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Peripheral blood lymphocyte apoptosis and circulating dendritic cells in patients with systemic lupus erythematosus: correlation with immunological status and disease-related symptoms

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Abstract We investigated in vivo the relationship between the degree of peripheral blood lymphocyte apoptosis and circulating dendritic cells (DC) as well as the immunological status in 45 patients with systemic lupus erythematosus (SLE). Apoptosis was detected by phosphatidylserine externalization and assays to detect caspase activation. The total DC count (tDC) and their myeloid, mDC1 (BDCA1+) and mDC2 (BDCA3+), and plasmacytoid, pDC (BDCA3+), subtypes were assessed. Moreover, several immunological parameters, such as complement proteins, interferons (IFN), tumor necrosis factor (TNF)-α, interleukin (IL)-4, and IL-6 levels were assessed. There were no significant differences in both the intensity of apoptosis and DC counts between active and inactive SLE as well as between untreated patients and those treated with steroids. The incidence of lymphocyte apoptosis correlated positively with T-cell count, both T-helper (p = 0.004) and cytotoxic T cells (p = 0.001), but not with B or natural killer (NK) cells. The intensity of apoptosis enhanced along with the increase in complement C3 (p = 0.016) and decrease in IFN-γ (p = 0.040) levels. The apoptotic cell count correlated with tDC (p = 0.031), mDC1 (p = 0.007), and pDC (p = 0.039) counts. Both tDC and mDC1 counts correlated positively with antinuclear antibody (ANA) titers (p = 0.017 and 0.032, respectively). Moreover, tDC correlated with C4 (p = 0.039) and pDC with both C3 (p = 0.032) and C4 (p = 0.007) levels. The DC counts correlated inversely with IFN-γ (tDC, p = 0.038; mDC1, p = 0.009), IL-6 (mDC2, p = 0.031), or serum IgG levels (tDC, p = 0.006; mDC1, p < 0.001; mDC2, p = 0.001). We found a positive correlation between lymphocyte apoptosis and peripheral blood DC count as well as the level of complement proteins in patients with SLE. The enhanced lymphocyte apoptosis was accompanied by the decrease in concentration of some cytokines, such as IFN-γ or IL-6, as well as serum IgG level. This finding may reflect pathogenetic events during development of the disease, which include a persistent signal derived from circulating apoptotic lymphocytes, mobilizing the complement system, and attracting peripheral blood DC.

Keywords Apoptosis · Complement · Dendritic cell · Immune system · SLE

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by B cell hyperactivity and defective T-cell function, with production of high-titer autoantibodies. So far, the pathogenesis of this disease has not been definitely elucidated; however, based on recent knowledge, impairment of at least three main mechanisms seems to be involved in SLE development: apoptosis, antigen presentation, and immune response.

Since the 1990s, a mounting body of evidence has been found that nucleosomes derived from apoptotic cells are antigens in SLE pathogenesis [1]. Persistent immunization resulting from impaired macrophage phagocytosis, reduced apoptotic cell clearance, and decreased production of immunosuppressive cytokines by defective macrophages was described [1–3]. Mechanisms of the impaired apoptosis of SLE lymphocytes have been extensively investigated. The increased expression of Fas/APO-1 and bcl-2 protein was found in SLE lymphocytes [4–6]. sFas levels seem to be increased secondary to tumor necrosis factor (TNF)-α concentration [7]. The alteration in the Fas signal transduction pathway leads to T-cell proliferation [8].
Defects in T-cell function observed in SLE may result from underlying defects in the function of antigen-presenting cells (APC), such as dendritic cells (DC). The DC are a group of bone marrow-derived cells responsible for the uptake, transport, processing, and presentation of antigens to T cells [9]. Thus, DC may play a crucial role in the pathogenesis of SLE providing costimulation to T cells. The SLE serum induces in vitro monocyte differentiation to DC, which is mediated mainly by interferon (IFN)-α [9–11]. On the other hand, plasmacytoid DC (pDC) are the major cellular source of IFN-α [9, 12]. Moreover, distinct abnormalities in the number of DC and their particular subsets were found in SLE patients, including significant reduction of CD11c+, myeloid DC (mDC) [13]. Accumulation of apoptotic cells in germinal follicles and attachment of apoptotic cell-derived nuclear antigens to the surface of follicular DC were described in SLE patients [14].

