody (Sigma-Aldrich). The viability of PMN and CD4⁺T was greater than 95% confirmed by trypan blue dye exclusion. The concentration of both cells was adjusted to 5×10⁶ cells/ml except particularly mentioned.

Measurement of GSH concentration

Soluble cellular GSH concentration was measured by BIOXYTECH GSH-400TM Colorimetric Assay Kit (OXIS International Inc. Portland, OR, USA). The detailed procedures are described in the manufacturer's instruction booklet. Briefly, the concentration of CD4⁺T and PMN was adjusted to 1×10⁷ cells/ml and the cells were sonicated immediately at 100w for 60sec. The GSH measurements include only soluble cellular form (GSH + glutathione disulfide). The detection limit of the assay is 0.5µm/ml.

Determination of GSH-Px activity

We used BIOXYTECH GPx-340TM Colorimetric Assay Kit (OXIS International Inc.) to measure GSH-Px enzymatic activity of CD4⁺T and PMN cell lysate. One milliunit (mU) of GSH-Px activity is defined as the activity that catalyzes the oxidation of 1 nmol NADPH/ml/min using an extinction molar coefficient of 6.22 × 10⁶ M⁻¹ cm⁻¹ for NADPH.

Determination of GSSG-R activity

We used BIOXYTECH GR-340TM Colorimetric Assay Kit (OXIS International Inc.) to measure GSSG-R activity of CD4⁺T and PMN cell lysate. The definition of mU of GSSG-R enzymatic activity was of the same as in GSH-Px activity.

Detection of GSH-Px mRNA expression in CD4⁺T and PMN by RT-PCR

Total cellular RNA was extracted from 1×10^7 /ml CD4⁺T or PMN using RNeasy Mini kit (Qiagen GmbH, Hilden, Germany). Detection of GSH-Px mRNA expression in CD4⁺T and PMN was conducted by RT-PCR reported in our previous study.¹⁸ The forward and reverse pair primers for human GSH-PX and glyceraldehyde-3-phosphate dehydrogenase (G3PDH, as internal control) were shown below.

GSH-Px: 5'-GGG GCC TGG TGG TGC TCG GCT-3' (sense)

5'-CAA TGG TCT GGA AGC GGC GGC-3' (anti-sense)

G3PDH: 5'-ACC ACA GTC CAT GCC ATC AC-3' (sense)

5'-TCC ACC ACC CTG TTG CTG TA-3' (anti-sense)

The amplified PCR products were 354bp for GSH-Px and 452bp for G3PDH

Western blot analysis of GSH-Px isomers

CD4⁺T and PMN at a concentration of 5×10^6 /ml were lysed and electrophoresed in 10% SDS-PAGE. The expression of GSH-Px isomers in cell lysate was detected by monoclonal anti-human GSH-Px antibody (MBL International, Woburn, MA, USA) and enhanced chemiluminescence protein detection kit (Amersham International Plc. Chalfont, Buckinghamshire, UK) after electro-transfer to nitrocellulose membrane.

Affinity-purified polyclonal anti-dsDNA autoantibodies from the sera of patients with active SLE

We prepared dsDNA-conjugated Sepharose 4B affinity column to purify polyclonal anti-dsDNA autoantibodies from active SLE sera as in our previous report.¹⁹ The dsDNA binding capacity of the purified antibodies was assayed by an anti-dsDNA ELISA kit (BioHyTech Co., Ramat Gan, Israel).

Morphological observation of cell apoptosis after incubation with anti-dsDNA autoantibodies

CD4⁺T or PMN at a concentration of 2×10^6 cells/ml were incubated with different concentrations of affinity-purified anti-dsDNA from 25 to 100 IU/ml for 24, 72 and 120h. After incubation, the cell suspension was cytospinned, fixed in 2% paraformaldehyde solution, and stained with H & E solution. The morphology of the cells in apoptosis with karyopyknotic/karyorrhexic nuclei and cell volume shrinkage were observed under light microscopic observation.

Statistical analysis

The results in the whole study are represented by mean+s.d. The statistical significance was assessed by non-parametric Wilcoxon signed rank test.

