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Altered redox state and apoptosis in the pathogenesis of systemic lupus erythematosus

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ABSTRACT

An altered redox status and increased lymphocyte apoptosis have been implicated in the development of systemic lupus erythematosus (SLE). In this study, we evaluated the relationship between glutathione (GSH) depletion, reactive oxygen species (ROS) and, the progression of apoptosis and their association with SLE severity. Significant low levels of intracellular glutathione, total thiol and altered redox state (GSH/GSSG) were found in SLE patients, in which lymphocyte apoptosis and activated caspase-3 expression in the lymphocytes were remarkably increased. The severity of disease was positively allied with the increased levels of lymphocyte apoptosis and caspase-3, but negatively with the decreased levels of total thiol, depleted intracellular glutathione and altered redox state (GSH/GSSG). The lymphocyte apoptosis and activated caspase-3 expression were negatively associated with intracellular levels of GSH and redox state and positively associated with the elevated levels of multiple oxidative stress markers; ROS and lipid peroxidation measured as malondialdehyde (MDA). These results suggest that GSH depletion and elevated oxidative stress trigger apoptosis and may be coupled with the severity of the disease.

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Introduction

Systemic lupus erythematosus (SLE) is a multifactorial chronic autoimmune disease with unidentified etiology. It is characterized by arthritis, cutaneous rash, and vasculitis, and involves central nervous system, renal and cardiopulmonary manifestations (Amital and Shoenfeld 2004). Several lines of evidence suggest that chronic immune activation in the disease is caused by depletion of intracellular glutathione through the oxygen-derived free radical production, known as oxidative stress (Kurien and Scofield 2003; Munoz et al. 2008; Li et al. 2009). Glutathione was significantly reduced in various components of blood including erythrocytes, monocytes, and plasma (Gergely et al. 2002a; Shah et al. 2011a), which leads to alter in redox state in SLE. The changes in the intracellular redox have been reported to be critical for cellular immune dysfunction, activation of apoptotic enzymes and progression of apoptosis (Li et al. 2012).

Apoptosis is a highly organized process, characterized by reactive oxygen species formation, changes in intracellular ionic homeostasis, cell shrinkage, loss of membrane lipid asymmetry, chromatin condensation and cell fragmentation (Green 2003; Bortner and Cidlowski 2002). A body of evidence has shown that excessive ROS production damages macromolecules, including DNA and proteins and can modulate expression of a variety of inflammatory molecules, exacerbating inflammation and tissue damage in SLE (Grisham 2004; Hassan et al. 2011). The primary targets of ROS are double bonds in polyunsaturated fatty acids in the cell membrane, which increase lipid peroxidation (LPO) and result in oxidative damage in SLE (Kurien and Scofield 2006; Perricone et al. 2009). An increase in protein oxidation markers (Morgan et al. 2009), MDA-modified proteins (Ben Mansour 2010), anti-SOD and anti-catalase antibodies (Mansour et al. 2008), correlating with SLE severity further support the role of oxidative stress in SLE.

Recently, the role of GSH depletion and altered redox state in the progression of inflammation and deregulation apoptosis

Abbreviations: CAT, catalase; C3, complement component 3; C4, complement component 4; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; ESR, erythrocyte sedimentation rate; Gpx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; FITC, fluorescein isothiocyanate; LPO, lipid peroxidation; MFI, mean fluorescence intensity; MDA, malondialdehyde; NBT, nitroblue tetrazolium; PBMCs, peripheral blood mononuclear cells; PI, propidium iodide; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SLEDAI score, systemic lupus erythematosus Disease Activity Index score; SOD, superoxide dismutase; TSH, total thiols.

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have been intensively studied in patients with autoimmune disease (Ballatori et al. 2009). However, the relationship of depletion of intracellular glutathione, altered redox state and apoptosis in SLE remains unclear. Also, there is lack of studies depicting the correlation of redox state and apoptosis together with severity of disease in SLE patients. Thus, we explored interactions among depletion of glutathione, altered redox state, and apoptosis with respect to the disease severity, which may have further implications in understanding the pathology and therapeutic management of the disease.

Materials and methods

Patients and controls

Patients for the study were selected from individuals attending out-patient Department of Internal Medicine at Postgraduate Institute of Medical Education and Research, Chandigarh, India. The study included 40 patients with SLE (36 females, 4 males) with mean age of 29.20 ± 8.50 years and the control group consisted of 40 healthy volunteers (36 females, 4 males) with mean age of 28.75 ± 7.60 years.

