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Differential reactive oxygen species production of neutrophils and their oxidative damage in patients with active and inactive systemic lupus erythematosus

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ABSTRACT

Objective: Increasing interest is given to the involvement of the innate immunity and especially Polymorphonuclear neutrophils (PMN) in the physiopathological process of inflammatory diseases such as systemic lupus erythematosus (SLE). Here, we investigated the oxidative burst and damages in SLE patients neutrophils, considering the two phases of the disease, the active and the remission/inactive states.

Methods: This study was conducted on 30 SLE patients and 23 healthy controls. The oxidative burst in neutrophils of SLE patients and controls was triggered by fMLP and TPA, while reactive oxygen species (ROS) production was evaluated using a chemiluminescence assay. Oxidative damages in neutrophils were assessed by measuring Free thiol groups level and carbonyl groups, as protein oxidative markers. The malondialdehyde (MDA) level informed about the lipid peroxidation (LPO) and the catalase activity indicated the antioxidant enzymatic activity.

Result: Compared to controls, SLE patients exhibited a significantly increased level of ROS production concomitantly to a decreased response time. Their Neutrophils were characterized by a decreased level of MDA and high levels of protein oxidation as evidenced by increased carbonyl groups and decreased SH levels. The catalase activity was higher in SLE patients' neutrophils compared to controls. When patients were clustered according to the disease activity, PMN of patients in active phase showed, paradoxically, a lower ROS production and exhibited higher oxidative damages than the inactive group.

Conclusion: Our results highlight an altered behavior of LES patients derived PMN particularly in the active phase of the disease. The evaluation of the redox status including the rate of ROS production could be a biological marker to follow the activity of the disease.

1. Introduction

The innate immunity is the first line of defense against microbial pathogens and foreign substances. Among the different effectors of innate immunity, neutrophils appear to be the most important cells of immune response mainly because they are the first white blood cells that are recruited to the inflammation sites. In response to a variety of stimuli, neutrophils adhere to and migrate through the endothelium to reach the inflamed tissue where they engulf invading microorganisms and destroy them through multiple oxidative and non-oxidative mechanisms [1–3]. The oxidative destruction of microorganisms in neutrophils is closely connected to the NADPH oxidase-dependent mechanisms in a phenomenon described as the respiratory burst [4]. It is characterized by an overproduction rate of superoxide anion, the precursor of a wide range of reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical, exerting different physiological and pathophysiological effects [5]. Despite their beneficial role in the destruction of pathogens, chronic and/or uncontrolled ROS production can provoke several oxidative

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damages in lipids membrane, DNA, proteins and other biological macromolecules leading to the onset of various autoimmune and inflammatory diseases. Indeed, several studies reported that neutrophils contribute significantly to tissue damage in acute disease processes, such as acute lung injury and spinal cord injuries, as well as in chronic disease processes, such as rheumatoid arthritis, systemic lupus erythematosus, chronic obstructive pulmonary disease, inflammatory bowel diseases, atherosclerosis, asthma etc. [3,6].

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by a large array of clinical symptoms, indicative of widespread immune- mediated damage [7]. SLE is one of the autoimmune diseases in which the oxidative stress seems to be involved in the onset as well as in the progression of the disease. The progression of SLE is characterized by the alternation of active and inactive periods, and by tissue damage mediated by the deposition of immune complexes (IC) and activation of the complement system, with subsequent recruitment of neutrophils [8]. PMN are the major type of cells involved in the inflammatory reaction, a special attention was given these last years to the physiology of PMN in SLE [9–11] and their implication in its pathogenesis, mainly via their proprieties of superoxide formation [12–14].

In a previous work, we observed an impaired oxidative profile in SLE patients compared to controls. To explain these facts, we speculated that the oxidative profile is the consequence of an over production of ROS accompanying the strong inflammatory reaction, one of the most important clinical features of the disease [15].

To understand more the role of neutrophils in SLE physiopathology, we studied here the redox statue of lupus derived neutrophils compared to healthy controls. First, ROS production rate was assessed in response to fMLP or TPA stimulation. Then, and in order to evaluate the outcome of the oxidative micro-environnement on PMN, different markers of oxidative stress were considered; namely LPO, protein oxidation and catalase activity.