RESULTS

Comparison of redox capacity in plasma and different normal blood cells

Glutathione is one of the most important endogenous molecules for modulating redox state in all animal cells.¹³⁻¹⁵ At first, we measured the intracellular GSH levels in different blood cells including CD4⁺T, PMN, RBC, platelets and plasma from normal individuals. We noticed that GSH is most abundant in the plasma and CD4⁺T followed by the order of PMN > RBC > platelet (Fig 1-A). Biologically, the reduced-form GSH is generated by the action of GSH-Px after reduction of the oxidative-form GSSG.²⁰ We found that GSH-Px enzymatic activity was much higher in CD4⁺T and plasma compared to PMN, platelets and RBC (Fig.1-B). Although the GSH-Px mRNA expression in normal CD4⁺T and PMN was not different (Fig.1-C), the composition of GSH-Px isomers in the two cells is quite different. As shown in Fig.1-D, CD4⁺T expressed mainly dimer (50kDa) and tetramer (100kDa) rather than monomer (25kDa) and trimer (75kDa) of GSH-Px isoforms. By contrast, PMN expressed mainly monomer and trimer, rather than dimer and tetramer of GSH-Px isoforms. These results are compatible with Misso et al.²¹ that normal neutrophils contain mainly monomer and trimer of GSH-Px isoforms. Since the GSH-Px activity of CD4⁺T is much higher than PMN, it seems possible that anti-oxidation activity of dimer and tetramer isoforms of GSH-Px is higher than monomer and trimer.

Comparison of redox capacity (GSH levels, GSH-Px and GSSG-R activity) of CD4⁺T and PMN between normal and SLE patients

The reduced-form GSH levels in SLE-CD4⁺T and SLE-PMN (Fig.2-A), and the GSH-Px enzymatic activity in SLE-CD4⁺T are significantly lower than their normal counterparts (Fig.2-B). However, the composition of the four GSH-Px isomers in SLE cells is not different from the normal counterparts (Fig.2-C). The GSSG-R activity, another redox modulating enzyme containing active dithiol moieties for protection and repair of protein sulfhydryls in an oxidative stress situation, was also not different in normal and SLE cells²⁰ (Fig.2-D). For determining whether decreased redox capacity in SLE cells is derived from anti-SLE medications (glucocorticoids, hydroxychloroquine and/or azathioprine) or disease activity *per se* in active SLE, the redox capacity of CD4⁺T and PMN was measured in 8 untreated active SLE patients. We found redox capacity in these non-treated active SLE cells was decreased similar to the treated group (Fig.3-A and B). In addition, we noted that the decreased redox capacity in the untreated active SLE group elevated after effective treatment (Fig.3-C). These results suggest that the reduced redox capacity in active SLE-CD4⁺T and -PMN is due to lupus disease activity, rather than the side effects of anti-SLE medications.

The effects of anti-dsDNA autoantibodies on GSH-Px activity and cell apoptosis of normal CD4⁺T and PMN

Polyclonal anti-dsDNA (25~100IU/ml) purified from active SLE sera significantly suppressed GSH-Px activity of normal CD4⁺T, but not PMN, after 24h incubation (Fig.4-A). These unique autoantibodies also enhanced apoptosis of normal CD4⁺T after 72h incubation (Fig.4-B) and PMN after 8h incubation (Fig.4-C). These results suggest that polyclonal anti-dsDNA is one of the pathological factors responsible for decreased redox capacity.

DISCUSSION

Defective cellular and innate immunities are documented in patients with active SLE patients.²² These defects attribute to premature senescence of immune cells through telomere shortening from different causes.^{3,23} The major factor leading to the premature senescence of SLE immune cells is believed from increased oxidative stress.^{4,7} Oikawa et al.^{4,6} demonstrated that H₂O₂ plus Cu²⁺ may induce the production of 8-oxo-dihydro- 2'-deoxyguanosine that causes predominant DNA damage at the 5' site of 5'-GGG-3' in the telomere sequence. Honda et al.⁵ showed that telomere shortening in the cells was the results of increased accumulation of single-stranded breaks in telomeric DNA. Bae et al.²⁴ further demonstrated that plasma superoxide dismutase and glutathione peroxidase activities were significantly lower in SLE than in controls whereas the plasma dialdehyde level was higher than controls. In addition, the antioxidant intake by SLE patients is also decreased. Many authors demonstrated that reactive oxygen species at a concentration much lower than require to inflict oxidative damage may impair cell signaling and gene expression.²⁵⁻²⁷ These redox-sensitive target molecules include transcription factors NF-κB, AP-1, NF-AT, PAX-8,²⁶⁻²⁸ GGG triplet sequence of telomere,⁴ and pyridine nucleotide of mitochondria.²³ Liu et al.²³ further demonstrated that reactive oxygen species are the key mediators leading to mitochondrial dysfunctions, telomere attrition and cell apoptosis. In our preliminary results, we found mitochondrial DNA 4977 deletion is more in SLE-CD4⁺T cells (18.2%) than normal-CD4⁺T cells (4.3%) (data not shown). Accordingly, it is worthy to evaluate the intracellular redox state of CD4⁺T and PMN and the role of anti-dsDNA autoantibodies on redox state in patients with active SLE. Several original observations were derived from the present study: (a) Decreased intracellular GSH levels in CD4⁺T and PMN (Fig.2-A), and decreased GSH-Px activity in CD4⁺T (Fig.2-B) of active SLE. (b) No difference in GSSG-R activity of CD4⁺T and PMN between normal and SLE patients (Fig.2-D). (c) No difference in the expression of GSH-Px isomers between normal and SLE cells that dimer and tetramer are major isoforms in CD4⁺T whereas monomer and trimer are major isoforms in PMN (Fig.2-C). (d) Anti-dsDNA antibodies suppress GSH-Px activity of normal CD4⁺T, but not PMN, via enhancing cell apoptosis (Fig.4-A, B & C). Because GSH-Px activity in PMN is quite low (Fig.1-B) and the life span of PMN is very short (6-8h *in vivo*), it is hard to get a significant difference between normal and SLE-PMN despite difference of intracellular GSH between them. These findings may suggest that increased oxidative stress in SLE immune cells by different causes leading to cellular and innate immune dysfunctions in the patients. The presence of anti-dsDNA in SLE serum is one of the pathological factors in reducing redox capacity of CD4⁺T and PMN. The affections of anti-dsDNA on reactive oxygen species production by normal CD4⁺T and PMN are now under investigation. For further clarifying the roles of anti-SLE medications on reduced redox capacity, 8 untreated active SLE patients were studied. We found a similar tendency of decreased redox capacity in these non-treated active SLE patients as in treated patients (Fig.3-A and B) although glucocorticoids facilitate protein degradation by a catabolic effect and immunosuppressants potently inhibit the protein and DNA synthesis of redox-related molecules. Besides, we noticed the abnormal redox capacity restored gradually to normal levels after effective treatment (Fig.3-C). These results indicate that SLE disease activity *per se*, rather than anti-SLE medications, reduced CD4⁺T and PMN redox capacity via inhibitory effect of anti-dsDNA autoantibodies on GSH-Px activity. These results are quite compatible with