Measurement of SLE Disease Activity Index (SLEDAI) score

The individual with SLE was diagnosed using the American College of Rheumatology (ACR) 1997 revised criteria (Hochberg 1997). These revised criteria are; malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder, hematologic disorder, immunologic disorder (anti-dsDNA, anti-Sm, and/or anti-phospholipid) and antinuclear antibodies. Any combination of 4 or more of 11 criteria, well-documented at any time during a patient's history, makes it likely that the patient has SLE (specificity and sensitivity are 95% and 75%, respectively). Disease activity was determined by using SLE Disease Activity Index (SLEDAI) score (maximum score of 105): mild score <10; moderate score 10–20; severe score >20 (Bombardier et al. 1992). The scale consists of 24 weighted attributes, grouped into nine domains by organ systems (weightings in brackets): central nervous system (8), vascular (8), renal (4), musculoskeletal (4), serosal (2), dermal (2), immunologic (2), constitutional (1) and hematological (1). If during the 10-day period prior to the assessment a patient full-fills an attribute, then the corresponding score is given. The sum of all weighted attribute scores comprises the final SLEDAI score (range, 0–105) with a score of 0 being no disease. The study protocol was approved by the Institute Ethics Committee, Postgraduate Institute of Medical Education and Research, Chandigarh, India and informed consent was obtained from all the patients and healthy subjects.

Inclusion and exclusion criteria

All the patients enrolled in the present study were non-smokers and non-alcoholics, not associated with any other autoimmune disease and under standard care treatment, which included prednisolone with or without combination of other drugs like; cyclosporine A and hydroxychloroquine. Individuals with incomplete lupus erythematosus (ILE) or SLE patients overlapping with some other disease or drugs induced lupus or SLE patients taking cytotoxic drugs were excluded from the study.

Blood sample collection

Venous blood samples obtained from patients and controls were collected into heparinized vacutainers (Becton Dickinson, USA). Erythrocyte hemolysate was prepared by the method of Lohr and Waller (1974) for the estimation of lipid peroxidation, GSH and antioxidant enzymes (SOD, CAT and GPx). Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by density gradient centrifugation using Ficoll-Histopaque-1077 (Sigma–Aldrich, USA) (Kay 1980) for determining ROS production, apoptosis, and caspase-3 activation in the lymphocytes.

ROS measurement

The PBMCs isolated from the blood samples were washed twice with phosphate-buffered saline (PBS) and cell viability was assessed by trypan blue exclusion method. ROS production was detected by flow cytometry using the dye 2′,7′-dichlorofluorescein diacetate (DCFH-DA, Sigma) according to the method of Sarkar et al. (2006). DCFH-DA is a stable non-fluorescent, cell permeable compound, which upon penetrating the cell is converted to the highly fluorescent 2′,7′ dichlorofluorescein (DCF) by intracellular esterases. The deesterified product is trapped and stable within the cell, and emits green fluorescence upon excitation at 488 nm, proportional to the intracellular level of ROS, which is commonly used to quantify ROS production in cells. To measure intracellular ROS, 2′,7′-dichlorofluorescein diacetate (10 mM) was added to the mononuclear cells (1×10^6 cells/ml) and incubated at 37 °C for 30 min in dark. Cells were then washed, resuspended in PBS, and kept on ice for an immediate detection of ROS by flow cytometry (FACS Calibur, Becton Dickinson, San Jose, CA). Data was acquired and analyzed using the CELL Quest software (Becton Dickinson).

Assay for lipid peroxidation

The quantitative measurement of lipid peroxidation was performed in the erythrocytes hemolysate according to the method of Buege and Aust (1978). The amount of MDA formed was measured by the reaction with thiobarbituric acid at 532 nm. The results are expressed as nmol MDA/mg protein using molar extinction coefficient of MDA–thiobarbituric chromophore ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

Determination of SOD activity

The superoxide dismutase activity was assayed in the erythrocytes hemolysate according to the method described by Kono (1978). The method is based on the generation of superoxide radicals produced by the oxidation of hydroxylamine hydrochloride that reduces nitroblue tetrazolium (NBT) to blue formazone dye. The reduction of nitroblue tetrazolium to blue formazon was measured at 560 nm under aerobic conditions. Addition of superoxide dismutase inhibited the reduction of nitroblue tetrazolium and the extent of inhibition was taken as a measure of enzyme activity. One unit of enzyme activity was defined as the amount of enzyme necessary to produce 50% inhibition in the rate of reduction of nitroblue tetrazolium (NBT). The enzyme activity is expressed as unit (U)/mg protein.

Determination of CAT activity

The catalase activity was assayed in the erythrocyte hemolysate by the method of Luck (1971). Hydrogen peroxide decomposition by CAT was monitored spectrophotometrically by recording the decrease in absorbance at 240 nm for 3 min. Results are expressed as U/mg protein using molar extinction coefficient of H_2O_2 ($71 \times M^{-1}\ cm^{-1}$).

GPx activity

The glutathione peroxidase activity was measured in the erythrocytes hemolysate by the method of Paglia and Valentine (1967).

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