Deficiency in the components of the classic pathway complement is also associated with the pathogenesis of SLE. In addition, development of the disease causes further consumption of complement, which enlarges the defect in apoptotic cell clearance [15, 16]. Impaired in vitro phagocytosis of iC3b-opsonized apoptotic cells by macrophages in SLE is associated with accelerated macrophage apoptosis [17]. The role of anionic surface phospholipids for anti-inflammatory clearance of apoptotic cells was indicated [18]. Thus, impaired clearance and opsonization with antiphospholipid antibodies are discussed as being responsible for SLE development.

Several studies have been performed either in vitro or in experimental models, focusing separately on particular, possible pathways of SLE pathogenesis, including lymphocyte apoptosis, antigen-presenting cell, or complement activation abnormalities. There were, however, no comprehensive clinical studies evaluating all those parameters under in vivo conditions. Therefore, we aimed to investigate in vivo whether any cross-talk exists between the intensity of spontaneous apoptosis of circulating lymphocytes and peripheral blood DC in patients with SLE. The other question was if there is any relationship between those two parameters and immune status markers, including complement activation, or the disease-related symptoms.

Materials and methods

The study was performed in 45 patients with SLE, 42 females and 3 males (mean age: 44 years, range: 22–66 years). Twenty-one healthy volunteers, sex- and age-matched, served as a control group. The SLE was diagnosed according to revised criteria of the American College of Rheumatology [19]. The study was performed on SLE patients available during the period of collecting samples, actually diagnosed or under observation, with inactive or active disease, untreated or treated with steroids. Patients treated with other immunosuppressive agents were excluded from the study.

The mean disease duration was 64 months (4 months to 25 years). Sixteen patients had never been treated with immunosuppressive agents. Twenty-nine of them were treated with prednisone at a dose of 5–20 mg/day. Disease activity was scored according to the Systemic Lupus Activity Measure (SLAM) [20]. The score 0–15 points was assumed as inactive disease and over 15 points as active disease [21]. The SLE patients and the controls showed no clinical signs of infection or neoplastic disease and received no other medications for at least 4 weeks prior to blood donation. All patients’ samples were collected by venipuncture for routine laboratory investigations. The study was performed in accordance with the Helsinki declaration. Informed consent was obtained from all patients participating in the study, and was approved by the local Ethics Committee.

Skin lesions

Together with other clinical features of SLE, the skin lesions accompanying SLE were also routinely assessed. Namely, the SLE-related skin involvement was defined as the presence of at least one of the following: malar rash, discoid rash, oral ulcers, and photosensitivity. Moreover, vasculitis and/or alopecia were taken into consideration as suggested additional dermato logic criteria of SLE [22].

Isolation of PBMCs

The peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized blood by centrifugation in the Histopaque-1077 (Sigma Diagnostic, St. Louis, MO, USA) density gradient. Isolated PBMCs were split; a portion of them were resuspended in phosphate-buffered saline (PBS, Sigma–Aldrich, Deisenhofen, Germany) for immunophenotypic study and apoptosis assessment. Simultaneously, samples of serum were collected and stored at −80°C.

Lymphocyte gating and immunophenotyping

The PBMC immunophenotyping was performed by standard double-color immunofluorescence measurement using flow cytometry (FACScan, Becton-Dickinson, Franklin Lakes, NJ, USA), using a combination of fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated monoclonal antibodies (MoAbs). Based on the side scatter (SSC) versus forward scatter (FSC) discrimination method and CD14 and CD45 RO antigen expression, the lymphocyte population was identified in PBMC for further analysis. Moreover, cell