Morgan et al.¹¹ that increase serum oxidation was correlated with elevation of anti-dsDNA titer. However, further investigation is necessary to confirm it. Reduced redox capacity seems to be a pivotal factor for diverse cellular and innate immunity defects. Many biological molecules critically important for cell signaling and gene expression, such as ion-transporters, mitochondria-mediated Ca²⁺ metabolism, cytokine production (IL-1, IL-2, IL-6, IL-8, TGF- β , TNF- α), and cell growth-related genes are sensitive to reactive oxygen species.^{25,29-34} Clinically, abnormal redox state had been reported in the body fluid and blood cells of some diseases, such as rheumatoid arthritis,^{20, 35,36} cardiovascular disorders^{37,38} and atopic asthma,^{21,39} owing to glutathione reductase/glutathione peroxidase imbalance. In the present study, we originally demonstrate that impaired intracellular redox capacity in CD4⁺T and PMN in patients with active SLE renders patients with immune hyporesponsiveness to mitogens, antigens and allogeneic cells.⁴⁰⁻⁴³ The manipulation of cell redox state may provide an alternative strategy for improving immune responses of some forms of cancer¹⁵ and also immune hyporesponsive- ness states. Tarp et al.²⁰ reported that the abnormal redox state in severe rheumatoid arthritis was correlated with decreased selenium concentration. However, the supplementation of the element failed to restore anti-oxidative capacity of the patients. In contrast, Maurice et al.³⁵ demonstrated that altered redox state is responsible for the hyporesponsiveness of rheumatoid synovial T cells. The supplement of GSH with glutathione precursor, N-acetyl-L-cysteine, enhances mitogen-induced proliferative responses and IL-2 production of synovial T lymphocytes. In contrast, patients with atopic asthma,^{21,39} ischemic heart disease³⁷ or stroke³⁸ do not exhibit distinct immune hyporesponsiveness to external stimuli, although reduced redox capacity was found in these diseases. Accordingly, it is possible that factors other than imbalanced redox capacity involve in T cell hyporesponsiveness in some specified autoimmune disorders including RA and SLE. Whether immune hyporesponsiveness in SLE can be restored by supplement with glutathione or its precursors is now under investigation.

It is conceivable that anti-dsDNA autoantibodies exert diverse immunological effects on patients with active SLE.⁴⁴⁻⁴⁷ In this study, we found a novel effect of anti-dsDNA in suppressing GSH-Px activity of CD4⁺T cells via accelerated cell apoptosis. We deduce that the GSH-Px-suppressing activity of anti-dsDNA auto-antibodies may derive from their activation-induced cell death as reported in our previous study.^{2,3,5,7} However, the real molecular basis of this novel activity needs further investigation.

In conclusion, we are the first authors to demonstrate impaired intracellular redox capacity of CD4⁺T and PMN in active SLE patients. Anti-dsDNA suppresses the GSH-Px activity of CD4⁺T and therefore and plays a role in reducing redox capacity of patients with active SLE.