2. Material and methods

2.1. Patients and controls

Twenty eight SLE patients were selected for this study. The diagnosis of SLE was based on the American College of Rheumatology (ACR) criteria [16]. Disease activity was assessed using the systemic lupus erythematosus disease activity index (SLEDAI) [17]. Among our patients, 11 were in a lupus active phase (SLEDAI≥ 6) and 17 others were in inactive phase. Sex, age, disease activity, serological findings and major medications of patients are mentioned in Table 1. All treated patients were under a weak dose of corticoid treatment (not over of 10 mg/day) and no one of them is receiving immunosupressive drugs for over 3months.

Twenty three healthy subjects(13 women and 10 men) with a mean age 30.6 years SD = 7,7 were chosen as a control group. All patients and controls are coming from south Tunisia and were recruited for the study after giving their consent. This study was approved by the ethical committee of the Habib Bourguiba Hospital of Sfax, Tunisia (protocol number of ethical committee, 4/12).

2.2. Polymorphonuclear neutrophils (PMN) isolation

Blood was drawn from the cubital median vein of patients and controls and placed in citrate-anticoagulated tubes. Neutrophils were isolated from whole blood using Granulosep purification kit (Eurobio, AbCys®) according to the manufacturer's instructions. After isolation, the viability and purity of neutrophils were verified and evaluated >95%. Neutrophils were used to perform the oxidative profile and ROS generation.

Table 1
Demographic and serologic data and major medications of SLE patients.

		Active lupus	Inactive lupus
Sex (F/M)		10/1	14/3
Age (mean ± SD)		34,8 ± 12,6	34,11 ± 9,4
SLEDAI (min/max)		6 to18	0
Anti-ANA	+	11	13
	–	0	4
Anti-dsDNA	+	8	7
	–	3	10
Anti-Nucleosome	+	7	9
	–	4	8
Anti-Histone	+	5	3
	–	6	14
Anti-Sm	+	5	2
	–	6	15
Low level complement	+	7	0
	–	4	17
Major medication	Treated	3 ≤ 10 mg prednisone/day ≤400 mg HQN/day	≤10 mg prednisone/day ≤400 mg HQN/day
	Newly diagnosed	8	

SD: standard deviation, +: presence, –: absence, HQN: hydroxychloroquine.

2.3. Measurement of ROS production by neutrophils

The measurement of neutrophil ROS generation was made by chemiluminescence using a microplate luminometer (Luminoskan Ascent, Thermo Electron Corporation®). luminol (5-amino-2, 3-dihydro 1, 4-phthalazinedione; Sigma chemical Co) was used as redox sensitive light emitting probe. The final reaction volume was fixed at 200 μl. Human neutrophils were seeded into white 96-well plates (Nunc) in Hank's Balanced Salt Solution (HBSS). The ROS production was evaluated at basal levels and after stimulation of PMN with fMLP (Formyl-Methionyl-Leucyl- Phenylalanine; Sigma Aldrich) or TPA (12- 0-tetradecanoylphorbol-13-acetate). The chemiluminescence signal was monitored for 10 min and the results were expressed as relative light units (RLU).

2.4. Assessment of oxidative profile in (PMN) lysates

2.4.1. Lipid peroxidation

MDA was used as marker of lipid peroxidation (LPO) in this study, it was evaluated using the thiobarbituric acid reactive species (TBARs) assay. Neutrophils lysates were diluted in 500 μl distilled water and 2 vol. of thiobarbituric acid agent were added (15% TCA and0.8% thiobarbituric acid in 0.25 Na HCl). The mixture was then heated at 95 °C for 15 min. After centrifugation at 3000 rpm for 10 min, the optical density of the supernatant was determined at 532 nm 1,1,3,3-Tetra-ethoxy propane (TEP) was used as a standard.

2.4.2. Catalase activity

Catalase activity was measured as described previously by Abei [18]. This method is based on the principle that the absorbance at 240 nm decreases because of dismutation of H_2O_2. The amount of H_2O_2 converted into H_2O and O_2 in 1 min under standard conditions is accepted as the enzyme reaction velocity. The number of catalase units was determined as follows: U/mL = [(3.45*slope)/0.05]*(1000/50 μl).

2.4.3. Free thiol group

Protein thiols were quantified spectrophotometrically using 5,5-dithionitrobenzoic acid (DTNB); 250 ml of freshly prepared 10 mM DTNB in 0.05 M phosphate buffer pH 8, were added to 50 μl of lysates in 1200 μl of0.05 M phosphate buffer. After incubation in the dark for15 min at room temperature, the release of 5-thiobenzoicacid was quantified by measuring the absorbance at