REFERENCES

- 1 Swaak AJG, Aarden LA, Staius VAN, Feltkamps T E W. Anti-dsDNA and complement profiles as prognostic guides in systemic lupus erythematosus. *Arthritis Rheum* 1979; 22: 226-235.
- 2 Yu C-L, Chang K-L, Chiu C-C, Chiang BN, Han S-H., Wang S-R. Alteration of the mitogenic responses of mononuclear cells by anti-dsDNA antibodies resembling immune disorders in patients with systemic lupus erythematosus. *Scand J Rheumatol* 1989; 18: 265-276.
- 3 Wu C-H, Hsieh S-C, Li K-J, Lu M-C, Yu C-L. Premature telomere shortening in polymorphonuclear neutrophils from patients with systemic lupus erythematosus is related to the lupus disease activity. *Lupus* (2007) (in press).
- 4 Oikawa S, Kawanishi S. Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. *FEBS Lett* 1999; 25: 365-368.
- 5 Honda S, Hjelmeland LM, Handa JT. Oxidative stress-induced single-strand breaks in chromosomal telomeres of human retinal pigment epithelial cells *in vitro*. *Invest Ophthalmol Vis Sci* 2001; 42: 2139-2144.
- 6 Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. *Ann NY Acad Sci* 2004; 1019: 278-284.
- 7 Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci* 2004; 117: 2417-2426.
- 8 Ames PR, Alves J, Murat I, Isenberg DA, Nouroozadeh J. Oxidative stress in systemic lupus erythematosus and allied conditions with vascular involvement. *Rheumatology* 1999; 38: 529-534.
- 9 Kurien BT, Scofield RH. Free radical mediated peroxidative damage in systemic lupus erythematosus. *Life Sci* 2003; 73: 1655-1666.
- 10 Frostegard J, Svenungsson E, Wu R et al. Lipid peroxidation is enhanced in patients with systemic lupus erythematosus and is associated with arterial and renal disease manifestations. *Arthritis Rheum* 2005; 52:192-200.
- 11 Morgan PE, Sturgess AD, Davies MJ. Increased levels of serum protein oxidation and correlation with disease activity in systemic lupus erythematosus. *Arthritis Rheum* 2005; 52: 2069-2079.
- 12 Viora M, Quaranta MG, Straface E, Vari R, Massella R, Malorni W. Redox imbalance and immune functions: opposite effects of oxidized low-density lipoproteins and N-acetylcysteine. *Immunology* 2001; 104: 431-458.
- 13 Holmgren A. Thioredoxin and glutaredoxin systems. *J Biol Chem* 1989; 264: 13963- 13966.
- 14 Holmgren A. Glutathione-dependent synthesis of deoxyribonucleotide. Characterization of the enzymatic mechanism of *Escherichia coli* glutaredoxin. *J Biol Chem* 1979; 254: 3672-3678.
- 15 Sen CK. Nutritional biochemistry of cellular glutathione. *J Nutr Biochem* 1997; 8: 660-672.
- 16 Bombardier C, Gladman DD, Urowitz MB et al. Derivation of the SLEDAI: A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-640.

- 17 Li K-J, Lu M-C, Hsieh S-C et al. Release of surface-expressed lactoferrin from polymorphonuclear neutrophils after contact with CD4⁺T cells and its modulation on Th1/Th2 cytokine production. *J Leukoc Biol* 2006; 80: 350-358.
- 18 Wu C-H, Hsieh S-C, Yu H-S et al. Anti-CD45 antibody enhances lipoxygenase pathway of human naïve mononuclear cells and cyclooxygenase pathway of neutrophils. *Inflamm Res* 2006; 55: 92-98.
- 19 Sun K-H, Liu W-T, Tsai C-Y, Tang S-J Yu C-L. Anti-dsDNA antibodies cross-react with ribosomal P proteins expressed on the surface of glomerular mesangial cells to exert a cytostatic effect. *Immunology* 1995, 85: 262-269.
- 20 Tarp U, Strengard-Pedersen K, Hansen JC, Thorling EB. Glutathione redox cycle enzymes and selenium in severe rheumatoid arthritis: lack of antioxidative response to selenium supplementation in polymorphonuclear leukocytes. *Ann Rheum Dis* 1992; 51: 1044-1049.
- 21 Misso NLA, Peroni DJ, Watkins DN, Stewart GA., Thompson PJ. Glutathione peroxidase activity and mRNA expression in eosinophils and neutrophils of asthmatic and non-asthmatic subjects. *J Leukoc Biol* 1998; 63: 124-130.
- 22 Tsokos GC, Liossis SN. Immune cell signaling defects in lupus: activation, anergy and death. *Immunol Today* 1999; 20:119-124.
- 23 Liu L, Trimarchi JR, Smith PJ, Keefe DL. Mitochondrial dysfunction leads to telomere attrition and genetic instability. *Aging Cell* 2000; 1: 40-46.
- 24 Bae S-C, Kim S-J, Sung M-K. Impaired antioxidant status and decreased diet intake of antioxidant in patients with systemic lupus erythematosus. *Rheumatol Int* 2000; 22: 238-243.
- 25 Sen SK. Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem Pharmacol* 1998; 55: 1747-1758.
- 26 Sen CK, Roy S, Packer L. Involvement of intracellular Ca²⁺ in oxidant-induced NF-κB activation. *FEBS Lett* 1996; 385: 58-62.
- 27 Sen SK, Packer L. Anti-oxidant and redox regulation of gene transcription. *FASEB J* 1996; 10: 709-720.
- 28 Kambe F, Nomura Y, Okamoto T, Seo H. Redox regulation of thyroid-transcription factors, Pax-8 and TTF-1, is involved in their increased DNA-binding activities by thyrotropin in rat thyroid FRTL-5 cells. *Mol Endocrinol* 1996; 10: 801-812.
- 29 Kourie JI. A redox O₂ sensor modulates the SR Ca²⁺ countercurrent through voltage and Ca²⁺-dependent Cl-channels. *Am J Physiol* 1997; 272: C324-C332.
- 30 Sen CK, Kolosova I, Hanninen O, Orlov SN. Inward potassium transport systems in skeletal muscle derived cells are highly sensitive to oxidant exposure. *Free Radic Biol Med* 1995; 18: 795-800.
- 31 Schenk H, Vogt M, Droge W, Schulze-Osthoff K. Thioredoxin as a potent costimulus of cytokine expression. *J Immunol* 1996; 156: 765-771.
- 32 Barcellos-Hoff MH, Dix TA. Redox-mediated activation of latent transforming growth factor-β1. *Mol Endocrinol* 1996; 10: 1077-1083.
- 33 Epsosito F, Cuccovillo F, Vanonic M et al. Redox mediated regulation of p21 expression involves a post-transcriptional mechanism and activation of the mitogen activated protein kinase pathway. *Eur J Biochem* 1997; 245: 730-737.
- 34 Irani K, Xia Y, Zweier JL et al. Mitogenic signaling mediated by oxidants in Ras-transformed

fibroblast. *Science* 1997; 275: 1649-1652.

- 35 Maurice MM, Nakamura, H, van der Voort EAM et al. Evidence for the role of an altered redox state in hyporesponsiveness of synovial T cells in rheumatoid arthritis. *J Immunol* 1997; 158:1458-1465.
- 36 Bazzichi I, Ciompi ML, Betti L et al. Impaired glutathione reductase activity and levels of collagenase and elastase in synovial fluid of rheumatoid arthritis. *Clin Exp Rheumatol* 2002; 20: 761-766.
- 37 Porter M, Pearson DJ, Suarez-Mendez VJ, Blann AD. Plasma, platelet and erythrocyte glutathione peroxidases as risk factors in ischaemic heart disease in man. *Clin Science* 1992; 83: 343-345.
- 38 Isshibashi N, Prokopenko O, Reuhl KR, Mirochnitchenko O. Inflammatory response and glutathione peroxidase in a model of stroke. *J Immunol* 2002; 168: 1926-1933.
- 39 Comhair SAA, Bhathena PR, Farver C, Thunnissen FBJM., Erzurum SC. Extracellular glutathione peroxidase induction in asthmatic lung: evidence for redox regulation of expression in human airway epithelial cells. *FASEB J* 2001; 15: 70-78
- 40 Landry M. Phagocyte function and cell-mediated immunity in systemic lupus erythematosus. *Arch Dermatol* 1977; 113: 147-154.
- 41 Hsieh S-C, Tsai C-Y, Sun K-H et al. Decreased spontaneous and lipopolysacchride stimulated production of interleukin-8 by polymorphonuclear neutrophils of patients with active systemic lupus erythematosus. *Clin Exp Rheumatol* 1994; 12: 627-633.
- 42 Malave I, Layrisse Z, Layrisse M. Dose-dependent hyporeactivity to phyto- hemmagglutinin in systemic lupus erythematosus. *Cell Immunol* 1975; 15: 231-236.
- 43 Kuntz M, Innes JB, Weksler M. The cellular basis of the impaired autologous mixed lymphocyte reaction in patients with systemic lupus erythematosus. *J Clin Invest* 1979; 63: 151-159.
- 44 Sun K-H, Yu C-L, Tang S-J, Sun G-H. Monoclonal anti-double stranded DNA autoantibody stimulates the expression and release of IL-1 β , IL-6, IL-8, IL-10 and TNF- α from normal human mononuclear cells involving lupus pathogenesis. *Immunology* 2000; 99: 352-360.
- 45 Hsieh S-C, Sun K-H, Tsai C-Y et al. Monoclonal anti-double stranded DNA antibody is a leukocyte-binding protein to up-regulate interleukin 8 gene expression and elicit apoptosis of normal human polymorphonuclear neutrophils. *Rheumatology* 2001; 40: 851-858.
- 46 Tsai, C-Y, Wu T-H, Sun K-H, Liao T-S, Lin W-M, Yu C-L. Polyclonal IgG anti-dsDNA antibodies exert cytotoxic effect on cultured rat mesangial cells by binding to cell membrane and augmenting apoptosis. *Scand J Rheumatol* 1993; 22: 162-171.
- 47 Yu C-L, Huang M-H, Tsai C-Y et al. The effect of human polyclonal anti-dsDNA autoantibodies on apoptotic gene expression in cultured rat glomerular mesangial cells. *Scand J Rheumatol* 1998; 27: 54-60.

FIGURE LEGENDS

- Fig.1: Comparison of intracellular reduced-form glutathione (GSH) levels, glutathione peroxidase (GSH-Px) activity, GSH-Px mRNA expression, and composition of GSH-Px isomers among CD4⁺T, polymorphonuclear neutrophils (PMN), red blood cells (RBC), platelets, and plasma from normal individuals (A) Intracellular GSH levels detected by commercial GSH Colorimetric Assay Kit. (B) GSH-Px activity detected by commercial GSH-Px Colorimetric Assay Kit. (C) GSH-Px mRNA expression in two different normal CD4⁺T and PMN by RT-PCR, lane 1: G3PDH (452bp, as internal control), lane 2: GSH-Px (354bp). The same experiment was also conducted in another two normal individuals with a similar tendency. (D) Different GSH-Px isomers distribution in normal CD4⁺T and PMN lysates (protein concentration 26mg/ml) detected by Western blot (2μl in lanes 1 and 4; 4μl in lanes 2 and 5; 10μl in lanes 3 and 6) probed by anti-human GSH-Px antibody. Four GSH-Px isomers are identified as monomer (25kDa), dimer (50kDa), trimer (75kDa) and tetramer (100kDa).
- Fig.2: Comparison of intracellular reduced-form GSH levels, glutathione peroxidase (GSH-Px) activity, distribution of GSH-Px isomers, and glutathione reductase (GSSG-R) activity in CD4⁺T and PMN lysates from normal and SLE patients. (A) Reduced-form GSH levels. (B) GSH-Px activity. (C) Western blot analysis of GSH-Px isomer distribution in two cases of CD4⁺T and PMN from normal and SLE patients. Normal and SLE-PMN express monomer (25kDa) and trimer (75kDa), rather than dimer (50kDa) and tetramer (100kDa). By contrast, reverse distribution of GSH-Px isomers was found in normal and SLE-CD4⁺T. Lane 1 and lane 2 are different cases of PMN. Lane 3 and lane 4 are different cases of CD4⁺T. (D) Intracellular glutathione reductase (GSSG-R) activity detected by commercial GSSG-R Colorimetric Assay Kit.
- Fig.3: Comparison of reduced-form GSH levels and GSH-Px enzymatic activity of CD4⁺T and PMN from 8 non-treated active SLE patients and well-matched normal individuals. (A) Intracellular GSH levels. (B) Intracellular GSH-Px enzymatic activity. (C) Intracellular GSH-Px enzymatic activity in CD4⁺T before and after effective treatments with glucocorticoids and immunosuppressants for 2 weeks. The connecting lines represent the same patient before and after treatment.
- Fig.4: The effects of polyclonal anti-dsDNA autoantibodies (anti-dsDNA) purified from active SLE sera on GSH-Px activity and cell apoptosis of normal CD4⁺T and PMN. (A) Effect of anti-dsDNA (100 IU/ml) and human non-specific IgG (10mg/ml) on GSH-Px activity of normal CD4⁺T and PMN after 24h incubation. (B) Anti-dsDNA (100IU/ml) increased CD4⁺T apoptosis than medium or non-specific human IgG (10mg/ml) after 72h and 120h incubation. (C) Anti- dsDNA (100IU/ml) increased PMN apoptosis than medium or non-specific human IgG (10mg/ml) after 8h incubation. The arrows indicate CD4⁺T and PMN with karyopyknotic/karyorrhexic nuclei, a typical change of cell apoptosis (original magnification 1000x in all pictures).

Fig.1

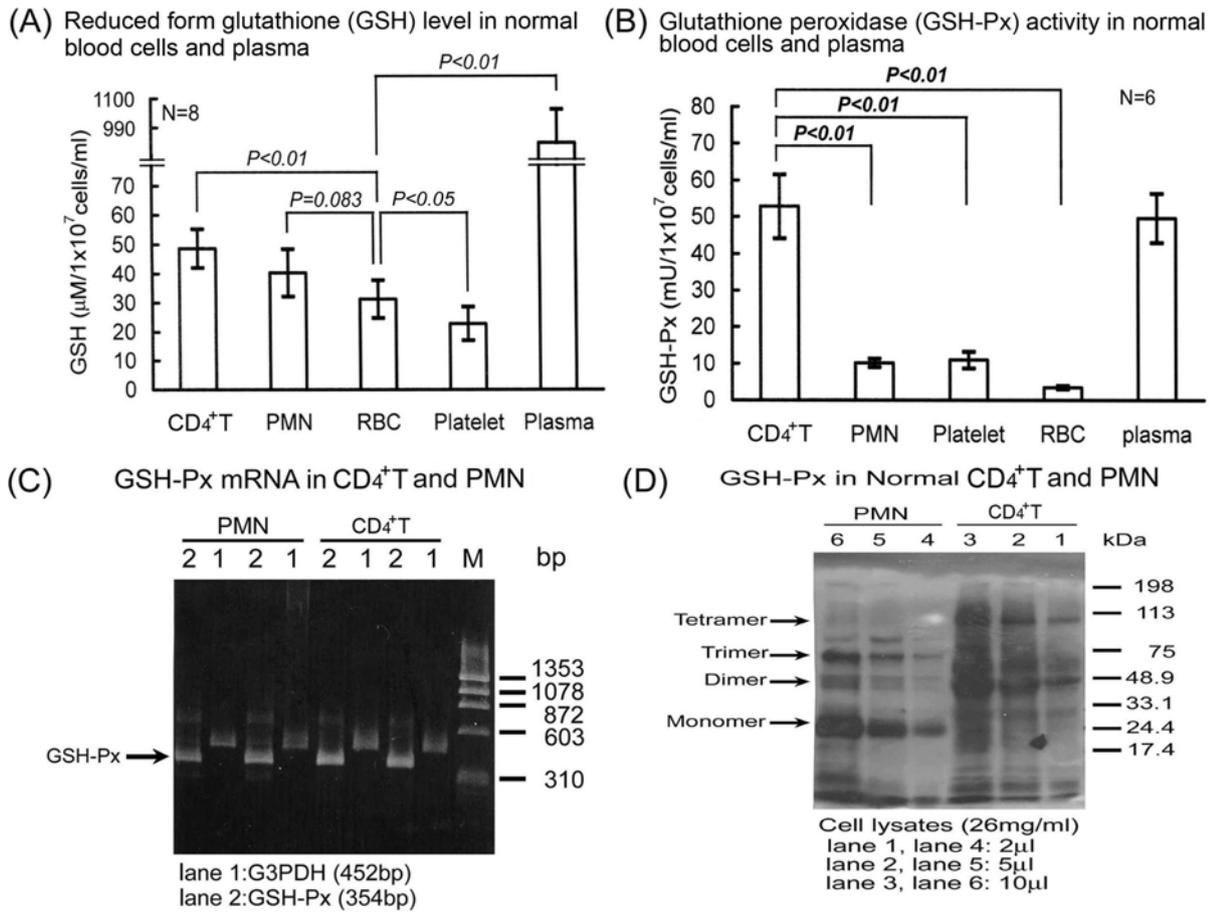


Fig.2

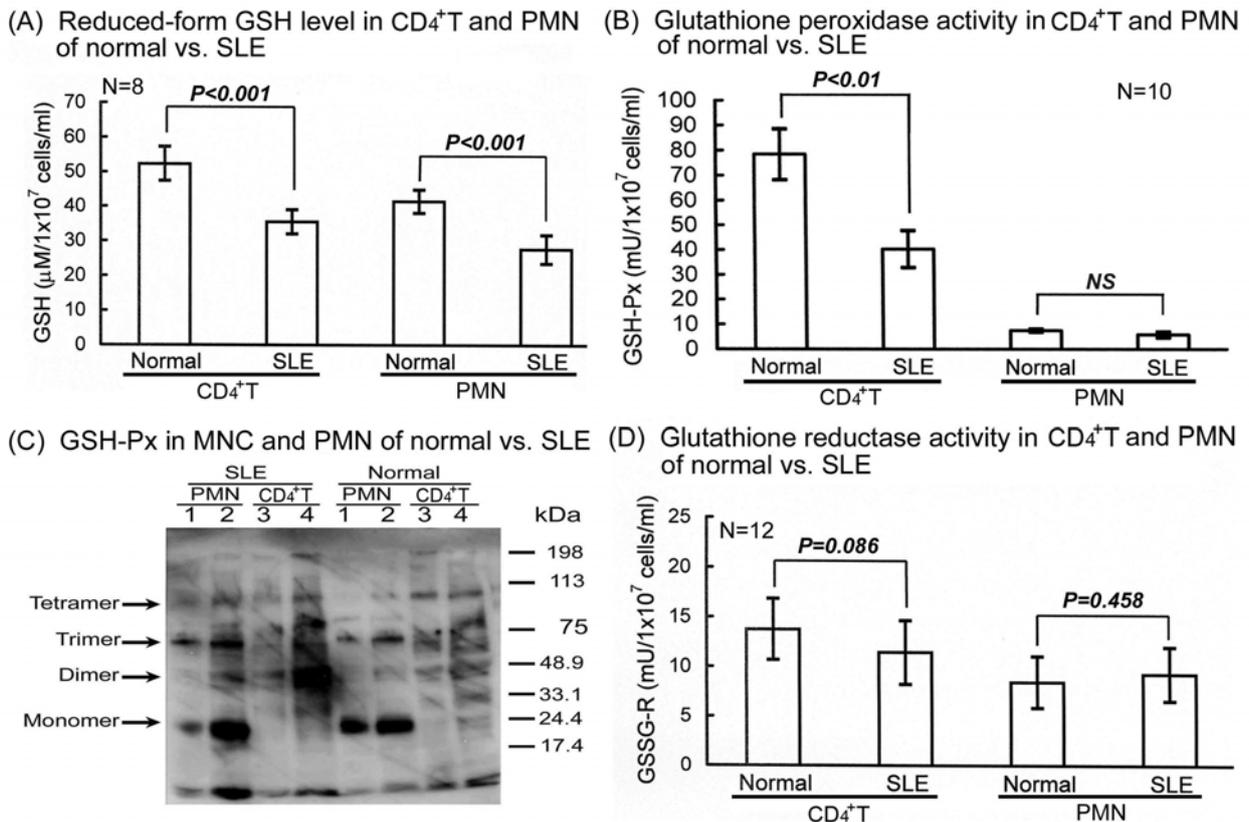


Fig.3

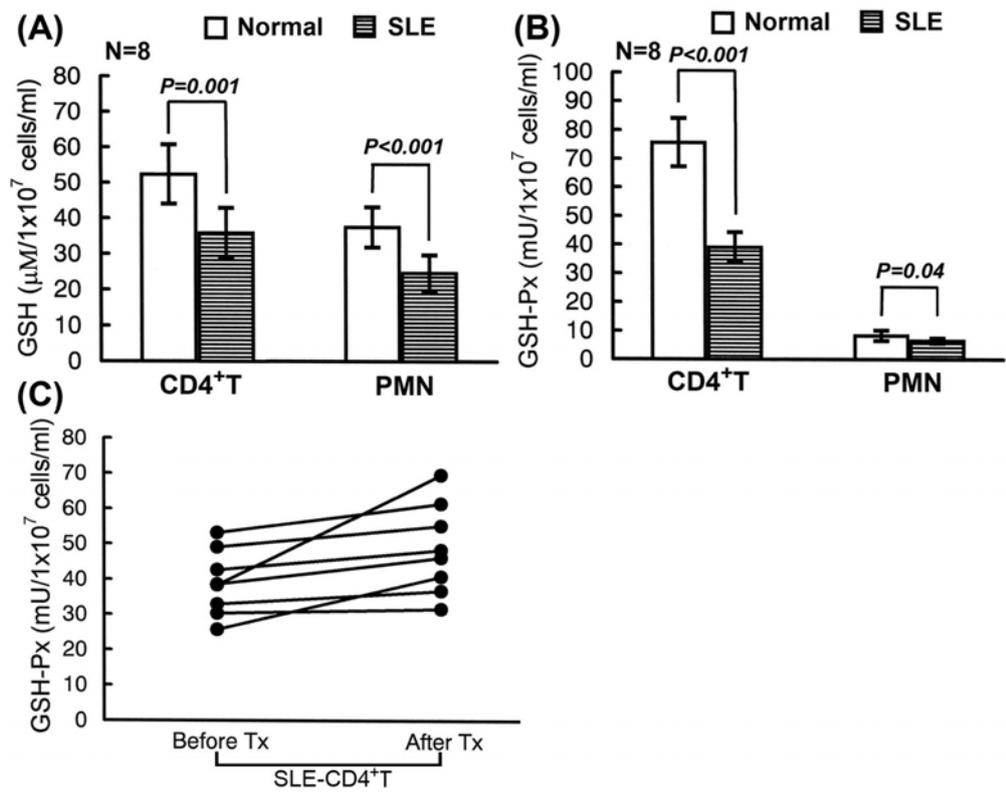


Fig.4

